Intellectual Merit: The human body is made up of approximately 50% non-human cells, mostly consisting of bacteria (~99%), followed by archaea, then eukaryota, collectively forming the human microbiome.[1,2] Over the past decade, research has revealed impacts of colon microbiota on human physiology, including roles in digestion, metabolism, immune system regulation, hormone signaling, and development.[3,4] Colon microbiota reside in a specialized niche—a layer of mucus secreted by the colon epithelium, heavily comprised of the gel-forming protein MUC2.[5] Adenomatous Polyposis Coli (APC) is a scaffolding protein that has long been studied as a tumor suppressor antagonist of the Wnt/β-catenin signaling pathway, with additional roles less defined. In Dr. Kristi Neufeld's lab at the University of Kansas, we have accumulated evidence suggesting multiple novel functions of APC, including in the expression of *MUC2*. Given this evidence, **I hypothesize that APC directly or indirectly promotes *MUC2* expression, and therefore has a role in colonic mucus generation, microbiome homeostasis, and colon function.** Uncovering roles of APC in *MUC2* expression would fill a critical knowledge gap not only in our understanding of the human microbiome and its homeostasis, but also in the cellular function of highly conserved APC-like orthologs found nearly universally across invertebrates and vertebrates.[6]

In a recent study, using Human Colon Epithelial Cells (HCECs), we found that reduction of APC led to a 75% decrease in IL-1R, an integral membrane receptor protein that, when bound to a ligand, promotes *MUC2* expression.[7] We found a similar trend in the IL-1R ligand, IL-1β. To further investigate the relationship between APC, IL-1 signaling, and *MUC2* expression, we modified a human colon cancer cell line, DLD-1, using CRISPR/Cas9. We inserted wildtype (WT) APC under control of a doxycycline (Dox) responsive promoter, which allowed us to treat cells with Dox and induce expression of WT APC. Using unmodified (parental) DLD-1s as a control, we found that IL-1β and WT APC individually increased *MUC2* expression 2-fold, but when expressed simultaneously, *MUC2* expression increased 15-fold (Fig. 1).[7] This evidence suggests that IL-1 signaling and APC act synergistically to promote *MUC2*. This evidence also suggests that IL-1R activation increases *MUC2* expression, which may occur through signaling of PKC-δ, a protein previously correlated with IL-1R activation and *MUC2* expression.[8] To investigate DNA-binding of APC that might be involved in *MUC2* promotion, we used a dataset from a recent ChIP-seq study[9] and found that APC binds to the promoter of the *MUC2* gene. Combined with our APC gain and loss of function studies, I created a simple model to reflect hypothesized interactions between IL-1R, APC, PKC-δ, and *MUC2* (Fig. 2).

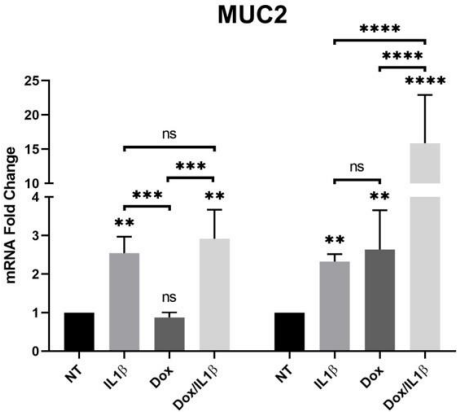

Figure 1. RT-qPCR for MUC2 in Parental and APC-Inducible DLD-1 cells. (Two-way ANOVA with Tukey's range test, **P <0.005, ***P<0.0005, ****P<0.0001)

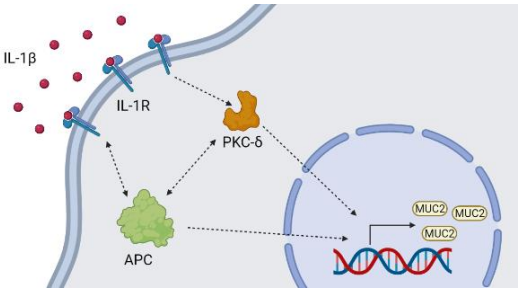

Figure 2. Model of relationships that would allow IL-1R and APC to increase *MUC2* expression individually and synergistically.*

Aim 1: Elucidate the Molecular Roles of APC in MUC2 Expression. As evidenced by our research, APC expression leads to an increase in *MUC2* mRNA levels and APC loss leads to reduction in IL-1R. However, whether APC induces translation of MUC2 or IL-1R is yet to be determined. With the help of Dr. Yoshiaki Azuma, a CRISPR/Cas9 expert at the University of Kansas, **I have designed and begun creation of two novel cell lines to uncover the molecular roles of APC**. By modifying HCEC and DLD-1 cells, we will be able to perform quick, cheap, efficient, and consistent 1) APC KO using an Auxin-Inducible-Degron (AID), 2) basal-level APC expression with no treatment, and 3) APC overexpression using Tetracycline-Inducible-Expression (TIE). The AID system uses OsTIR1, an auxin-responsive ubiquitin ligase, to polyubiquitinate AID-tagged proteins for degradation in the proteosome.[10] I am tagging endogenous WT APC with an AID for APC KO. The TIE system initiates transcription of a gene through

a tetracycline (TET)-responsive promoter[11] and will be paired with the endogenous AID-tagged APC. I will use *MUC2* promoter/Luciferase Reporter Assays (LRAs) and our in-lab luminometer to confirm response to various levels of cellular APC. An increase in luciferase indicates that APC increases MUC2 promoter activity, a neutral result indicates no effect of APC, and a negative result indicates APC reduces MUC2 promoter activity. This design can be repeated with reporters for all my candidate gene promotors, including IL-1R, MUC2, synergism between APC and IL-1β for MUC, etc. Regardless of my findings, these results will direct my further studies on APC and the other proteins of interest in this system, including investigation on protein localization, protein modifications, and signaling pathways. I will use co-immunoprecipitation followed by mass spectrometry performed at KU's Core Mass Spec Lab to identify signaling proteins, florescence microscopy using our in-lab microscope to understand protein localizations and treatment phenotypes, SDS-PAGE and western blots for exposing signaling pathway patterns, mobility shift assays for uncovering post-translational modifications and protein activation, and much more to understand my proteins of interest and their signaling pathways.

Aim 2. Monitor Impacts of APC on Colon Microbiome Homeostasis. In another recent study, we created transgenic mice with an APC allele containing mutations in the protein's Nuclear Localization Sequences (APC-mNLS), preventing APC from entering the nucleus, and thus, inhibiting potential direct APC-driven nuclear promotion of gene expression. In this study, we uncovered that APC-mNLS mice had a >90% decrease in *MUC2* expression following colon epithelial injury compared to control mice.[12] I hypothesize that APC-mNLS mice have lower levels of MUC2, reducing the amount of mucus habitable by microbiota in the colon and thereby decreasing microbiome diversity and microbiota count. Using this mouse strain, with our extensive mouse care facilities and IACUC approval (137-01), I will collect fecal and colonic mucus swab samples of both mutant mice and control littermates for Zymo Research's Microbiome Analysis Service,[13] which provides publication-ready data of a list of microbiomic data, including absolute microbiota counts, multi-kingdom accounts of microbiome diversity within and between samples, and more. In addition to these data, I will use immunohistochemistry to visualize MUC2 in the colon epithelium using anti-MUC2 antibodies, comparing mutant and control littermates. This will provide comprehensive evidence of any changes to the microbiome and the colonic mucus layer in response to loss of nuclear APC, providing insight into APC's physiological function and connection to the microbiome *in vivo*.

Broader Impacts: The proposed research will expand our understanding of non-Wnt functions of APC, the function of APC in the generation of the colon mucus layer, the impact of APC on the microbiome, and provide insight on the function of APC orthologs across the animal kingdom. These data have the potential to uncover numerous novel functions of APC and better our understanding of microbiome homeostasis in relation to physiological function, a field of study that is still in its infancy. The cell lines I will create and data I will collect will be powerful research tools for myself, the Neufeld lab, and other researchers studying APC and colon microbiomics. I will communicate the conclusions of my research through publications, as well as through posters and oral presentations at regional and national conferences. I will also continue to develop the Rural Scientist Initiative (RSI) outlined in my personal statement; the NSF GRF would allow me to dedicate more time to the RSI while attending graduate school. Development of the RSI includes presenting this research to rural students along with information on scientific careers to ~15-30 of the 70 high school students at Norwich High School per year, based on student interest. I will continue communicating my experience as a scientist from several underrepresented communities to these students, encouraging rural students to pursue careers in scientific research.

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