Carly Anderson

Title:

Modulation of dendritic spine density in CA1 hippocampal neurons by GluN3A containing NMDA receptors

Abstract:

NMDA receptors (NMDARs) are a subset of ionotropic glutamate receptors that mediate excitatory synaptic transmission within the central nervous system. These receptors are composed of four subunits, two of which are obligatorily GluN1, the remaining two are either GluN2A-D or GluN3A-B subunits. These different GluN2 and GluN3 subunits have distinct spatial and temporal expression in the central nervous system, suggesting they play distinct physiological roles. Dendritic spines are protrusions off the neurons that form excitatory postsynaptic terminals. During early development synapse and spine proliferation occurs at high levels, during which GluN3A-containing NMDARs are thought to act as a repressor of spine maturation and thereby promote spine elimination. Thus, Dendritic spine density is higher in GluN3A KO mice, and there are several neurological diseases which display a notable change in the average number of dendritic spines, including Huntington's disease, Alzheimer's disease, schizophrenia, and autism spectrum disorder. Understanding the relationship between GluN3A-containing NMDARs, spine density, and the impact on neurons may advance our understanding of these diseases. We have shown that overexpression of GluN3A decreases the average dendritic spine density in hippocampal CA1 neurons and is investigating effect of known NMDAR modulators on spine density

Jasper Aquino

Title:

Methoxyamino and 2-aminopyridyl functionalized scaffold as tool for systematic investigation of β-glucan binding and Dectin-1 activation

Abstract:

Tuberculosis (TB) – an infectious disease caused by Mycobacterium tuberculosis (Mtb) – remains as a global epidemic. To address this epidemic, a protective vaccine is needed. In recent years, synergistic Th1 and Th17 cells have emerged as the key players for a vaccine-induced protection against TB. There is currently no vaccine approved for humans that elicits a Th17-mediated response. Dectin-1, a pattern recognition receptor (PRR) of β-1,3-glucans (βglucans) primarily from fungi cell walls, is known to induce a Th17-mediated response. The Th17 response upon binding of β-glucan and activation of Dectin-1 is determined by the β-glucan properties namely: size, structure (branching), multivalency and 3D structure. However, the mechanism of action is poorly understood. Our project seeks to develop a molecular tool for a systematic investigation of these properties of β-1,3-glucans and probe their effect in the binding and activation of Dectin-1

Elizabeth Arrigali

Elizabeth Arrigali[1], Monica Serban[*1]
Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT, United States

Title:

Novel Topical Therapeutics Against Hearing Loss

Abstract:

Introduction: Regardless of the etiology of hearing loss, mechanistically, inflammation and oxidative stress are major players in inner ear structures damage. Therefore, treatments targeting reactive oxygen species and inflammation represent a viable approach for the prevention of noise induced hearing loss. Our group has synthesized several dual acting antioxidant/anti-inflammatory therapeutics that show promise in protecting inner ear cells against oxidative damage.

Experimental methods: Hyaluronan-antioxidant conjugates (HAO) were prepared by chemical conjugation of HA and antioxidants. The conjugates were chemically characterized via 1H-NMR and HPLC. The materials were tested for cytocompatibility with mouse inner ear cells with a MTS colorimetric assay. Cell proliferation rates of conjugate-treated cells determined via CyQUANT assay. The oxidative protection properties of HAO were assessed using a fluorescent (CMH2DCFDA) reagent. The mechanism of oxidative protection of the conjugates was assessed microscopically and via NADP/NADPH assays.

Results and discussions: HAO conjugates were successfully synthesized, with structures and purity confirmed by 1H-NMR analysis and HPLC. When HEI-OC1 and SV-k1 cells were treated with HAOs, the metabolic activity in
both cell types significantly increased. Interestingly, this observation did not correlate with an increase in cell numbers, suggesting that HAOs directly impact the mitochondrial activity of the cells. As mitochondrial overdrive

is one of the issues leading to hearing loss, we next investigated if HAO treatment increases the reactive oxygen species (ROS) content of the treated cells; however, HAO treated cells displayed similar ROS levels with the untreated control cells. In contrast, when cells were treated with HAO then oxidatively stressed with hydrogen peroxide, the conjugates seem to have protective effects on both HEI-OC1 and SV-k1 cells. Our additional data indicate that HA helps with the internalization of the antioxidant thus providing protective effects.

Conclusions: Our data so far highlights the practicality of chemically conjugating HA with an antioxidant. We anticipate that our ongoing experiments will support our hypothesis that HA conjugation with antioxidants can protect inner ear cells from oxidative damage and can enhance the drug's permeability across the round window membrane.

References/Acknowledgements: M.A. Serban, Synergistic hyaluronan-based anti-inflammatory and anti-oxidant therapeutics, US 62/571,011.

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Disclosure of Interest: None

James Bosco

Title:

GLH Protein at the Heart of P Granule Network

Abstract:

Germ granules are cytoplasmic ribonucleoprotein assemblies conserved across the vast majority of metazoans from worms to humans. One key component of germ granules are Vasa-family DEAD box RNA helicases. Vasa mutation or knockdown compromises germ cell specification, survival and function leading to sterility in various organisms. C. elegans has four Vasa homologs, GLH-1, GLH-2, GLH-3, and GLH-4, all of which localize to germ granules called P granules. GLH proteins contribute to the integrity of P granules in C. elegans germline (Kuznicki et al., 2000; Spike et al., 2008; Marnik et al., 2019). We asked if this function of GLH family proteins might be related to an ability to directly bind some core or transient P granule components by testing protein-protein interactions in vitro. Surprisingly, we found a diverse protein-protein interaction network for GLH-4 that includes dimerization with all four GLH family members, other core P granule components (PGL-1 and PGL-3) as well as P granule associated proteins FBF-2, DLC1, and MEG-4. We demonstrate that the C terminal DEAD box helicase region of GLH-4 is where GLH-4 is binding to each one of these P granule proteins. We find that PGL-1 exclusively binds to GLH-4, this binding interaction is independent of RNA, and that GLH-4 can bridge binding between GLH-1 and PGL-1. Taken as an aggregate, these results demonstrate a network of protein interaction within P granules that puts GLH-4 at the nexus for P granule organization and function.

Sofia de Mare

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Title:

Cryo-EM studies of fibronectin for structural determination

Abstract:

Fibronectin (FN) is a major component of the extracellular matrix (ECM) and has important roles in many physiological processes such as cell adhesion and migration, blood coagulation and wound healing. Compact, soluble FN molecules are secreted and assembled into elongated, insoluble FN fibrils in the ECM by the cells. Although studied extensively for decades, the structure of the intact FN molecule and structural details of intramolecular interactions and conformational changes involved in fibril formation are unknown. By using cryo-EM we aim to determine the structure of FN. FN is a ~500 kDa disulfidelinked dimer composed of multiple copies of FN type I, II and III domains. For our studies, FN is recombinantly expressed in HEK239T cells and purified by its intrinsic gelatin-binding domain using gelatin Sepharose affinity resin. Cryo-EM grids with FN frozen in vitrified ice were prepared, and we found that single particles of FN, as well as FN fibrils of varying thickness can be imaged by cryo-EM. Single molecules of FN displayed a variety of conformations, consistent with previous research findings. With the recent advances in cryo-EM technology, multiple conformations of a protein can successfully be processed and classified for structural determination. The size of FN and its propensity to form fibrils make FN an interesting target for cryo-EM studies. Structural information about FN is highly desired as it would provide insights essential for answering fundamental questions about FN function and fibril formation, and its role in physiological and pathophysiological processes.

Mikhail Drobizhev

Title:

Resource for Multiphoton Characterization of Genetically-Encoded Probes

Abstract:

Two-photon absorption spectra of molecules are different from onephoton absorption spectra because the two processes are described by different quantum mechanical rules and expressions. Our Resource supported by the NIH BRAIN Initiative makes it possible to measure two- and three-photon absorption spectra of fluorescent molecules in solutions as well as their multiphoton absorption cross sections in the spectral range from 680 - 1300 nm. This information is necessary for optimum selection and improvement of properties of fluorescent probes and biosensors for multiphoton microscopy of biological tissues.

Mary Ellenbecker

Authors: Nicholas J Day, Mary Ellenbecker, Xiaobo Wang and Ekaterina Voronina

Title:

DLC-1 promotes germ granule integrity in *C. elegans* embryo

Abstract:

P granules are RNA-protein complexes located in the germline of *Caenorhabditis elegans* that are important for RNA regulation, germ cell identity and fertility. P granules are present throughout the *C. elegans* germline lifecycle, during which they undergo several dynamic transitions. For example, P granules are perinuclear during most of germline development but become cytoplasmic during oocyte maturation and during embryogenesis P granules segregate asymmetrically with the P cell lineage that produces the primordial germ cells. Here we identify dynein light chain (DLC-1) as an important determinant of P granule formation and subcellular localization in the *C. elegans* embryo. DLC-1 is a bimolecular hub that interacts with a variety of cellular proteins and functions in diverse processes from dyneindependent transport to allosteric regulation of ribonucleoprotein complexes. We used an *in silico* motif scanning approach to search for new DLC-1 binding partners, identifying several P granule components as a result. Direct interaction between DLC-1 and PGL-1, PGL-3, GLH-4 and MEG-4 was established using a biochemical binding assay *in vitro*. We confirmed that DLC-1 is in complex with P granule components PGL-1 and PGL-3 in the germline and embryo *in vivo* using proximity ligation assay. Loss of *dlc-1* disrupts assembly of embryonic P granules as assessed by localization of multiple P granule components but does not impact the levels of PGL-1 or PGL-3 protein expression. Our findings shed light on DLC-1 contribution to germ granule phase separation.

Dominick Faith

Dominick R. Faith1, Amelia K. Schmidt1, Alexa D. Fitzpatrick4, Caleb M. Schwartzkopf1, Laura K.Jennings1, Alison Coluccio1, Devin J. Hunt1, Lia A. Michaels1, Dave W. Dorward2, Jenny Wachter3, Patricia A. Rosa3, Karen L. Maxwell4, and Patrick R. Secor1

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Title:

A Filamentous Bacteriophage Protein Inhibits Type IV Pili to Prevent Superinfection of Pseudomonas aeruginosa

Abstract:

Pseudomonas aeruginosa is an opportunistic pathogen that causes infections in a variety of settings. Many P. aeruginosa isolates are infected by filamentous Pf bacteriophage integrated into the bacterial chromosome as a prophage. Pf virions can be produced without lysing P. aeruginosa. However, cell lysis can occur during superinfection, which occurs when Pf virions successfully infect a host lysogenized by a Pf prophage. Temperate phages typically encode superinfection exclusion mechanisms to prevent host lysis by virions of the same or similar species. In this study, we sought to elucidate the superinfection exclusion mechanism of Pf phage. Initially, we observed that P. aeruginosa that survive Pf superinfection are transiently resistant to Pf-induced plaquing and are deficient in twitching motility, which is mediated by type IV pili (T4P). Pf utilize T4P as a cell surface receptor, suggesting that T4P are suppressed in bacteria that survive superinfection. We tested the hypothesis that a Pf-encoded protein mediated superinfection exclusion by suppressing T4P by expressing Pf proteins in P. aeruginosa and measuring plaquing and twitching motility. We found that the Pf protein PA0721, which we termed Pf superinfection exclusion (PfsE), promoted resistance to Pf infection and suppressed twitching motility by binding the T4P protein PilC. We went on to identify aromatic residues on PfsE that are critical for PilC binding. Because T4P play key roles in biofilm formation and virulence, the ability of Pf phage to modulate T4P via PfsE has implications in the ability of P. aeruginosa to persist at sites of infection.

Ariel Frederick

Title:

Effect on Intrinsic Peroxidase Activity of Substituting Coevolved Residues from omega-loop C of Human Cytochrome c into Yeast Iso-1-Cytochrome c

Abstract:

Apoptosis is a key process in various human diseases. It is initiated in the mitochondria, where changes in the local environment cause cytochrome c (cytc) to become competent for peroxidase activity. Human cytc has 20- to 30-fold lower peroxidase activity than yeast cytc. Many residues that have evolved between yeast and human are found in Ω-loop C, which is one of the most dynamic regions of cytc. This Ω-loop is thought to affect the dynamics of opening the heme crevice, and thus peroxidase activity. Residues present in human are larger and more hydrophobic than in yeast cytochrome c, so mutations have been made in yeast to reflect that. By mutating residues S40, N63, V57, and A81I, which are larger and more hydrophobic in human cytc, both individually and pairwise in yeast iso-1-cytc, we expect to see corresponding changes in peroxidase activity with minimal changes in overall structure. Circular dichroism was used to monitor guanidine unfolding and assess changes in global stability between each of the variants. The alkaline transition was monitored to estimate the effect of these mutations on Ω-loop D stability. Peroxidase activity measurements were conducted using stopped-flow with guaiacol as a substrate. Our results indicate that single mutations usually have small effects, and that as more mutations are added the peroxidase activity lowers. Based on the crystal structure of the N63T mutant and kinetic data from imidazole binding, we hypothesize that Ω-loop C may make specific hydrogen bond contacts that affect heme crevice dynamics and thus peroxidase activity.

Miyuki Hayashi

Title:

Characterization of nucleocapsid-RNA interactions in Rift Valley fever virus infection

Abstract:

Rift Valley fever virus (RVFV) is a negative sense, single-stranded RNA virus prevalent in Africa and the Arabian Peninsula. The nucleocapsid protein (N) in RVFV is an RNA-binding protein that is necessary for viral transcription, replication, and the production of nascent viral particles. We have conducted crosslinking, immunoprecipitation, and sequencing to characterize host and viral RNAs interacting with N during infection. Moreover, multiple reaction monitoring mass spectrometry was used to quantify N. Our result shows that N binds mostly to host RNAs at the early stage of infection, which results in reduced specific infectivity of nascent particles. The expression of N plateaus after 10-hour post-infection whereas the viral RNA concentration continues to increase. Taken together, these results suggest a regulated mechanism in the N expression and viral RNA expression important for the production of infectious particles.

Eric John

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Title:

Synthetic Anion Transporters Utilizing Hydrogen Bond Enhanced Halogen Bonds

Abstract:

Anion transport supports vital life functions. Disfunctions in chloride channels result in channelopathies like cystic fibrosis and Bartter syndrome. To better understand anion transport and related diseases, synthetic chemists have developed a variety of abiotic systems to mimic natural transporters. These will act as biophysical tools to selectively transport anions. Until recently, both natural and synthetic systems have employed hydrogen bonds (HBs) for anion transport. However, unlike amino acids used in nature, chemists are not limited to these noncovalent interactions. Our approach leverages the unique characteristics of the halogen bond (XB) to obtain a superior synthetic transporter. Compared to the HB, XB donors feature favorable lipophilicity, relative pH-insensitivity, and a more selective interaction due to their soft, linear nature. These properties are combined with our new strategy, the intramolecular hydrogen bond-enhanced halogen bond1-3 which preorganizes structure and enhances XB strength. Together, they motivate our pursuit of building XB foldamer transporters. Cellular transport will be mimicked with simple fluorescence assays involving Large Unilamellar Vesicles (LUVs) and a pH sensitive dye (HPTS). Foldamers which provide quick transport at low concentrations will be tested in vitro. By studying the transport ability and selectivity of these foldamers future studies for rational design of improved synthetic anion carriers and channels will be improved.

Allison Kelly

Title:

Design and Synthesis of Triazole Trehalose Tuberculosis Vaccine Adjuvants

Abstract:

Mycobacterium tuberculosis (Mtb) is a multidrug resistant bacterium that causes tuberculosis and is extremely prevalent in the world's population. Currently, there is only a vaccine given to adolescents but the immunity wanes over time, leaving no protection in adulthood. The toxicity of Mtb is due to the main component of the cell wall – mycobacterial cord factor trehalose 6,6'-dimycolate (TDM). The TDM in the cell wall inhibits immune cells to engulf the bacterium as well as makes the cell wall impermeable to potential therapeutics. These compounds are recognized by C-type lectin receptors (CLR), specifically our research focuses on Mincle. The Mincle-dependent pathway is a combination of a Th1- and Th17- immune response, proposing a potential target for adjuvant development. There has been a synthetic analog of TDM that has been developed, trehalose dibehenate (TDB), however is too toxic to be utilized. Other adjuvants have been established from TDM; however, structures not containing a long lipid chain did not induce an immune response. The compounds synthesized for this research contain trehalose, two 5-membered heterocyclic rings containing three nitrogens, and ether lipids to improve water solubility. These compounds differ in the carbon chain length of the ether lipid ranging from 5-10 carbons. These molecules will allow us to probe the Mincle specific pathway and further gain understanding into how to target a combined Th1 and Th17 immune response. The design and synthesis of Triazole Trehalose Tuberculosis Vaccine Adjuvants will be presented.

James Lotti

Title:

Teasing apart Binding Affinity and efficacy for NMDA receptor ligands

Abstract:

Binding affinity is defined as the strength of interaction, in this case between a receptor and a ligand. Efficacy, on the molecular level, is the ability of bound ligand to induce conformational change. The efficacy of an agonist for NMDA receptors, for example, would be a measure of the ability of bound agonist to induce a conformational change to open the channel pore. Determining binding affinity free from influence of efficacy in wild type NMDA receptors is challenging, since the receptor rapidly undergoes conformational changes following ligand binding that in turn alter affinity. To determine binding affinity and efficacy, we define a model representing the mechanism of NMDA receptor agonist activation and allosteric modulation. Fitting this model to our data enables measurements of binding affinity and efficacy for agonists and allosteric modulators, thereby providing quantitative insights to drug action that are required for rational drug design of more potent and efficacious ligands.

Michelle Nemetchek

Title:

Distinct coactivator structural classes reveal a new paradigm of nuclear receptor activation

Abstract:

Glitazones, also called thiazolidinediones or TZDs, are a commonly prescribed class of anti-diabetes drugs that target the nuclear receptor PPARγ. These effective insulin sensitization drugs are marred by side effects that can lead to worse outcomes in patients. Efforts to develop non-TZD moiety PPARγ ligands are promising, yet curious: though agonistic like TZDs, some ligands cause PPARγ to bind transcriptomic coregulator proteins to different extents, favoring some over others. The mechanism of these ligands (which we term “biased agonists”) has never been explained.

Through novel crystal structures reported here and many others deposited in the Protein Data Bank, we demonstrate that this bias is dependent on the type of structural cap that resides N-terminal to the nuclear receptor-binding motif. This cap controls how the coregulator interacts with PPARγ; namely, whether and how the coregulator binds to the helix 4 of PPARγ. These capping motifs and their importance are rigorously defined here by crystal structure, fluorescence anisotropy, isothermal titration calorimetry, and molecular dynamics. We also demonstrate the distinct transcriptional effects of these biased ligands through RNAseq. Particularly thrilling is that the structural groups of coregulator interaction motifs defined here apply to almost all proteins in the nuclear receptor family due to their conserved structure. These data illuminate how genes can be selectively controlled through nuclear receptors, and will inform the design of pharmaceutical drugs with fewer side effects.

Precious Ann Nepomunceno

Precious Ann Nepomuceno[1], Sofia de Mare[1], Tung-Chung Mou[2], Stephen Sprang[2], Klara Briknarova[1]
[1]Department of Chemistry and Biochemistry, [2]Center for Biomolecular Structure and Dynamics, University of Montana

Title:

The Effect Of Interdomain Interactions On The Structure And Stability Of The Eleventh Type Iii Domain From Human Fibronectin

Abstract:

Fibronectin type 3 (FN3) domains have a β-sandwich structure composed of three-stranded (β-strands A, B and E) and four-stranded (β-strands C, D, F and G) β-sheets. They have a high degree of similarity despite low sequence identity. In this study, we found that the β-strand G in the crystal structures of multi-domain protein constructs containing 11th FN3 was shifted by one residue compared to 11th FN3 alone. We also observed multiple conformations in the 1H15N HSQC NMR spectra of two to three-domain protein constructs containing 11th FN3. In addition to this, changes in the sequence in the C-terminal portion of 11th FN3 due to alternative splicing resulted in dramatical changes in the 1H-15N HSQC NMR spectra. 11th FN3 and its neighboring domains have very distinct stabilities when by themselves. Stability studies using tryptophan fluorescence in the presence of increasing amounts of guanidine hydrochloride of multidomain protein constructs containing 11th FN3 were done; however, the data still needs to be analyzed further to be able to draw concrete conclusions.

Emily Osterli

Title:

COP9 signalosome component CSN-5 promotes accumulation and function of stem cell regulators FBF-1 and FBF-2

Abstract:

RNA-binding proteins FBF-1 and FBF-2 (FBFs) are required for stem cell maintenance in C. elegans, although the mechanisms by which FBFs protein levels are regulated remain unknown. Using a yeast twohybrid screen, we identified an interaction between both FBFs and CSN-5, a component of the COP9 (constitutive photomorphogenesis 9) signalosome. This highly conserved COP9 complex can affect protein stability through a range of mechanisms including deneddylation, deubiquitination, and phosphorylation (Wolf et al., 2003). Mapping protein-protein interactions between FBFs and CSN-5 suggested that the MPN (Mpr1/Pad1 N-terminal) metalloprotease domain of CSN-5 interacts with the RNA-binding domain of FBFs at physiologically relevant (micromolar) concentrations. Furthermore, these conserved domains of the human homologs PUM1 and CSN5 interact as well, thus identifying a protein complex conserved in evolution. We discovered that CSN-5 promotes the accumulation of FBF-1 and FBF-2 proteins in C. elegans stem and progenitor cells. Phenotypic analysis results were consistent with csn-5 contributing to FBF function since csn-5 germlines are masculinized (produce only sperm similar to fbf-1/2 loss of function) and show reduced numbers of stem cells. Similar phenotypes were observed in worms mutant for another COP9 holoenzyme component, csn-6. Curiously, phenotypes of csn-2 mutant were clearly distinct, where oocytes were still forming and stem cell numbers were not as affected, suggesting that csn-5's effect on FBFs might be independent of the COP9 holoenzyme. Investigating CSN-5 contribution to FBF protein activity and stem cell maintenance will have implications for human stem cell biology and improve our understanding of diseases such as cancer.

Mariah Rayl

Title:

Molecular Dynamics Simulations Reveal Ligand Dependent Variation in PPARγ-Coregulator Interactions

Abstract:

Peroxisome-proliferator activated receptor gamma (PPARγ) is a major drug target for insulin sensitization in patients with type II diabetes mellitus. PPARγ alters transcription in a ligand dependent manner by recruiting coactivators containing an LXXLL motif. Unfortunately, the FDA approved PPARγ agonists induce serious side effects by increasing binding to many coactivators. Biased ligands that recruit specific coregulators are required for better patient outcomes. Recent work in the Hughes lab uncovered the structural feature on the coregulator that allows for biased recruitment. The mechanism by which ligands induce this bias is still unknown.

This work uses both accelerated and conventional molecular dynamics simulations to analyze protein movements and interactions with different ligands and coactivator peptides. Simulations include two coactivators from different classes: PGC1α and MED1. Both coactivators contain the canonical LXXLL motif, but PGC1α contains a different capping residue N-terminal to the motif than MED1. Each PPARγ-coregulator structure includes one of three ligands with differing structures: GW1929, rosiglitazone, and MRL24. Rosiglitazone is an FDA approved agonist, GW1929 is an agonist, and MRL24 is a partial agonist. In simulations the coregulator binding surface remains stable with all three ligands but hydrophobic interactions and hydrogen bonding differ based on both the ligand and the coregulator. This work provides a framework to interrogate the ligand dependent effects on PPARγ-coregulator interactions.

Mike Rothfuss

Title:

High accuracy achieved predicting stabilizing surface mutations in UBA(1)

Abstract:

Despite monumental advances in protein structure prediction our ability to predict the effects of mutations on protein stability remains limited. Restricting our scope to solvent accessible mutation sites, we have developed an extremely fast method to calculate the relative stabilities of amino acid substitutions within a protein's structure. The stability of UBA(1) was successfully doubled using 4 solvent accessible mutations that were predicted with unprecedented physical accuracy ($R^2=0.97$, slope=0.97). UBA(1) variants were crystallized to verify and analyze their structures at atomic resolution. Thermodynamic and kinetic folding experiments were performed to determine the magnitude and mechanism of stabilization. Our method has the potential to enable rapid, rational optimization of natural proteins to enhance their stability, shelf-life, and yield.

Elizabeth Sather

Title:

Biased Agonism in Farnesoid X Receptor

Abstract:

Farnesoid X Receptor (FXR) is a metabolic nuclear receptor. Also known as a bile acid receptor, FXR is a ligand-activated transcription factor responsible for regulating bile acid, lipid and glucose metabolism. This regulation occurs through co-activator recruitment after ligand binding.

We hypothesized that FXR agonists cause FXR to favor binding some coactivators more than others, which is known as biased agonism. Our lab previously established biased agonism in another metabolic nuclear receptor, PPARγ. To test this hypothesis, we tested the affinity of two different agonist-FXR complexes for two different coactivator peptides. The two agonists we used are 1) obeticholic acid and 2) GW4064. The co-activators we used are 1) Steroid Receptor Coactivator 2 (SRC2) and 2) Mediator Subunit 1 (MED1). Because FXR can be found in cells as a monomer or heterodimer with RXRα, we use a FXR Ligand Binding Domain (LBD) monomer as well as FXR-RXRα LBD heterodimer.

Luke White

Title:

Innate immune protein RIOK3 is regulated at the splicing level by TRA2-B during Rift Valley fever virus infection

Abstract:

Rift Valley fever virus (RVFV) is an emerging pathogen that has a potential to cause severe disease in humans and domestic livestock. Propagation of RVFV is negatively regulated by the actions of full length RIOK3, a protein involved in the cellular immune response to viral infection. During RVFV infection, RIOK3 mRNA is alternatively spliced to produce an isoform that inhibits interferon beta signaling. We identified splicing factor TRA2-B as a key regulator governing the relative abundance of RIOK3 splicing isoforms. Using RT-PCR, minigenes, and biotin proximity ligation, we identified TRA2-B binding on RIOK3 pre-mRNA as necessary for canonical splicing of RIOK3 mRNA. Intriguingly, TRA2-B mRNA is also alternatively spliced during RVFV infection. These results suggest that splicing modulation as a potential immune evasion strategy employed by RVFV and/or a cellular mechanism to prevent excessive immune response.