QCB EVENING

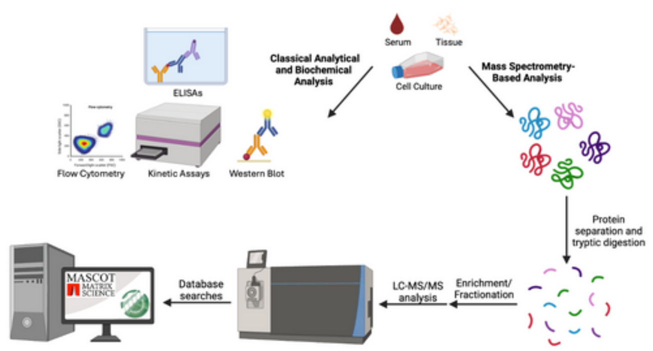
OCTOBER 15TH @ 5:45PM IN SI001

Allan Gramillo | Fuqua Lab

In the phytopathogen Agrobacterium tumefaciens biofilm formation relies on stable surface attachment via its polarly localized adhesin, the unipolar polysaccharide (UPP). Limiting phosphorus (Pi) stimulates UPP-dependent surface attachment in A. tumefaciens. The large monotopic phosphoglycosyl transferase (Lg-PGT, 518 aa), UppE is active under replete and limited Pi conditions. In Pi limitation a small monoPGT called UplP (UPP production in limited Pi; 242 aa), can direct UPP production in a uppE null mutant, but fails to restore full wild type stimulation of attachment observed in Pi limitation. Both UppE and UplP, contain a similar core with a single re-entrant membrane helix and a cytoplasmic catalytic center, but only Lg-PGTs contain a 4TM-RF domain: a four transmembrane helical bundle and a cytoplasmic Rossman fold domain. Truncation of the 4TM-RF from UppE, decreased its ability to fully drive surface attachment, similar to UplP under limited Pi. These observations suggested that the 4TM-RF domain might be required to stimulate surface attachment by UppE under low Pi. We created a chimeric protein by fusing the UppE 4TM-RF domain to UplP. Ectopic expression of the 4TM-RF-UplP chimera increased surface attachment similar to WT in limited Pi. However, at high Pi levels 4TM-RF-UplP expression caused production of a highly viscous, muco-elastic exopolysaccharide (EPS). We hypothesized that the chimeric protein may be perturbing precursor pools that impact other A. tumefaciens EPS pathways. Expression of the 4TM-RF-UplP in a series of different EPS mutants revealed that disabling the UPP or the chvAB genes encoding synthesis of beta-1,2-glucan, abolished the muco-elastic phenotype, but was not affected by the other A. tumefaciens EPSs. Mutation of the UplP catalytic site (D136N) disabling glycosyl transferase activity abolished this muco-elastic phenotype.

Cayla Rose Collins | Dann Lab

Protein tyrosine sulfation is a key post-translational modification (PTM) tied to many biological processes such as inflammation, hemostasis, visual functions, and viral infection. Tyrosine sulfation is mediated by the Golgi-resident enzymes tyrosylprotein sulfotransferase (TPST), catalyzes the transfer of the sulfonate group from Adenosine 3'-phosphate 5'-phosphosulfate to the hydroxyl group of tyrosine. Advancement in the understanding of sulfation within the proteome is largely hindered due to the complexity in identification and characterization of the proteome for this modification via mass spectrometry (MS). Many methods used for enriching sulfotyrosine have utilized the same toolkits for phosphotyrosine enrichments: immobilized metal oxide affinity chromatography, or antibody/protein domain-based enrichment strategies. Efforts to identify novel sulfotyrosine substrates using enrichment and standard bottom-up workflow strategies have yielded few new identifications of novel sulfotyrosine containing substrates. We are developing a biotin proximity-based labeling approach known as BioID as an alternative strategy for identifying novel sulfotyrosine substrates. This approach relies on fusing a promiscuous biotin ligase (BirA) to the TPSTs. In addition to the proximity-labeling work, we are interested in validating and characterizing identified TPST substrate through a fluorescence-based sulfation assay using active TPSTs.



RSVP for food & drinks!

Postdoc & Graduate Students ONLY!

SCAN ME!