Notable Specific Aims examples in this handout

- Using subheadings (Parrish)
- Dividing an Aim into parts (Parrish, Wahlby, Ratner, Hudson, Desai, Jeong)
- Making intro paragraphs interesting (Hudson, Ferrara, Ley, Brownson)
- Bold type for key sentences (Jansson)
- Overview diagrams (Ratner, Chackerian, Jeong)
- Overall Impact (Ratner, Ley)
- Handling immediate objections (Coaker)
- Using white space (Goga, Striepen)

Specific Aims Checklist

Specific Aims gives reviewers an overview of your project. With funding so tight, NIH isn't in the market for boring projects, so make this page exciting, especially the intro at the top.

Introduction: these things are usually essential to your first one or two paragraphs

- [ ] Your overall goal for this project
- [ ] Your research subject and its relation to health
- [ ] Your experimental system or model
- [ ] New knowledge this project will produce
- [ ] Keep the intro less than 400 words; intros of less than 200 words have been successful
- [ ] If you know reviewers will have some kind of knee-jerk objection to your project, include a short justification for your project that you can expand upon in your Research Plan.

Additional things to include in the opening paragraphs, if useful

- [ ] This is a fundamental problem with implications for many studies outside this field
- [ ] The barrier to scientific or medical progress that this project will overcome
- [ ] What is innovative about your approach
- [ ] What is novel about the subject you study, making it a unique and promising research topic
- [ ] You will use techniques invented in your lab
- [ ] No one has applied these techniques to this problem before
- [ ] Relevant background info, especially if reviewers will find it surprising and fascinating
- [ ] Why your project is more challenging than your reviewers may realize
- [ ] What you will do in your Aims
- [ ] Your central hypothesis
- [ ] What you achieved with your current funding that justifies this project
- [ ] New goals, experiments or methods that are based upon achievements supported by your current funding that will enable you to achieve even more in the future
- [ ] Your team of collaborators is uniquely qualified to make this project succeed

Considerations for writing your Aims

After the introduction, write your Aims one by one. Most projects have two or three.

- [ ] Summarize each Aim in a <u>short</u> sentence in bold.
- [ ] If an Aim has subaims, try writing them as a list: A, B, C, etc.
- [ ] Alternately, try describing your aim (after the short sentence in bold) with sentences beginning with subheadings: Challenge, Approach, and Impact.
- [ ] If an Aim has a hypothesis, try writing it in *italics* to make it stand out.
- [ ] State the results your Aim will achieve
- [ ] Be brief; a single aim can be described in 50 – 150 words

At the bottom of the page

- [ ] State your project's Overall Impact in 40 words or less. Overall Impact <u>summarizes your entire grant</u> in just a few words to tell NIH how science will advance if your work is funded.

Finally…

- [ ] Will reviewers think Specific Aims is interesting and exciting?
- [ ] Is your project clearly focused on a testable hypothesis?
- [ ] Will reviewers agree with the Aims you've chosen? (If they won't, your grant is dead on arrival.)
- [ ] Will they think you're proposing more work than is doable in the grant period?
- [ ] Are unnecessary words omitted so there's white space between paragraphs?
- [ ] Consider replacing words with an overview diagram; if the diagram won't fit in Specific Aims, try putting it near the top of the first page of your Research Plan.

This Specific Aims page is from a R01 awarded to Jae-Wook Jeong of MSU in 2016. It's an excellent model for writing Specific Aims.

The opening sentence gets right to the point: "This proposal is about...."

The proposal's preliminary data is connected to better understanding of mechanisms of health and disease and the desire for better treatments.

SPECIFIC AIMS

This proposal is about the role of a chromatin modifying factor in regulating uterine epithelial proliferation in response to hormonal signals. Our preliminary data strongly suggest AT-rich interactive domain-containing protein 1A (ARID1A) has a key role in implantation and decidualization, and that ARID1A expression is lost in endometriosis, a disorder characterized by overproliferation of the endometrium. This is significant for understanding both normal implantation and its dysregulation in endometriosis. Further, this proposal offers the potential to discover new therapies for infertility and endometriosis: (1) by identifying the downstream targets of ARID1A; and (2) by testing whether resveratrol, a phytoestrogen that has successfully inhibited epithelial proliferation of human cancers[85], can reverse uterine epithelial proliferation.

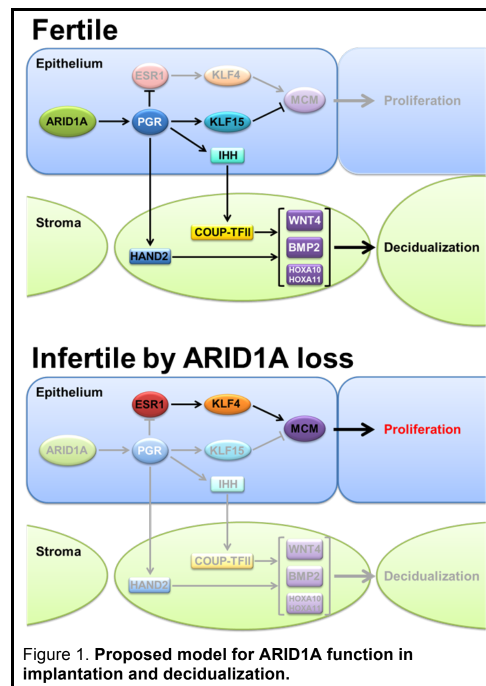


Figure 1. **Proposed model for ARID1A function in implantation and decidualization.**

Pointing out a gap in our knowledge

ARID1A belongs to the SWI/SNF family and is required to activate transcription of genes normally repressed by chromatin[4,5]. ARID1A loss is uniquely associated with endometriosis-associated ovarian carcinomas[6-8]. However, how ARID1A works in the female reproductive track in both health and disease is unclear.

The proposal's experiments are summarized. Particularly novel aspects are called to reviewers' attention.

Our experiments will comprehensively test the interactions between ARID1A and progesterone receptor (PGR), identify the gene targets of ARID1A, and test the ability of resveratrol to reverse uterine epithelial proliferation caused by ARID1A loss. There is strong innovation in the novelty of our hypotheses and our cutting-edge technical approaches. In particular, our experiments will employ the first low cost animal model that closely resembles human endometriosis.

Aims in bold face and subaims in indented lists make easy reading. Notes about the methods to be used (which many writers include) have been deferred to the Research Plan.

Aim 1. Determine the role of ARID1A in suppressing epithelial cell proliferation for uterine receptivity.

A. Determine if ARID1A negatively regulates E2-induced epithelial cell proliferation through PGR interactions
B. Characterize transcriptional regulation of P4 target genes by ARID1A
C. Evaluate ARID1A loss in tissues of infertile women with endometriosis compared to controls

Aim 2. Determine the importance of ARID1A loss in decidualization, infertility and endometriosis.

A. Determine whether ARID1A loss causes a decidualization defect in conditional *Arid1a* KO ($Pgr^{cre/+}Arid1a^{f/f}$; $Arid1a^{d/d}$) mice and human endometrial stromal cells
B. Determine if ARID1A loss causes P4 resistance in endometriosis using a mouse model that realistically resembles human endometriosis
C. Determine if ARID1A loss causes endometriosis-related infertility using a mouse model

Aim 3. Evaluate the ability of resveratrol to restore uterine function in cases of infertility and endometriosis due to ARID1A loss.

A. Determine if resveratrol overcomes aberrant epithelial proliferation and implantation defects in $Arid1a^{d/d}$ mice
B. Determine effect of resveratrol on establishment of endometriotic lesions and infertility
C. Determine effect of resveratrol in a Xenograft model using human endometrial tissue

Overall Impact ends Specific Aims by summarizing the entire grant in just two sentences.

OVERALL IMPACT: We will clarify how ARID1A mediates P4 inhibition of E2 signaling in the uterus, and test using mice and human tissues whether a phytoestrogen, resveratrol, can help treat infertility and endometriosis. Our experiments will employ the first low cost animal model that closely resembles human endometriosis.

This Specific Aims page from Boris Striepen is for a competing renewal R01. It's important to tie what he proposes to do here to the success he had with his previous R01

Describes significance to health

Ties to PIs long-term goals

Describes previous research (for a renewal)

Ties project to previous studies

Ties to preliminary data

Describes previous research

States goals of aim

Specific Aims:
Apicomplexa are responsible for a number of important human diseases including malaria, toxoplasmosis, cryptosporidiosis and cyclosporidiosis. Management of these diseases rests heavily on chemotherapy but anti-parasitic drug treatment faces multiple challenges. These include poor overall potency, restriction to certain life-cycle stages, unwanted side effects, and rapidly emerging multiple drug resistance. A constant stream of new drugs and potential drug targets is required to stay abreast of the threat posed by these pathogens. One of the most promising sources of such parasite specific targets is the apicomplexan plastid or apicoplast. The apicoplast is unique to the parasite and its function is essential to parasite survival. This organelle is a holdover from a free-living photosynthetic past. The structure and biology of the apicoplast is remarkably complex as it is derived from the endosymbiotic marriage of two eukaryotes: a red alga and an auxotrophic protist. The goal of this application is to unravel the complexity of this biology in mechanistic detail. We hypothesize that the photosynthetic past of Apicomplexa and the continued presence of a plastid has profound and lasting implications for their current metabolism and cell biology. Further we believe that discovering and characterizing this biology in its molecular detail will lead us to important insights into the biology of Apicomplexa, the evolution of the eukaryotic cell, and ultimately to novel targets for anti-parasitic interference. In our current funding period we have conducted genetic studies on proteins involved in apicoplast replication, protein import, and metabolism that were identifiable as plastid proteins in part based on their similarity to plant chloroplast proteins. We did so in a gene-by-gene fashion characterizing a limited number of proteins in considerable depth using a genetic approach. This has been an excellent strategy that served us well, we will continue to use this approach to dig deeper into mechanism in the current application. However, we also feel that we might have harvested the lower hanging fruit of candidates with a lot of function left unassigned. We therefore will complement this approach with a broader effort to define a comprehensive set of plastid proteins to continue to feed our pipeline of hypothesis-driven mechanistic experiments with strong candidate genes.

Specific Aim1: Dissect the mechanism of apicoplast protein import. The bulk of the ~500 apicoplast proteins is nuclear encoded and post-translationally imported across four membranes. We (and others) have described three mechanistically distinct candidate protein translocons that reside in the three inner membranes of complex plastids. In the current funding period we will focus on a newly discovered mechanism that was derived from the ER-associated degradation system (ERAD) of the algal endosymbiont. We will use conditional gene disruptions and complementation assays to establish the importance of individual components and to define the energy source of the translocation process.

Specific Aim2: Understand the function of the apicoplast ubiquitination pathway. The ER-localized ERAD pathway goes hand in hand with the ubiquitination and subsequent proteasomal degradation of translocated proteins. Our preliminary data indicates that aspects of this protein modification pathway are still present in the apicoplast. What is the enzymatic machinery involved in this process? What are its substrates? And most importantly, what is the molecular function of apicoplast ubiquitination? A combination of genetic and biochemical approaches will be used to answer these important questions.

Specific Aim 3: Discover a comprehensive set of apicoplast proteins and characterize their function. Mining comparative and functional genomic information we have assembled an extensive list of proteins for which we hypothesize a role in apicoplast biology. We will establish the localization of their protein products for a comprehensive set of these candidate genes by epitope tagging. In the previous funding period we have found conditional null mutants to be highly informative to study apicoplast protein function and we have developed phenotypic assays to detect defects in apicoplast protein import, apicoplast division, and apicoplast metabolism. We will apply this genetic approach to a prioritized list of validated candidates. To increase the throughput of our analyses we will develop and test a new mutagenesis approach based on promoter replacement.

Shows significance

Using subheadings to begin the intro paragraphs makes it easier for reviewers to understand what this proposal is about and it's importance to science.

Gives science background

States aim and hypothesis

Using bold and italics and breaking the aim into steps make the aim easier to read and understand

States aim and hypothesis

States aim and hypothesis

Capsid structures, variation and flexibility. This project brings together the skills of laboratories at Cornell University and at Pennsylvania State University Medical Center to provide a detailed understanding of the roles of structural variation and flexibility in the parvoviral capsid, and their effects on receptor and antibody binding and the controls of cell infection and host range. These are fundamental problems that apply to all animal viruses, where the capsid must protect the genome in the environment, interact with host molecules including cell receptors and antibodies, and undergo a series of regulated structural transitions during cell entry to eventually release the genome for replication. Viral capsid binding to host receptors and antibodies can have varying and often unpredictable effects on infection, and those interactions also control many other replication steps. Where these virus-host interactions are specific they can control the viral host ranges.

A model for understanding virus cell infection and host range control through differential receptor binding. We study two viruses that differ in host range due to 3 or 4 capsid protein mutations that control specific receptor binding. Canine parvovirus (CPV) arose around 1976 as a variant of feline panleukopenia virus (FPV), and caused a pandemic of disease during 1978 and 1979. That virus has continued to circulate worldwide as a serious canine pathogen, and has also evolved new antigenic, receptor binding, and host range variants. FPV and CPV both can bind the feline transferrin receptor 1 (TfR) to infect cat cells, and CPV gained the host range for dogs by gaining the ability to bind the canine TfR. This new binding property was associated with increased flexibility of 2 or 3 of the surface loops in the capsid that allowed CPV to accommodate a glycan on the canine TfR binding domain. Flexibility in the capsid is controlled by variation in hydrogen bonds, by cleavages of the VP2, and by ion binding. Later steps in infection also involve changes in the capsid structure that release the viral DNA or protein domains of VP2 and VP1. These viruses are targeted by antibodies that can differ in their binding sites and in their ability to neutralize the virus. We will examine a set of antibodies that detect specific capsid structures, examining the effects of antibodies on TfR binding, and seeking to understand the mechanisms of neutralization.

Aim 1. To define the structural variation in parvovirus capsids, and to determine the effects on capsid functions and DNA release. *Hypothesis: That the capsids of parvoviruses undergo structural variation that is important for infection. That occurs through the binding or release of divalent ions, by site-specific proteolysis, or by variation in specific intra- or inter-chain bonds.*

a) Further define the structural flexibility in the capsid through analysis of the structures and to identify sources of variation using specific peptide and protease analysis.
b) Determine the functions of specific capsid structures by preparing mutants with altered inter-chain bonds, divalent ion binding sites, or protease cleavage sites.
c) Compare the functions of capsid structures in mutant or naturally variant viruses to reveal the structures and interactions that are critical for capsid stability, TfR binding, and the processes of cell infection.

Aim 2. To define the structural interactions between various parvovirus capsids and variants of the transferrin receptor or artificial receptors. *Hypothesis: That specific binding of capsids to the feline or canine TfRs is required for successful cell infection, and those interactions are controlled by viral structures varying in structure and flexibility.*

a) Determine the interactions of the feline and canine TfRs with different parvovirus capsids, examining cryoEM structures of receptor-capsid complexes at moderately high resolution. By correlating residues on the capsid and TfR that affect binding, identify the interacting structures.
b) Identify functional sites on the capsids by selecting for mutants of CPV or FPV by growth on TfRs with mutant binding domains, or on receptors with artificial binding ligands.
c) Prepare capsids with insertions that bind alternative cell receptors, and test for cell infection.
d) Examine how flexibility of capsid loops controls interactions with different host TfR - in particular receptors with additional glycans within the attachment face of the receptor.

Aim 3. Use antibodies to probe the capsid structure, and also to determine how binding to overlapping sites leads to variable neutralization. *Hypothesis: That antibodies can be used to detect variant structures in the viral capsid, and that the specific position and orientation of binding controls the likelihood of competition with the TfR, and neutralization of infection.*

a) Examine antibodies with known capsid binding sites for their effects on TfR binding, including the effects of cleavage with proteinases or after other asymmetrically or symmetrically displayed modifications.
b) Determine the effects on viral functions of antibody variants engineered with increased binding affinities. Identify sites on the virus that do not bind antibodies but that bind TfR, for example those subunits with cleavages in surface loops.

States how the research fits into the field

Specific Aims

Note how there are no references on this page. While your Research Plan must have references, references are optional in Specific Aims.

Microscopy has emerged as one of the most powerful and informative ways to analyze cell-based high-throughput screening (HTS) samples in experiments designed to uncover novel drugs and drug targets. However, many diseases and biological pathways can be better studied in whole animals–particularly diseases that involve organ systems and multicellular interactions, such as metabolism and infection. The worm *Caenorhabditis elegans* is a well-established and effective model organism that can be robotically prepared and imaged, but existing image-analysis methods are insufficient for most assays.

We propose to develop algorithms for the analysis of high-throughput *C. elegans* images, validating them in three specific experiments to identify chemicals to cure human infections and genetic regulators of host response to pathogens and fat metabolism. Novel computational tools for automated image analysis of *C. elegans* assays will make whole-animal screening possible for a variety of biological questions not approachable by cell-based assays. Building on our expertise in developing image processing and machine learning algorithms for high-throughput screening, and on our established collaborations with leaders in *C. elegans* research, we will:

Uses bold headers and strong organization

Walhby's organization of her aims by this Challenge, Approach, Impact formula make her aims, and their impact on science, easier to understand.

Aim 1: Develop algorithms for *C. elegans* viability assays to identify modulators of pathogen infection

Challenge: To identify individual worms in thousands of two-dimensional brightfield images of worm populations infected by Microsporidia, and measure viability based on worm body shape (live worms are curvy whereas dead worms are straight).

Approach: We will develop algorithms that use a probabilistic shape model of *C. elegans* learned from examples, enabling segmentation and body shape measurements even when worms touch or cross.

Impact: These algorithms will quantify a wide range of phenotypic descriptors detectable in individual worms, including body morphology as well as subtle variations in reporter signal levels.

Aim 2: Develop algorithms for *C. elegans* lipid assays to identify genes that regulate fat metabolism

Challenge: To detect worms versus background, despite artifacts from sample preparation, and detect subtle phenotypes of worm populations.

Approach: We will improve well edge detection, illumination correction, and detection of artifacts (e.g. bubbles and aggregates of bacteria) and enable image segmentation in highly variable image backgrounds using level-set segmentation. We will also design feature descriptors that can capture worm population phenotypes.

Impact: These algorithms will provide detection for a variety of phenotypes in worm populations. They will also improve data quality in other assays, such as those in Aims 1 and 3.

Aim 3: Develop algorithms for gene expression pattern assays to identify regulators of the response of the *C. elegans* host to *Staphylococcus aureus* infection

Challenge: To map each worm to a reference and quantify changes in fluorescence localization patterns.

Approach: We will develop worm mapping algorithms and combine them with anatomical maps to extract atlas-based measurements of staining patterns and localization. We will then use machine learning to distinguish morphological phenotypes of interest based on the extracted features.

Impact: These algorithms will enable addressing a variety of biological questions by measuring complex morphologies within individual worms.

This paragraph near the end of Specific Aims summarizes Walhby's entire grant. This is her grant's Overall Impact statement.

In addition to discovering novel anti-infectives and genes involved in metabolism and pathogen resistance, this work will provide the *C. elegans* community with (a) a versatile, modular, open-source toolbox of algorithms readily usable by biologists to quantify a wide range of important high-throughput whole-organism assays, (b) a new framework for extracting morphological features from *C. elegans* populations for quantitative analysis of this organism, and (c) the capability to discover disease-related pathways, chemical probes, and drug targets in high-throughput screens relevant to a variety of diseases.

Highlights PI and team

Primary collaborators

Gary Ruvkun and **Fred Ausubel**, MGH/Harvard Medical School: Development, execution, and follow-up of large-scale *C. elegans* screens probing metabolism and infection. **Polina Golland** and **Tammy Riklin-Raviv**, MIT Computer Science and Artificial Intelligence Lab: Illumination/bias correction, model-based segmentation, and statistical image analysis. **Anne Carpenter**, Broad Imaging Platform: Software engineering and support.

The first sentence identifies the problem this grant deals with and the knowledge gap it addresses. The next three explain in different ways the cost of not solving this problem. Then the next sentence is about what they propose to do overall. The final sentence mentions their qualifications to do this work.

Specific Aims

While intensive applied behavior analysis intervention is an evidence-based practice for increasing the social competence and communication of young children with ASD (80), little is known about effective methods for preparing young children with ASD to be academically successful (e.g., competent readers). To date, there are no published studies that examine the emergent literacy skills of children with ASD or preschool literacy interventions to prepare young children with ASD to benefit from subsequent classroom literacy instruction. The consequences for failing to develop reading proficiency are dire for all children, including children with ASD. Reading is a pivotal skill; without the ability to read, there is a strong likelihood that, as they grow up, children with ASD will encounter a lack of post-secondary educational opportunities, employment, and participation in citizenship. Reading is also a functional skill; without literacy skills needed for independent living, leisure time activities, and supported employment, it will be difficult for adults with ASD to lead self-sufficient lives. We propose that investigating effective preschool interventions supporting emergent literacy for children with ASD will lead to improved future academic outcomes for these students. Building on our expertise in both developing preschool interventions for young children with ASD and reading interventions for children with disabilities, we will explore the following.

Aims are highlighted with words in bold and then presented in a way that's easy for reviewers to understand through this Approach, Hypotheses, Impact formula.

Aim 1: Determine the immediate treatment effects for preschool children with ASD (Years 1-3)

- **Approach:** Using randomized control trials, examine the efficacy of Dialogic Reading (DR) intervention compared with regular preschool instruction controls (Year 1), Phonological Awareness (PA) intervention compared with controls (Year 2), and compare the efficacy of each treatment (DR and PA) (Year 3).
- **Hypotheses:** We expect that the children with ASD will develop better oral language skills in the DR condition than controls, and that they will develop better PA skills in the PA condition compared with controls. We do not expect them to differ in alphabetics due to group membership. It is an empirical question as to which outcomes DR and PA show benefits for in children with ASD (Year 3).
- **Impact:** Establish which of the two preschool interventions are effective in improving the emergent literacy of young children with ASD.

Aim 2: Determine treatment mediators and moderators for preschool children with ASD (Years 1-3)

- **Approach:** Determine whether the magnitude of treatment effects changes after treatment fidelity and pretests are accounted for. We will also determine whether the treatment effects differ (responsiveness to intervention) for children with lower or higher pretests.
- **Hypotheses:** Given our use of random assignment, we do not believe that any full mediators will be present. Further, careful monitoring of treatment implementation will yield high fidelity overall and little predictive utility for fidelity to mediate treatment. More importantly, examination of treatment moderators (testing treatment interaction terms) will inform which children benefit from each type of intervention.
- **Impact:** Determine optimal conditions for DR and PA intervention effects for young children with ASD.

Aim 3: Determine the longer term (kindergarten) treatment effects for children with ASD who receive preschool reading intervention (Years 2-4)

- **Approach:** Determine whether treatment effects are present one year post-intervention and whether effects depend on child characteristics, such as pretests (mediators and moderators).
- **Hypotheses:** We expect children in the DR condition (Years 1 and 3) to exhibit higher performance on reading comprehension, listening comprehension, and oral language compared with children in the control or PA groups. Alternatively, we expect children in the PA condition (Years 2 and 3) to have higher performance in nonsense word decoding and word reading compared to the other two conditions. We have no set hypothesis related to text reading fluency outcomes because both language (DR) and word reading (PA) have been shown previously to relate to this outcome.
- **Impact:** Determine which preschool literacy intervention better prepares children with ASD for formal reading instruction in kindergarten. This is a crucial outcome of the project because reading comprehension skills have been particularly difficult for children with ASD to acquire.

Aim 4: Determine which preschool pretest characteristics predict longer term (kindergarten) outcomes for children with ASD (Years 2-4)

- **Approach:** Determine which pretest characteristics directly and uniquely predict outcomes in kindergarten by testing their predictive utility, both individually and combined.
- **Hypotheses & Impact:** Results will contribute important new information as to which preschool skills are strong indicators of kindergarten reading skills for children with ASD, irrespective of group assignment.

The two intro paragraphs of this R01 clinical trial proposal are a sort of summary (Who, What, When, Where, Why and How) of the trial.

The Primary Aim is organized differently than the Secondary Aims, and there's an Exploratory Aim at the bottom. Organizing Aims differently doesn't matter -- as long as reviewers can understand what you intend to do and why it's important.

2. Specific Aims.

Within the Kaiser Permanente Northern California (KPNC) health system, we propose a randomized controlled clinical trial of diet and physical activity (PA) to help overweight/obese pregnant women achieve appropriate gestational weight gain (GWG) for their prepregnancy body mass index (BMI) and weeks of pregnancy, as recommended by the Institute of Medicine (IOM).[13] Participants will be randomly assigned to a lifestyle intervention or usual medical care (**200 women in each arm; 25% White, 25% African Americans, 25% Asians and 25% Hispanic**). The sample will be selected from among women with body weight measured by a KPNC provider no more than 12 months prior to conception in their electronic medical record (EMR).

Women will be enrolled at 10 wks of pregnancy. The intervention will begin at 12 wks. It will be delivered via 2 in-person counseling sessions and 11 telephone contacts with study dieticians trained in motivational interviewing techniques and operating from the KPNC Perinatal Center. The intervention will be evaluated for possible translation and adoption by the health care system. Study measurements will be assessed by trained study personnel at 10 and 32-wks of pregnancy, at delivery and at 6 and 12-months postpartum.

PRIMARY AIM 1. We propose to develop and evaluate a diet and PA intervention tailored to pregnant women and feasible for a health plan setting with the **primary goal** of helping overweight/obese women achieve appropriate total GWG according to their prepregnancy BMI and weeks of pregnancy.

Hypothesis. As compared with usual care, a lifestyle intervention that starts at 12 wks of pregnancy will result in a greater proportion of women with appropriate total GWG, and infants whose birthweight is appropriate for gestational age (AGA).

Primary outcomes will be the proportion of women with appropriate Total GWG, as recommended by the IOM,[13] and Rate of Maternal GWG (in kilograms per week).

Secondary outcome will be the proportion of infants with AGA birthweight.

SECONDARY AIMS.

2. To assess whether during pregnancy women assigned to the lifestyle intervention have more favorable changes in percent of calories from fat, minutes of PA per week and body fat mass compared to usual care. We hypothesize that the intervention will result in more favorable behaviors and less body fat mass.

3. To assess whether at 12 months postpartum women assigned to the lifestyle intervention have more favorable changes in weight and body fat mass compared to usual care. We hypothesize that appropriate GWG will result in less pregnancy weight retention and less body fat postpartum.

4. To assess whether at 12 months infants of women assigned to the intervention are more likely to have more appropriate anthropometrics than infants of women assigned to usual care independently of infant diet. We hypothesize that appropriate maternal GWG will results in a greater proportion of infants with anthropometric measurements between the 10th and 90th percentiles according to the WHO Standards.[14;15] These associations are partially mediated by AGA birthweight.

Exploratory AIMS (**Figure 1**).

5. To explore the associations between GWG intervention and women's cardiometabolic profiles and infants' cardiometabolic profiles and body fat mass, by assessing whether:

a.

b. Women assigned to the intervention are more likely to have infants with more favorable cord blood levels of cardiometabolic markers and *lower fat mass* than infants of women in usual care. We hypothesize that:

i.

ii.

Intro paragraph #1 explains the problem this grant deals with.

Intro paragraph #2 justifies a randomized study to help solve the problem.

Intro paragraph #3 states this grant's objective.

Aims 1 and 2 are organized by hypotheses.

Specific Aims ends with this grant's Overall Impact; that is, a brief summary of how the grant's success will have a strong impact toward solving the problem.

SPECIFIC AIMS AND HYPOTHESES

In this project, we implement and evaluate an electronic health record (EHR)-linked, Web-based clinical decision support (CDS) system that identifies and and gives patients and their primary care providers personalized, evidence-based CDS and follow up to reduce cardiovascular (CV) risk of heart attacks or stroke. Strong, consistent evidence shows that pharmacologic and lifestyle management of adults with prediabetes improves CV risk factors, reduces CV events, slows progression to diabetes, and is cost-saving.[4 14] Despite this, clinical care of these patients shows generally poor recognition of prediabetes, very low rates of metformin use, and poor control of concomitant CV risk factors.[1,2,6,13,15-18] These problems are magnified in rural areas, where providers are overworked and patients often have limited access to primary and subspecialty care and are, on average, older, poorer, less healthy, and less health literate than patients in urban areas.[19-21]

To improve implementation of evidence-based prediabetes care, we believe a randomized study of the implementation and impact of a prediabetes CDS intervention on CV risk in prediabetes patients (20-75 years old, BMI ≥25 kg/m^2, current smoker, uncontrolled blood pressure [BP] or lipids) is now justified based on these considerations: (a) over 75% of rural primary care providers (PCP) now use EHR systems[22]; (b) in a previous randomized trial, we showed that CDS in primary care clinics significantly improved BP and glucose control in adults with diabetes; (c) this CDS system elicits evidence-informed patient treatment preferences using tested human-computer interfaces; (d) the CDS system is minimally disruptive of clinic workflow with 80% use rates in targeted diabetes patients and 95% provider satisfaction; (e) treatment and prioritization algorithms are Web-based and therefore easily updated and highly scalable to any medical group that uses EHRs; and (f) this approach is very cost-effective, and may be cost-saving at scale, making it highly sustainable.

The objective of this project is to systematically and pragmatically improve care and CV risk factors in predominantly rural prediabetes patients at high risk for developing diabetes and CV events by implementing a Web-based CDS system that integrates with local EMR systems and presents the patient and PCP with personalized, evidence-based drug and lifestyle treatment recommendations. To accomplish this objective, we address the following specific aims and hypotheses:

Specific Aim 1: Cluster-randomize 30 primary care clinics with 450 PCPs and over 11,000 high-risk adult prediabetes patients to one of two study arms: (a) Usual care (UC) clinics or (b) Intervention (CDS) clinics, which use an EHR-linked, Web-based Prediabetes CDS system designed to improve prediabetes care.

Hypothesis 1 (H1): Relative to those treated at UC clinics, eligible 20- to 75-year-old prediabetes patients at high CV risk treated at CDS clinics will have significantly more favorable trajectories in CV risk estimates over a median 24-month follow up.

Hypothesis 2 (H2): Relative to those treated at UC clinics, eligible 20-75-year-old prediabetes patients with uncontrolled CV risk factors at CDS clinics will have significantly more favorable trajectories in specific CV risk factors, including BP, lipids, smoking, HbA1c, and weight over a median 24-month follow up.

Hypothesis 3 (H3): Relative to those treated at UC clinics, eligible 20- to 75-year-old prediabetes patients at high CV risk treated at CDS clinics will have significantly more favorable patterns of metformin and statin use over a median 24-month follow up.

Specific Aim 2. Assess the cost and cost-effectiveness of the CDS intervention from the health system (payer) perspective through utilization analyses and microsimulation modeling at the patient level.

Hypothesis 4 (H4): After controlling for demographics and baseline clinical status, eligible patients with prediabetes treated in UC clinics versus CDS clinics will have significantly lower healthcare costs from the index date over a median follow up of 24 months.

Hypothesis 5 (H5): After controlling for demographics and baseline clinical status, eligible patients treated in CDS clinics versus UC clinics will have significantly lower rates of major CV events and mortality in simulation analysis using state-of-the-art cost-effectiveness microsimulation modeling methods.

Specific Aim 3: Describe critical facilitators and barriers for the prediabetes CDS implementation process, outcomes, and future dissemination using a mixed-methods approach.

Project results will provide a template for implementation of personalized CDS tools in rural and urban health settings, resulting in more efficient, effective rural healthcare that can be applied across many clinical domains, incorporates patient treatment preferences, and could substantially and sustainably improve the quality of CV care and clinical outcomes of millions of Americans with prediabetes in medically underserved areas.

Intro paragraph #1 states the problem this grant will deal with. Note the mentions of recommendations of medical organizations as one way to emphasize the importance of the problem.

Intro paragraph #2 explains their long term goal, overall objective, and working hypothesis.

Primary and secondary Aims are briefly stated, followed by a hypothesis for each one.

The final paragraph in Specific Aims explains the impact that achieving these Aims will have. This is the grant's Overall Impact statement.

A. SPECIFIC AIMS

Despite the high prevalence of falls in older adults, falls can be prevented. In fact, there are currently several effective exercise-based fall prevention interventions designed for community use.[1] Of these, Tai Chi is recommended by the American Geriatrics Society,[2,3] CDC,[1] Administration on Aging (AoA),[4] and other fall prevention advocates such as the National Council on Aging.[5] However, there has been no consideration of the comparative efficacy and cost implications of these fall prevention programs, making it difficult, from the perspective of policy making and programming, to choose the most appropriate program for any particular setting. This lack of information represents a significant gap between the urgent need for effective fall programs and the economic constraints imposed upon community service providers and organizations as a result of increasing health care costs and inadequate funding for preventive services.

This study addresses this important public health and policy decision-making gap, as well as the practical barriers to broad community dissemination of evidence-based falls prevention programs. Our long-term goal is to create an effective, low-cost, scalable community-based Tai Chi program to help public health practitioners and community-based organizations address the problem of falls among older adults and to help older adults maintain their health and independence in the community, thereby reducing the drain on health care spending. Moving toward achieving this public health goal, the overall objective of this study is to conduct a comparative efficacy study and cost-effectiveness analysis using Tai Chi: Moving for Better Balance (TCMBB),[6,7,8] a fall prevention program recommended by the CDC[1] and rated by AoA[4] as meeting the highest criteria for evidence-based interventions. On the basis of prior findings and recent program cost information, our central working hypothesis is that TCMBB will reduce falls and will do so at a lower cost (i.e., be more cost-effective) than an alternative evidence-based fall prevention program. We plan to rigorously test our central hypothesis and thereby accomplish the objective of this application by pursuing the following three aims:

Primary Aim 1: *To compare two distinct types of fall prevention programs (TCMBB, Strength Training) for reducing falls among older adults at risk of falling.*

Hypothesis: *On the basis of prior work, we hypothesize that participants receiving either Tai Chi or Strength Training will show a significant reduction in falls compared to those receiving a Stretching control. We further predict that, relative to the Stretching control, both Tai Chi and Strength Training will result in improvements in secondary outcome measures of balance, gait, and physical performance.*

Primary Aim 2: *To determine the cost-effectiveness of the two exercise programs relative to Stretching, a proxy for a minimum practice standard.*

Hypothesis: *On the basis of our prior work, we predict that, compared to Strength Training, the TCMBB program will be more cost effective, relative to Stretching, in terms of the cost per fall prevented and quality adjusted life year (QALY) gained.*

Secondary Aim: *To determine the sustainability of the effects of our TCMBB intervention.*

Hypothesis: *We hypothesize that, compared to the Stretching control, reductions in falls and improvements in performance-based measures observed as a result of the Tai Chi intervention will be maintained at 6-month post-intervention follow-up. A secondary hypothesis is that participants in Tai Chi will be more likely to continue to exercise.*

With respect to anticipated outcomes, the work proposed in Primary Aim 1 is expected to generate ***new*** *information about the effectiveness of our improved training approach in reducing falls. The results should aid community-service and health care providers and health plan decision-makers by providing information on the comparative effectiveness of two fall prevention programs recommended by the CDC.[1] Achieving Primary Aim 2 will be of great public health significance as it will allow us to fill an important knowledge gap regarding the economic point at which the low-tech TCMBB may be offered by service providers, practitioners, policy makers, and health care planners, as a potential high "return on investment" program to address the increasing need for efficacious, but cost-effective and community-based, falls prevention programs. By successfully achieving Aims 1 and 2, we will thus shed light on the real-world impact of our TCMBB program. If the Secondary Aim is attained, it will enhance our understanding of the sustainability of TCMBB beyond the supervised exercise delivery period and further increase the economic and health impact of this intervention on long-term community services.*

The first paragraph explains the importance of the health problem this grant will deal with. The last sentence explains why solving this problem faces obstacles that some reviewers may not be aware of.

Paragraph #2 compactly describes: 1) the project will be in phases; 2) why this project is significant; 3) that the team is ideal for the job; 4) why this project is innovative; 5) what the impact of this project's success will be. A little bit of underlining and bold text help reviewers pick these things out.

The project's primary goal.

Aim 1 is organized by Rationale and Research Questions; Aim 2, by Rationale and Hypotheses.

1. SPECIFIC AIMS

Through application of existing knowledge, much of the cancer burden is preventable.[11, 12] State-level practitioners are in ideal positions to affect programs and policies related to cancer control. Yet sparse knowledge exists regarding effective approaches for dissemination of research-tested interventions among practice audiences.[13-16] This proposal seeks to reduce the burden of cancer by increasing adoption of evidence-based programs and policies (EBPPs) among public health practitioners and their partners to improve the effectiveness of cancer control. Cancer control practitioners are people who direct and implement population-based intervention programs in agencies or in community-based coalitions (described in detail in section C.5.). Many cancer control practitioners are unaware of research-tested interventions, lack the needed skills to adapt and use them, or face institutional barriers to use of EBPPs.[10, 17-20]

The proposed project applies state-of-the-art methods in the dissemination of EBPPs among cancer control practitioners in public health departments. This project includes two overlapping phases. In Phase 1, we will refine and finalize measures to assess the effectiveness of dissemination of EBPPs. Phase 2 uses a group-randomized effectiveness trial to evaluate the active dissemination of already proven EBPPs in *14 states (7 intervention, 7 control)*. The active dissemination activities include: conducting dissemination workshops, fostering institutional changes, and using knowledge brokers (i.e., a masters-trained individual available for technical assistance). This study is **significant** by addressing cancer risk factors with high burden, where intervention knowledge on EBPPs is substantial, yet not commonly applied, and where a large reduction in cancer mortality is feasible if this knowledge was more widely taken up into practice and policy.[21, 22] It builds on our research team's **extensive experience** in 1) developing state-level partnerships, 2) training practitioners in use of EBPPs, and 3) conducting dissemination research studies that inform the current proposal. *This research is **innovative** by working in real world settings, augmenting Diffusion of Innovations theory with a theory from outside of health (Institutional Theory), developing new dissemination measures,* and using social network analysis to better understand the flow of knowledge. Our results will **impact** the field by enhancing abilities to: 1) conduct active dissemination in practice settings, 2) speed up the translation of cancer prevention knowledge into practice, and 3) measure dissemination of EBPPs.

Our primary goal is to increase the dissemination of EBPPs to control cancer, focusing on the uptake of effective approaches among state-level practitioners.

Specific Aim 1: Develop and test self-reported and objective approaches for assessing the dissemination of evidence-based interventions to control cancer in public health settings.

Rationale: Our recent work shows not only that there are sequential stages to dissemination but also that they can be accurately measured.[20, 23, 24] Yet these stages do not adequately take into account institutional and policy-related factors. Also lacking are objective measures of adoption of evidence-based practices.

Research Questions:

1.1: In which ways can dissemination stages and institutional factors be measured reliably through self-report?

1.2: In which ways can dissemination attributes (e.g., adoption of EBPPs) be measured reliably through objective methods (record audits)?

Specific Aim 2: Evaluate the effectiveness of active dissemination methods designed to increase the uptake of EBPPs to control cancer among public health practitioners.

Rationale: Despite extensive pilot work and smaller scale studies,[8, 10, 25] there has not been a large-scale study of active dissemination approaches for cancer control among public health practitioners in the United States.

Hypotheses:

2.1: Active dissemination activities will be associated with higher rates of awareness, adoption, implementation, and maintenance of EBPPs.

2.2: (Interaction effect) Associations will be modified by institutional (presence of incentives, high turnover) and individual-level (training in key public health disciplines) factors.

2.3: States with higher dissemination rates will have more dense and less centralized social networks than states with lower dissemination rates.

2.4: Data on project costs will show that the dissemination activities are feasible for the budgets of most state-level public health agencies.

Note that "Specific Aims" at the top is in blue letters. There's no rule that everything has to be in black.

Gives long-term goal

Ties project to previous studies and techniques used

States 2 aims

This intro paragraph basically restates Aims 1 and 2 in the middle of the page, but with a bit more detail.

The sentence at the end of this paragraph, "In addition to..." is the grant's Overall Impact statement. A better place for this sentence might have been the bottom of the page.

Defines the aims's objectives

Note the brevity of Ratner's aims.

The overview diagram helps reviewers to understand Aims 1 and 2. But it's placed here at the cost of 250 to 300 words. Adam Ratner decided he would probably be more persuasive with reviewers by using the diagram and so the text he gave up was worth the trade-off.

Gardnerella vaginalis: toxin production and pathogenesis

SPECIFIC AIMS

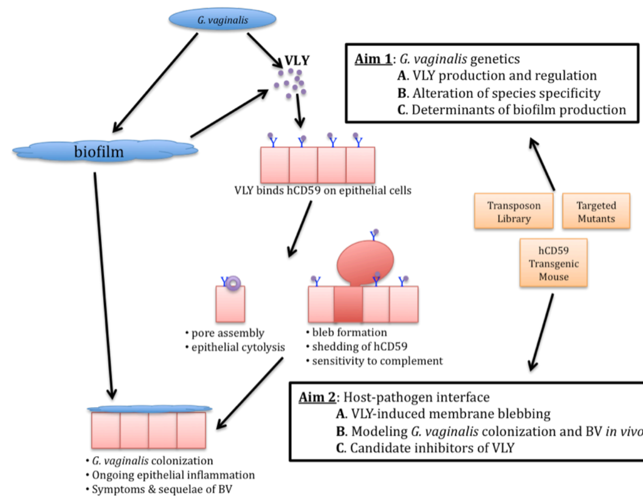
Our overall goal is to define the role of vaginolysin (VLY), a novel, human-specific toxin produced by *Gardnerella vaginalis*, in the pathogenesis of bacterial vaginosis (BV). We propose the following research plan directed at elucidating both genetic mechanisms of control of VLY production by *G. vaginalis* and the specific role of VLY in the interaction of *G. vaginalis* with host cells. Using techniques developed in our laboratory, we will perform the first genetic screens in *G. vaginalis*, define genes required for toxin production and other virulence properties, and construct defined mutants of *G. vaginalis* that express a validated, non-species-selective VLY toxin chimera (**Aim 1**). We will also determine the role of VLY at the host-pathogen interface by characterizing VLY-specific responses of vaginal epithelial cells, including bleb formation and increased host cell sensitivity to complement. Both of these responses are unique to VLY and its interaction with the hCD59 receptor, and we hypothesize that they are important to the pathogenesis of BV (**Aim 2A**). Finally, we will take advantage of our knowledge of the VLY-hCD59 interaction to develop novel in vivo models of *G. vaginalis* pathogenesis. Using defined mouse lines transgenic for hCD59 as well as VLY mutants that lack species selectivity, we will manipulate both host and pathogen to perform a detailed analysis of the role of VLY in vivo (**Aims 2B-C**). In addition to vastly expanding our knowledge of *G. vaginalis* pathogenesis and of VLY, this research program will address the potential to manipulate the VLY-hCD59 interaction in order to develop therapeutic strategies and in vivo models for bacterial vaginosis.

Aim 1: **Define determinants of *Gardnerella vaginalis* virulence using new genetic techniques.**

A. Determine genes required for production and regulation of VLY.
B. Construct and evaluate specific *G. vaginalis* strains with altered species specificity.
C. Determine genes required for biofilm formation in *G. vaginalis*.

Aim 2: **Determine the role of VLY in *G. vaginalis* at the host-pathogen interface in vitro and in vivo.**

A. Determine the role of VLY-induced membrane blebbing as a mechanism for protection of vaginal epithelial cells from toxin pores and as a pathway sensitizing cells to complement.
B. Define the role of the VLY-hCD59 interaction in *G. vaginalis* pathogenesis in vivo.
C. Evaluate candidate inhibitors of the VLY-hCD59 interaction in vivo.



Paragraph #1 introduces the general topics of the grant, VLPs, but not the specific problem the grant will deal with. That is introduced in paragraph #2.

SPECIFIC AIMS

Virus-like particles (VLPs) make excellent vaccines. They are non-infectious, often easier to produce than actual viruses, and, because the regularity of their capsid structure presents viral epitopes as dense, highly repetitive arrays that strongly stimulate B cells, they are highly immunogenic. VLPs can be used as the basis for vaccines targeting the virus from which they were derived [the Hepatitis B virus and Human Papillomavirus (HPV) vaccines are two examples], but they also can be used as platforms to display practically any epitope in a multivalent format. Foreign peptides displayed on VLPs by genetic fusion or by other methods (such as chemical conjugation) exhibit the same high immunogenicity as unmodified VLPs. Even self-antigens, which are normally subject to the mechanisms of B cell tolerance, are immunogenic when displayed at high density on the surface of VLPs. Several VLP-based vaccines that target heterologous foreign antigens derived from pathogens and self-antigens involved in chronic diseases are currently in clinical trials [9].

A major hurdle in developing new vaccines using VLPs is that there is often difficulty in both identifying relevant target epitopes and then presenting them to the immune system in a context that mimics their native conformation. Our idea is to create a new VLP-based platform that will facilitate this process, serving both for epitope affinity-selection and as an immunogen. This platform is based on VLPs derived from an RNA bacteriophage, MS2. We have made substantial progress in demonstrating the suitability of this platform for phage display and have shown that recombinant MS2 VLPs are highly immunogenic [11].

Paragraph #3 explains the background leading up to this proposal and the barrier to progress this proposal intends to overcome.

The development of this system is motivated, in part, by the identification of broadly neutralizing monoclonal antibodies (mAbs) against infectious agents, such as HIV, Human Papillomavirus (HPV), and Hepatitis C virus (HCV), and mAbs that have been used to treat chronic diseases, such as arthritis, cancer, and allergy, as well as by the limitations of these mAbs, which are often prohibitively expensive, and usually have limited practical prophylactic value. The existence these mAbs indicates that it may be possible to identify immunogens capable of eliciting similar antibody responses. However, the filamentous phages that form the basis of the most widely used display methods to permit affinity selection of epitopes from peptide libraries (i.e. phage display) do not support the presentation of foreign peptides at the high densities required for potent immunogenicity. This typically means that epitopes identified by conventional phage display must be produced synthetically and then linked to a carrier that displays them in a structural context unrelated to the one in which they were selected. In their new environments they seldom maintain the same affinity they showed originally and frequently lose the ability to induce antibodies whose characteristics mimic those of the selecting antibody.

Paragraph #4 explains the innovation this proposal brings and the impact this proposal will have when it succeeds.

The intent of this proposal is to exploit a phage display system that we have recently developed [11]. In this system epitopes are identified in the same structural context as which they will be presented to the immune system as a vaccine; combining, for the first time, the selective power of phage display and the high immunogenicity of VLPs (shown, schematically, in Fig. 1). The ability to optimize peptides for specific antibody binding by affinity selection, and to do it on the same structural platform used for immunization should greatly increase the likelihood of obtaining a molecular mimic able to induce the desired antibody response. The goal of this proposal is to develop methods that will allow us to exploit this system and rapidly identify new vaccine candidates. As such, the specific aims for this proposal are:

Note the brevity of the Aims.

Aim 1. To develop methods for affinity selection using model monoclonal antibodies recognizing linear and conformational epitopes.

Aim 2. To assess the affinity and immunogenicity of selected recombinant phage.

Chackerian uses an overview diagram to help reviewers understand his vaccine discovery strategy. The text in the diagram is so small, though, that he's forcing reviewers to greatly enlarge the diagram in order to read it. The text in Fig. 1 is probably 8 or 9 point.

A strategy for vaccine discovery using MS2 VLP peptide display.

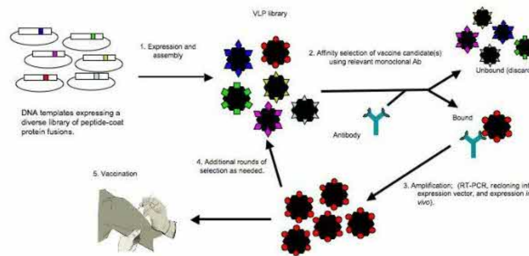


Fig. 1. Our scheme is based on the ability of the coat proteins of RNA bacteriophages both to display foreign peptides and to encapsidate the same mRNAs that serve as templates for their synthesis, thus establishing the needed linkage of genotype and phenotype. The process entails the synthesis of coat protein-peptide fusions from plasmids in bacterial cells. VLPs are extracted from cells and subjected to affinity selection for binding to specific antibodies. Finally, RNA is extracted from the selected VLPs and subjected to reverse transcription and PCR to recover and amplify the encapsidated sequences, which are then cloned into our expression vector and reintroduced into bacteria, where they serve as templates for another round of synthesis, assembly and selection. The process is repeated through as many cycles as needed and, in the end, the selected sequences are cloned for high-level bacterial expression of the selected VLPs, each of which is potentially a vaccine lead.

If you don't have enough room for an overview diagram in Specific Aims, try placing it at the beginning of your Research Plan, as Thomas Jansson did here.

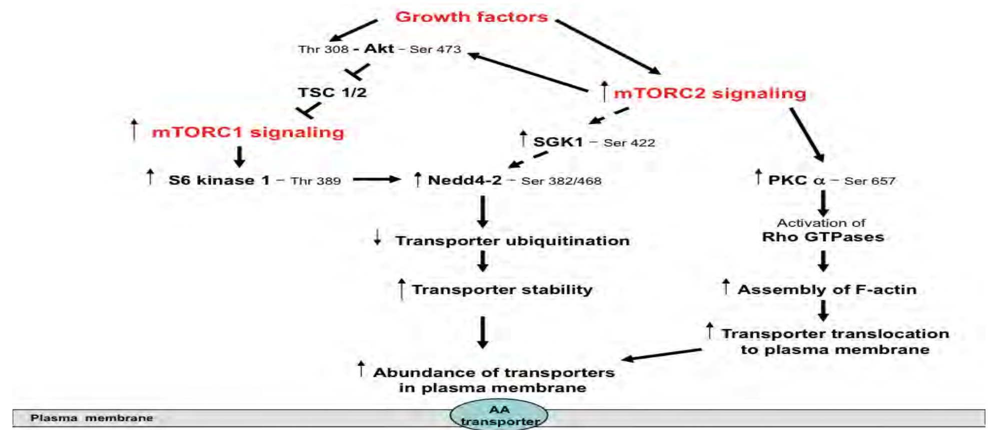


Fig 1. **Central hypothesis.** The figure shows the proposed effects of activation of trophoblast mTOR signaling on plasma membrane distribution of amino acid transporters. We propose that both mTORC1 and mTORC 2 regulates amino acid transporter activity by affecting the plasma membrane trafficking of transporters, thereby regulating trophoblast amino acid transport. Specifically we propose that the molecular mechanisms involved are distinct in that mTORC1 activation phosphorylates the E3 ubiquitin ligase Nedd4-2, which decreases transporter ubiquitination resulting in increased amino acid transporter expression at the cell surface. We further hypothesize that he primary mechanism by which mTORC 2 affect amino acid transporter trafficking is by activation of the actin skeleton mediated by PKCα phosphorylation. Since mTORC2 recently was reported to activate SGK1 [20-22], which is known to phosphorylate Nedd4-2 [23, 24] this link will also be investigated. *Abbreviations*: mammalian target of rapamycin complex 1 and 2; **mTORC1 and mTORC2**, tuberous sclerosis complex ½; **TSC1/2**, p70 S6 kinase; **s6 kinase 1**, Protein Kinase C-α; **PKCα**, Serum and Glucocorticoid-regulated Kinase 1; **SGK1**, Neuronal precursor cell-expressed, developmentally down regulated gene 4 isoform 2; **Nedd4-2.**

3. RESEARCH STRATEGY

a. Significance. This proposal is **significant** because:

1. The activity of key placental amino acid transporters is decreased in IUGR [3-5, 8] and up regulated in fetal overgrowth [9], suggesting that altered placental nutrient transporter activity contributes to abnormal fetal growth [10-12]. Since this work focuses on mechanisms by which placental amino acid transport is regulated, it addresses questions critical to the understanding of how important pregnancy complications develop.

2. Placental mTOR signaling activity is decreased in IUGR [1, 2] and preliminary data show an activation of placental mTOR signaling in fetal overgrowth [25]. Our preliminary data demonstrates that mTORC 1 and mTORC2 signaling has a profound impact on trophoblast amino acid transporter activity, suggesting that we have identified important mechanisms for the regulation of placental amino acid transport and fetal growth.

3. Functional data (nutrient transport activity) will be obtained in primary human trophoblast cells, using growth factors in physiological concentrations, which contributes to the physiological relevance of the proposed studies. Furthermore, the systematic utilization of gene silencing approaches in cultured human primary trophoblast cells will allow us to obtain specific mechanistic information on mTORC 1 and mTORC2 signaling pathways in the human placenta, contributing to the significance of the work.

4. In addition to playing a role in abnormal fetal growth, regulation of nutrient transporters has been implicated in many other diseases, including cancer [26]. However, the authors of several recent reviews have highlighted the existence of a major gap in knowledge with respect to the mechanisms regulating nutrient transporters. For example, Edinger concludes '*Despite the clear implications for human disease, there are large gaps in our knowledge of how nutrient transporter expression is regulated'* [27] and '*.. virtually nothing is known about how nutrient transporter internalization and trafficking is regulated in mammalian cells*' [28]. Thus, the proposed research is significant because it addresses a major gap in knowledge and mechanisms shown to regulate amino acid transporters in human primary trophoblast cells are likely to be relevant for other human cells.

b. Innovation. The proposed research is **innovative** because

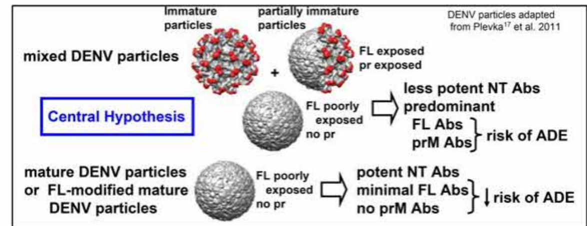
1. A number of the molecular links that we propose in Fig 1 have not been clearly demonstrated previously, **in any mammalian tissue**, and are therefore novel. These include the regulation of amino acid transporter by altered f-actin assembly and mTORC1 regulation of Nedd4-2.

Another example of putting an overview diagram at the beginning of the Research Plan when it won't fit in Specific Aims.

Abbreviations: Abs: antibodies ADE: antibody-dependent enhancement CR: complex-reactive (CR)
DENV: dengue virus DENV1, DENV2, DENV3, DENV4: dengue virus type 1, 2, 3, 4 DIII: domain III of envelope protein
DHF/DSS: dengue hemorrhagic fever/dengue shock syndrome E: envelope EM: electron microscopy
FRNT: focus reduction neutralization test FL: fusion loop GR: group-reactive M: membrane
mAbs: monoclonal Abs NT: neutralizing prM: precursor membrane pr: precursor RI: recognition index
VLPs: virus-like particles TS: type-specific WNV: West Nile virus WT: wide type

2. RESEARCH STRATEGY

A. SIGNIFICANCE



Despite considerable effort and progress in developing tetravalent live-attenuated DENV vaccine candidates, one of the major unmet challenges is the difficulty in eliciting balanced NT Abs against all four serotypes, and to substantially lower the risk of ADE, mediated mainly by cross-reactive and weakly or non-NT Abs[4,5,20,21]. Studies of human Abs after DENV infections have shown the immunodominance of cross-reactive and weakly or non-NT anti-E Abs against FL over the type-specific and potent NT Abs, and the presence of cross-reactive and weakly or non-NT anti-prM Abs[9-14]. The mechanisms underlying such immunodominance and whether it can be modulated to induce potent NT Abs without cross-reactive and weakly or non-NT Abs, thus reducing the risk of ADE, remain unknown. Lacking such information, vaccine strategies to induce protective NT Abs without cross-reactive and weakly or non-NT Abs to mitigate the risk of ADE are not possible.

Our proposal will contribute to the detailed understanding of immunodominance of the DENV E protein and demonstrate that such immunodominance can be modulated by the maturation status and epitopes of DENV particles. Specifically, we propose that mature and FL-modified DENV particles, compared with pr- and FL-expressed mixed DENV particles can elicit potent NT Abs, less cross-reactive and weakly or non-NT Abs thus reducing the risk of ADE. *The contribution is significant because it represents a vaccine strategy that can induce NT Abs and minimize the risk of ADE; this strategy is essential for a safe and effective DENV vaccine*[20,21]. Our proposed studies are highly translational in that they can be applied to the current DENV vaccine candidates including live-attenuated tetravalent vaccines in clinical trials, inactivated-virus vaccines and particle-based subunit vaccines in preclinical stages as the second-generation DENV vaccine to induce better humoral immune responses against DENV[4,5,37]. Moreover the proof-of-concept that epitope-modified mature DENV particles can redirect Ab responses to protective rather than non-protective or infection-enhancing epitopes can serve as a paradigm of epitope-modified vaccines for other infectious diseases, in which humoral immune responses after natural infections are often directed toward non-protective epitopes or result in pathogenic consequences[38-40].

B. INNOVATION

It is known that after primary DENV infection, individuals develop life-long protection against the infecting DENV serotype, which correlates with the appearance of NT Abs against the infecting serotype[41-45]. It is generally believed that presenting the DENV particles in its native form during natural infection would provide protection against that serotype[4,5]. Since DENV particles consist of a mixture of mature, immature and partially mature particles in tissue cultures[15-17], immunization of tissue culture-prepared mixed DENV particles, which have been widely employed in all current tetravalent live-attenuated virus and inactivated-virus vaccine candidates[4,5], will unavoidably induce predominantly cross-reactive, weakly or non-NT anti-FL Abs and also anti-prM Abs, similar to the characteristics of immunodominance generated during natural DENV infections[9-14,46]. These anti-FL and prM Abs have been shown to cause ADE in vitro and/or in vivo[12,14,29,31,47], which is the major risk for severe dengue diseases including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) during natural secondary DENV infection[7,8,48-50], and one of the safety concerns for the most advanced tetravalent live-attenuated DENV vaccines when balanced NT Abs response was not achieved[4,5,20,21,51,52].

The proposed research is innovative because it represents a new strategy for DENV vaccine design by using mature and epitope-modified particles as immunogen, which is a substantive departure from the status quo, namely the use of mixed DENV particles prepared from tissue culture in all current DENV particle-based vaccine candidates. Our preliminary studies (see Approach) strongly suggest that this novel approach for DENV vaccine

Note how carefully Jansson chose which sentences in his introduction to put in bold. If a reviewer scans just the sentences in bold, there's a little story -- namely, there's a rationale for believing mTOR signaling in the placenta is important, and mTOR's regulatory molecular mechanisms are unknown.

2. SPECIFIC AIMS

Abnormal fetal growth increases the risk for perinatal complications and predisposes for the development of obesity, diabetes and cardiovascular disease later in life. Fetal growth is strongly dependent on nutrient availability, which is determined by placental nutrient transport. The activity of key placental amino acid transporters is decreased in intrauterine growth restriction (IUGR) [3-5, 8] and up regulated in fetal overgrowth [9], suggesting that changes in the activity of placental nutrient transporters may directly contribute to abnormal fetal growth [10-12]. Mechanistic information on the regulation of placental nutrient transporters is currently lacking. The mammalian target of rapamycin (mTOR) signaling pathway responds to changes in nutrient availability and growth factor signaling to control cell growth [13-15]. We recently reported that trophoblast mTOR signaling is a positive regulator of amino acid transporters [1, 16, 17] and that placental mTOR activity is markedly decreased in human IUGR [1]. **These observations are consistent with an important role for placental mTOR signaling in regulating placental amino acid transport and fetal growth.** mTOR exists in two complexes, mTOR Complex 1 (mTORC1) and mTORC2 [15, 18]. mTORC1 phosphorylates S6 kinase 1 (S6K1) and 4E-BP1 (eukaryotic initiation factor 4E binding protein 1), which mediates many of the down-stream effects of mTOR [14]. mTORC2 phosphorylates Akt, Protein Kinase C-α (PKCα) and Serum and Glucocorticoid-regulated Kinase 1 (SGK1) and influences the actin skeleton [18, 19]. **However, the molecular mechanisms by which mTOR regulates nutrient transporters in the trophoblast, or in any other cell type, are largely unknown.** The *central hypothesis* in this mechanistic proposal is that both mTORC1 and mTORC 2 regulate placental amino acid transporter activity by affecting the plasma membrane trafficking of transporters (**Fig 1**). We further propose that the molecular mechanisms involved are distinct in that mTORC1 activation phosphorylates the E3 ubiquitin ligase Nedd4-2, which decreases transporter ubiquitination resulting in increased amino acid transporter expression at the cell surface whereas mTORC 2 activation stimulates the actin skeleton mediated by PKCα (**Fig 1**). This central hypothesis is based on compelling preliminary studies (**Figs 2-13**). We propose four specific aims:

Aim 1 **Determine the role of mTORC1 and 2 in regulating placental amino acid transporter activity.** Our *working hypothesis* is that mTOR regulates the activity of placental amino acid transporters, mediated by both the mTORC1 and mTORC2 signaling pathways. We will measure the activity of System A and System L amino acid transporters, and glucose transporters in primary human trophoblast cells subsequent to siRNA mediated silencing or activation (by silencing endogenous upstream inhibitors) of mTORC1 and/or mTORC2.

Aim 2**. Establish the effect of mTOR signaling on trophoblast amino acid transporter trafficking.** We *hypothesize* that mTORC1 and 2 regulate trophoblast amino acid transporter activity by affecting the trafficking of transporters between intracellular stores and the plasma membrane. Our approach will be to study the cellular distribution of amino acid and glucose transporter isoforms in cultured human primary trophoblast cells subsequent to siRNA mediated silencing or activation (by silencing endogenous upstream inhibitors) of mTORC1 and/or mTORC2. Cellular fluorescence imaging, subcellular fractionation and protein expression studies will be used to assess changes in subcellular localization of transporter isoforms.

Aim 3**. Identify the mechanisms by which mTOR regulates plasma membrane trafficking of trophoblast amino acid transporters.** We will test the *hypotheses* that (i) mTORC1 promotes transporter plasma membrane trafficking and cellular amino acid uptake by phosphorylating Nedd4-2 mediated by S6K1, which decreases amino acid transporter ubiquitination, resulting in increased amino acid transporter expression at the cell surface whereas (ii) mTORC 2 activation stimulates the actin skeleton mediated by PKCα. We will determine Nedd4-2 and PKCα phosphorylation, and transporter ubiquitination in cultured human primary trophoblasts subsequent to siRNA mediated silencing or activation of mTOR. We will directly assess the role of S6K1, Nedd2-4, PKCα, SGK1 and the actin cytoskeleton in regulating transporter membrane trafficking and cellular amino acid uptake using gene silencing approaches and inhibitors of actin polymerization.

Aim 4. **Determine the activity of the signaling pathway linking mTOR to amino acid transporter trafficking in IUGR and fetal overgrowth.** We *hypothesize* that the activity of mTOR signaling and the pathways identified in Aim 3 to link mTOR to amino acid transporter trafficking as well as the expression of amino acid transporter isoforms in the syncytiotrophoblast microvillous plasma membrane (MVM) are decreased in IUGR and increased in fetal overgrowth. Placentas from pregnancies with normal fetal growth, IUGR and fetal overgrowth will be collected and the expression of phosphorylated S6K1 and 4EBP-1 (readouts for mTORC1 activity), Ser457-Akt (mTORC2), Nedd2-4, PKCα, and SGK1 will be determined. MVM will be isolated and the activity and expression of System A and L isoforms will be measured and transporter expression in MVM and placental homogenates will be compared.

Turning Aims into questions may provoke reviewers' curiosity

A. SPECIFIC AIMS

Many bacteria move in a directed fashion, toward favorable and away from unfavorable conditions. This process, called chemotactic motility or chemotaxis, is observed in a number of bacterial pathogens but little is known about its role during infection. To swim in a directed manner, bacteria must accurately sense conditions in their environment. The long-term goal of my research is to understand the role of flagellar motility in colonization and pathogenesis, a goal that is achievable in the ulcer-causing bacterium, *Helicobacter pylori*. *H. pylori*, like many bacteria, moves via organelles called flagella. These structures must be present and functional for infection to occur [1, 2] The streamlined chemotaxis system of *H. pylori*, with only four chemoreceptors, makes it an excellent choice for analyzing how chemotaxis is used in the animal host.

Our preliminary work, as well as that of others, suggests that *H. pylori* uses chemotaxis to colonize the mouse stomach [3, 4]. The major hypothesis of this grant proposal is that *H. pylori* uses chemotaxis for initial colonization and long-term persistence, and that this chemotaxis responds to specific host cues.

Three specific aims in the proposal address this hypothesis:. (i) Define at what points chemotaxis is used during infection. (ii) Identify which chemoreceptors are used in vivo and what cues they sense. (iii) Determine what roles the multiple copies of the CheW and CheV signal transducing proteins play during *H. pylori* chemotactic signal transduction.

Specific Aim I: How and when is chemotactic motility used in vivo?
Data from my lab and that of Wren and coworkers suggest that chemotaxis-deficient *H. pylori* are defective in colonization, persistence or both [4]. To dissect when chemotaxis is required, we will: (1) Construct *H. pylori* strains defective specifically in chemotaxis. (2) Determine how well these mutant strains establish infection and persist in the host. (3) Ascertain whether chemotaxis is required for maintaining long-term infection by using regulatable promoters to eliminate chemotaxis after colonization. By testing how elimination of chemotaxis affects a resident infection, we will establish whether this process can serve as a potential drug target for human infection.

Specific Aim II: What chemoreceptors are needed for infection, and to what do they respond?
Chemoreceptors translate information about a bacterium's surroundings into a swimming response. *H. pylori* contains four chemoreceptors [5, 6]. To pinpoint which chemoreceptors are used in vivo and what compounds they sense, we propose three experiments. (1) Genetically alter individual and multiple chemoreceptors. (2) Determine which chemoreceptors are required for stomach colonization using *H. pylori* strains missing one or more chemoreceptors. (3) Expose *H. pylori* to various signals in vitro to assess its chemotactic response, and utilize chemoreceptor mutants to match each chemoreceptor to a cue or set of cues.

Specific Aim III: What roles do the multiple CheVs play during *H. pylori* chemotactic signal transduction?
The chemotactic signal transduction system of *H. pylori* differs from that of *E. coli* by the presence of three CheV hybrid proteins. Each of these proteins is composed of one CheW (coupling protein) domain and one CheY (response regulator) domain. In other microbes, CheV was shown to be redundant with CheW for chemotaxis [7]. In *H. pylori*, we find that CheW is required for in vitro chemotaxis (Terry and Ottemann, unpublished results) although it is somewhat dispensable for infection. We interpret these findings to suggest that redundancy of CheV and CheW is effective in vivo but not in vitro. To analyze the roles of the *H. pylori* CheV proteins, we propose to generate strains that lack the genes encoding the CheVs and CheW (singly and in combinations) and analyze their functions in vitro and in vivo, and determine with which receptors they interact.

This statistics research grant by Eli Stahl makes great use of white space, bold face text and subaim lists. It's inviting to read.

SPECIFIC AIMS

Schizophrenia (SCZ) is a common, complex psychiatric disorder that affects as much as 1% of the population, over two million people in the United States. SCZ entails debilitating comorbidities ranging from unemployment to early death. There is no cure, only palliative treatment with moderate success.

In high-profile psychiatric genetic studies of ever larger sample sizes (N>35,000 cases), over a hundred common variants have been definitively linked to SCZ risk. Utilization of new functional genomic information such as chromatin accessibility, tissue-/cell-specific expression and proteomic assays have helped to glean biological insights from these results, and could enable the detection of associations below standard but statistically conservative genome-wide thresholds.

Rare variants, including large insertions and deletions and gene-disrupting single nucleotide changes, contribute to SCZ risk, often with greater penetrance than common variants. However, it is often difficult to pinpoint the individual causal mutations and impacted genes, and to characterize these variants' effect sizes and penetrances. ***We propose to develop hierarchical Bayesian statistical models to infer causal genes and variants and to model their effect sizes, by integrating large-scale DNA sequence and external data. We will develop the method in concert with large-scale analyses of SCZ case/control and trio family whole exome sequences.***

Here we will develop innovative, integrative analyses of hierarchical models incorporating functional annotation and genomic data, and borrowing information across genes that individually have only very sparse rare variant observations. The proposed framework will also incorporate additional phenotypes (including family history and measures of cognitive ability) to estimate the effect sizes of functional mutations in the context of the genetic background and environmental exposures. Models built under this framework can then provide quantitatively sound bases for thinking about future study design and power analysis. Furthermore, the same analytic framework can be leveraged for genomic risk prediction that integrates multiple classes of genomic and clinical data.

SPECIFIC AIM 1: DEVELOPMENT AND APPLICATION TO SCZ OF THE CORE HIERARCHICAL BAYESIAN MODEL

1A: Develop a hierarchical Bayesian framework for inference of causality of genes and rare variants, given summary statistics from genetic studies and gene- and variant-level annotations. Analyze a simple generative model for rare functional DNA sequence variants; evaluate in simulations with realistic genetic architecture and population genetic parameters.

1B: Apply the method for SCZ discovery genetics, leveraging extant data from large-scale exome-sequencing, GWAS datasets, in conjunction with brain expression quantitative trait loci, chromatin accessibility and histone modifications. Calculate posterior probabilities of causality for all genes and variants, distributions of effect sizes, and statistical enrichment for each annotation used.

SPECIFIC AIM 2: EXTENSION TO INCLUDE INDIVIDUAL-LEVEL DATA

2A: Extend the hierarchical model to include individual-level genotypic data, to jointly model rare variant genetic architecture with common-variant GWAS in the same samples.

2B: Extend the hierarchical model to include epidemiological measures in the same samples. Assess alternative genetic models: liability-scale additive effects, endophenotype meditation models and gene-environment interactions.

SPECIFIC AIM 3: USING THE MODELS IN DOWNSTREAM APPLICATIONS

3A: Use models derived from 1B to seed power analyses for sequencing studies of family and case/control samples.

3B: Leverage the analytic framework and models from 1A and 1B for use in genomic risk prediction. Test in cross-validation and in new samples.

Core methods will be distributed in open-source, freely available software.

Note how brief the introduction at the top can be. In just three sentences Ruth Ley explains the health problem she deals with, what previous research has shown, and her goal for this R01 grant.

Specific Aims:

There is growing evidence that the gut microbiota exerts an influence on human health, and that metabolic dysfunction is often associated with disordered gut microbiota. Studies have documented extensive genetic differences among individuals, including heritable variation for a wide variety of metabolic attributes that are related to public health, including proclivity toward obesity and diabetes. The goal of this project is to identify variation in human genetic factors that influence the composition of gut bacteria, which may consequently influence individual disease susceptibility. To achieve this objective, we propose the following specific aims:

Note how brief the Aims in bold are. Ley then chooses to describe her approach to each Aim at some length.

(i) Characterize the gut microbiomes of 4,000 twin pairs.
We plan to characterize the fecal bacterial diversity of 8,000 volunteers in the UK Twin registry. These 8,000 individuals are either monozygotic (MZ) or dizygotic (DZ) twins who have been genotyped with the Illumina 300 or 600 HapChips, and have provided extensive information regarding their health and dietary habits. We have developed an efficient, high-throughput laboratory pipeline to process fecal samples, isolate DNA and multiplex samples to generate large 16S rRNA libraries from which we obtain 300,000 reads using the Illumina HiSeq 2000 platform. The pipeline includes bioinformatics components to identify and quantify bacterial species composition of the gut microbiomes. A subset of the subjects will be resampled at five time points (1.5 M reads) to assess turnover of the microbiome in this population.

(ii) Establish the heritability of the gut microbiome.
Once we determine the phylogenetic assignment and abundances of bacterial species in the fecal samples, we will apply quantitative genetics methods to determine which aspects of the gut microbiota are heritable. Attributes that we will consider include estimates of phylogenetic diversity, which provide an indication of species richness, and calculation of microbiotic ecological indices, such as the Simpson Index, which combine measures of taxonomic richness and evenness. We will also ascertain the relative abundances of specific bacterial taxa, including those with known taxonomic classifications as well as uncultured taxa that are known from sequence data alone. The heritability of these measures will be quantified using the genome-wide Single Nucleotide Polymorphism (SNP) data to estimate the proportion of the genome that is shared Identical-by-Descent (hereafter, IBD) between the DZ twin-pairs (this varies from about 30 to 70%). The regression of squared difference in phenotypic metrics on the proportion of IBD sharing (Haseman-Elston regression) provides a robust estimate of heritability of the respective microbiota metric. Positive results, including the relative abundance of specific taxa will be validated subsequently by qPCR of fecal samples.

(iii) Determine regions of the human genome responsible for the differences in gut microbiomes.
All the subjects in the UK Twin registry have been genotyped with well characterized SNPs that have been widely studied within the Illumina family of SNP genotyping platforms. This sample is more than adequate for a well-powered Genome-Wide Association Study (GWAS) that will probe the entire genome for statistical associations between genetic variants and gut microbiota composition. First, complementary to our analysis of heritable differences in gut microbiota from Specific Aim 2, the GWAS analysis will be applied to identify regions of the genome that confer differences in phylogenetic diversity, species richness, and the abundance of specific bacterial taxa. We will initially constrain the analysis to candidate genes (*e.g.*, innate immunity genes) and subsequently will include all genes. These tests will be based on IBD sharing of genomic regions between DZ twin pairs, and by fitting general linear models to test for positive associations between IBD sharing and phenotypic similarity. MZ twin data will be used in these tests as well (having 100% sharing). In a second approach, DZ twin microbiomes will be clustered by similarity, and we will search for regions of high IBD sharing in the genomes of twins with similar microbiomes. These data will provide powerful tests of nongenetic familial effects. Factors such as obesity, diet, and smoking in these twins will be incorporated into the models. Validation will be achieved by testing association of identified heritable components in extensive non-twin samples.

The final paragraph is her Overall Impact, explaining how success will have a strong, sustained impact in her field.

Impact of the results: The results of this research will impact the nascent field of human-microbiome interactions by providing researchers with target interactions between human genes and the microbiome. This genetic screen will reveal targets for subsequent mechanistic studies regarding host-microbial interactions, biomarkers for disease susceptibilities, and therapeutic targets for use in disease prevention. The communities that are impacted range from the biomedical research community to the millions of Americans suffering from increasingly prevalent chronic diseases linked to microbiome-induced inflammation.

In contrast to Ruth Ley's Specific Aims above (brief intro, long Aims) Cathryn Nagler takes the opposite approach (long intro, brief Aims).

The intro includes: the problem the grant deals with; why the focus in the field changed; Nagler's earlier work and preliminary data; this grant's central hypothesis; a summary of what Aims 1 and 2 will accomplish; what each Aim will do.

SPECIFIC AIMS

Both its rising incidence and the potential for fatal anaphylactic reactions have made allergic responses to food an increasingly important public health problem. Although there is clearly a heritable component to susceptibility to food allergy, variants identified to date explain only a small fraction of the overall genetic contribution to disease risk and cannot account for a dramatic increase in disease prevalence in a short timeframe. The focus has therefore shifted toward gene-by-environment interactions. Several hypotheses have been offered for how the environment may be interacting with the immune system to promote allergic disease. Increasing attention has focused on the role of the commensal bacteria that occupy the body's mucosal surfaces. Earlier work from our laboratory showed that mice unable to signal via TLR4, the receptor for bacterial lipopolysaccharide (LPS), exhibit enhanced allergic responses to food. We hypothesized that commensal bacteria were the source of the TLR4 ligand and demonstrated that neonatal administration of a cocktail of broad-spectrum antibiotics (Abx) induced an allergic response in TLR4 sufficient mice equivalent to that seen in TLR4 mutant mice. In the preliminary data presented in this revised application we have established a novel gnotobiotic model of food allergy and show that a defined bacterial consortium, derived directly from the intestinal microbiota of healthy mice, protects against systemic hyperreactivity to a food allergen. We demonstrate that bacteria in the Clostridia class selectively induce a barrier protective response that includes activation of the IL-23/IL-22 axis, the induction of anti-microbial peptides, and the expansion of intestinal Tregs and IgA secreting B cells; part of this response is TLR4-dependent. *We hypothesize that a Clostridia-containing microbiota is sufficient to elicit a barrier protective response that protects against allergic responses to food.* In the two **Aims** proposed we will examine how allergy protective bacterial populations deliver signals to their hosts at both the cellular and molecular level. **Aim 1** will determine which cellular interactions with commensal bacteria are necessary and sufficient to induce a barrier protective response. We have used Cre-Lox technology to generate mice with targeted mutations in MyD88 signaling in CD11c^{+} dendritic cells (DC) and in intestinal epithelial cells (IEC). We will also examine whether TLR4 signaling is required by the Tregs themselves**. Aim 2** will examine the downstream consequences of these host-bacteria interactions. Microarray analysis of intestinal epithelial cells highlighted two novel genes/pathways selectively upregulated in the epithelium of Clostridia colonized mice; the anti-microbial peptide *Reg3b* and a target gene for the aryl hydrocarbon receptor (Ahr). Both pathways have already been intimately linked to the regulation of intestinal immunity. The induction of epithelial *Reg3b/g* is known to require epithelial cell intrinsic MyD88 dependent signals. Interestingly, the Clostridia-induced barrier protective response is impaired in allergy susceptible *Tlr4*$^{-/-}$ mice. The induction of both *IL-23* and *Reg3b/g* expression in the colon of colonized mice is TLR4 dependent, further justifying the importance and novelty of the experiments proposed in **Aim 1**. Seven new figures of preliminary data also strongly support the new experiments proposed in **Aim 2a**, which will examine how Ahr-mediated signals and IL22 contribute to a barrier protective response that prevents an allergic response to food. Finally, **Aim 2b** will examine how Clostridia induced activation of the innate and adaptive immune system impacts epithelial tight junction protein expression and function.

Aim 1. To examine the cellular basis for the TLR4-dependent induction of a barrier protective response by the commensal microbiota

a. Characterization of mice with targeted mutations in MyD88 signaling.
b. Protection against allergy by transfer of Foxp3^{+}Tregs from *Tlr4-/-* mice

Aim 2. To examine the bacteria protective pathways induced by specific commensal species that protect against food allergy.

a. Examination of the role of Clostridia induced Ahr-mediated signals in protection against an allergic response to food.
b. Examination of the mechanisms by which Clostridia-mediated alterations in innate and adaptive immune system activation impact epithelial barrier function

SPECIFIC AIMS

Excessive weight gain during pregnancy is a consistent predictor of postpartum weight retention.[12,13] However, more than 45%[14,15] of women exceed guidelines established by the Institute of Medicine (IOM) for gestational weight gain (GWG).[16] Compared to normal weight women, women who begin pregnancy overweight or obese are more likely to gain weight excessively during pregnancy[17] and retain a larger amount of gestational weight postpartum.[18,19] In addition to the relationship of GWG to postpartum weight, large gestational weight gains increase the risk of negative pregnancy outcomes.[20,21] Given the strong relationship of GWG to postpartum weight retention and negative obstetric outcomes, preventing excessive GWG can improve obstetric outcomes and lead to improvements in the treatment of obesity for women. To date, however, efforts to prevent excessive GWG have not been successful.[22] Thus, there is a need to identify modifiable behaviors related to GWG that will support the development of effective interventions to prevent excessive GWG.

One modifiable mechanism that may explain excessive GWG is loss of control over eating (LOC). LOC refers to the ingestion of food with an associated experience of being unable to control one's eating, and is the core psychopathology of binge eating disorders.[23] LOC also occurs in women without eating disorders,[24] and is particularly common among overweight and obese individuals.[25-27] Episodes of overeating with and without LOC are associated with weight gain. However, LOC may be particularly related to excessive GWG because of the relationship of LOC to increased daily calorie intake and more frequent overeating episodes.[5-7] LOC also relates to higher levels of depressive symptoms,[28] which themselves are associated with weight gain and obesity.[29,30] Moreover, because effective interventions to reduce LOC and related psychopathology differ from general weight control interventions,[31] understanding the relationship between LOC and GWG may lead to improved interventions to prevent excessive GWG. Given that LOC is prevalent among overweight and obese women, related to weight gain and modifiable, it is important to determine the contribution of LOC to GWG.

Accordingly, we propose to assess LOC and psychosocial distress among overweight/obese women throughout pregnancy to elucidate the contribution of LOC to excess GWG. Specifically, the effect of depressive symptoms, perceived stress and social support, which may affect GWG[1,4] and relate to LOC will be examined. Overweight/obese pregnant women (N = 300) will be assessed at the end of the first trimester, monthly through the remainder of the pregnancy and, to examine postpartum weight retention, at 6 months postpartum. LOC, psychosocial distress and other conceptually relevant factors will be assessed in person at the beginning of pregnancy and six months postpartum and by telephone throughout pregnancy. Total GWG will be abstracted from medical charts and women will be weighed at 6 months postpartum.

In this grant a distinction is made between Specific Aims 1 and 2 and an Exploratory Aim 3. My interpretation of this is that Michele Levine regards Aims 1 and 2 as things she is sure to accomplish, and Aim 3 as something of higher risk, but worth NIH support because of what it will mean for her field if it succeeds.

The Specific Aims are to:

1. **Determine the contribution of loss of control over eating (LOC) to excessive GWG among overweight/obese women.**
   Hypothesis 1a: Prepregnancy LOC will be associated with an increased likelihood of exceeding IOM guidelines for GWG and with larger total GWG.
   Hypothesis 1b: Increases in the frequency of LOC during pregnancy will be associated with an increased likelihood of exceeding IOM guidelines for GWG and with larger total GWG.
2. **Determine the separate and combined effects of LOC and related psychosocial factors (depressive symptoms, perceived stress and social support) on GWG among overweight/obese women.**
   Hypothesis 2a: Women with greater psychosocial distress (i.e., depressive symptoms, perceived stress or poor social support) during pregnancy will be more likely to exceed IOM guidelines for GWG and have larger total GWG.
   Hypothesis 2b: The effect of LOC on GWG will persist after controlling for psychosocial distress.

An exploratory aim is to:

3. **Examine the relationship among LOC, depressive symptoms, social support, perceived stress and 6 month postpartum weight retention, independently of GWG.**
   Exploratory Hypotheses: After controlling for GWG, women with LOC or in whom depressive symptoms, perceived stress or poor social support is greater prior to or during pregnancy will retain more weight at 6 months postpartum than those who do not have LOC or have less psychosocial distress. In addition, after controlling for GWG, women with postpartum LOC or increased frequency in LOC episodes between pregnancy and 6 months postpartum will retain more weight 6 months postpartum than those without postpartum LOC or in whom the LOC frequency does not change.

This is an example of Specific Aims written knowing it must address an objection sure to arise as soon as reviewers understand what the project is about: namely, why is this project about plant research asking NIH for money? Isn't NSF the place for that? Laurel Coaker wisely begins to deal with this objection here in Specific Aims rather than delaying until later pages. As the last sentence in the first paragraph ("Furthermore,...") states, insights from this research will have implications for human disease. Without sentences like this here, in her abstract, and probably elsewhere, Coaker would be giving reviewers an excuse to kill this grant and send it to NSF.

A. SPECIFIC AIMS

Plants are constantly exposed to infectious microorganisms. However, the development of disease is the exception rather than the rule due to the evolution of highly coordinated passive and active defense systems in higher plants. There are two active branches of the plant immune system [3,5]. One branch consists of extracellular receptors recognizing conserved microbial features and functions to inhibit initial pathogen colonization. The second branch consists of intracellular receptors that recognize the presence of pathogen proteins present inside plant cells during infection. Despite the importance of the innate immune system, scientists still have a limited understanding of the composition and regulation of immune complexes in plants. The PI's laboratory investigates how the plant immune system recognizes bacterial pathogens. To date there is only one gene that can regulate both branches of the plant immune system: *RIN4* [6,7,8,9]. *RIN4* is highly conserved among all land plants, yet the mechanisms it uses to regulate defense signaling are largely unknown. RIN4 is a central player in regulating innate immunity at the membrane and its presence in more than one protein complex indicates that RIN4 is an important signaling molecule to study the regulation and activation of protein complexes controlling plant immunity. Our *long term goal* is to elucidate the signaling overlap between both branches of the plant innate immune system. This proposal seeks to understand how plant immune signaling unfolds by investigating RIN4-mediated cellular signaling cascades. *The central hypothesis of the proposed experiments is that RIN4 protein complex constituents will be key components controlling innate immune signaling.* A mechanistic understanding of how plant immune signaling unfolds will lead to innovative strategies to control and prevent plant disease. Furthermore, fundamental insight into how protein complexes are assembled, activated, and regulated at the membrane can also be applied to other eukaryotic systems, including human disease.

Using immunoaffinity chromatography, we have purified RIN4 associated proteins (RAPs) from the plant *Arabidopsis*. Purified proteins were identified by mass spectrometry, enabling the detection of RAPs in the absence and presence of pathogen stimulus. RIN4 associates with a different set of proteins during immune signaling, indicating that it may function as an adapter, transferring the signal of pathogen perception to intracellular signaling pathways. One RAP is a plasma membrane H^+-ATPase (AHA) that is expressed in guard cells, which make up stomatal pores. Our results indicate that RIN4 functions in concert with AHA to regulate leaf stomata during the innate immune response, thus blocking the entry of bacterial pathogens into the leaf interior. The discovery that RIN4 is a molecular link between immune signaling and stomatal movement provides an explanation for how this important defense regulator can act to control immunity at the level of pathogen invasion. Recently, we have also shown that two additional RAPs play a role in plant innate immunity.

The Specific Aims of this application are:

1) Elucidate the mechanism RIN4 uses to regulate plasma membrane H^+-ATPase enzymatic activity. RIN4 is posttranslationally modified during pathogenesis and we are unable to detect an interaction between modified RIN4 and AHA1. We will test the hypothesis that RIN4's phosphorylation status controls its interaction with AHA1, leading to the regulation of stomatal apertures during innate immune defenses.

2) Investigate the spatial and temporal components of the RIN4 protein network. We hypothesize that RIN4 is an adapter protein for multiple protein complexes and exists in distinct pools within plant cells. We will generate a RIN4 interactome map by conducting targeted yeast-two hybrid with RIN4 and RAPs as well as RAPs with one another. We will also analyze complex assembly *in planta* in different sub-cellular regions and tissues using a combination of Blue-Native PAGE and bimolecular fluorescence.

3) Functionally characterize *Arabidopsis* RAPs. We have reproducibly identified 15 novel RAPs. Several of these RAPs are differentially regulated during infection and some have been implicated in immune signaling in either plants or vertebrates. Available RAP knockout lines will be analyzed for altered disease phenotypes. Two informative RAPs that can interact with RIN4 and are involved in defense signaling will be characterized in-depth using a combination of genetics, cell biology, and biochemistry.

Targeting the MYC Oncogene with CDK Inhibitors

A. SPECIFIC AIMS

The MYC oncogene is either amplified or over-expressed in some of the most prevalent and difficult to treat human malignancies including breast and liver cancers and lymphomas. To date there is no therapeutic strategy to specifically target the MYC pathway without also harming normal cells. However, we recently discovered that if cancer cells overexpress *MYC*, then they are killed by inhibitors of cyclin dependent kinases (CDKs)[4]. Importantly we showed that normal cells are not sensitive to such killing and that this context-dependent cell killing (called synthetic-lethality) appears specific for cells that over express *MYC*. Understanding the molecular events that increase the sensitivity of cancer cells to CDK inhibitors should allow for more rapid development of these compounds as therapeutics. We propose innovative ways of using mouse and human cells to investigate how CDK inhibition selectively kills cells that over-express MYC. Our long term objective is to understand the biology of this synthetic-lethality and to develop inhibitors useful for the treatment of human malignancies. We propose that CDK inhibitors may have broad application for the treatment of human cancers in which *MYC* is over-expressed.

Note the nice way in which Goga used white space to make his central question and central hypothesis easy to notice.

Central Question: How does MYC over-expression interact with CDK inhibition to kill malignant cells while sparing normal cells?

Central Hypotheses: Inhibition of CDK1 is central to the synthetic lethal interaction with MYC over-expression. CDK1 inhibition alters the function of BIM, Survivin, and possibly other factors. These alterations are lethal to cells that overexpress MYC.

Aim I: Determine whether specific inhibition of CDK1, CDK2 or both is required for the synthetic-lethal effect of CDK inhibition on malignant cells that over-express MYC.

CDK1 and CDK2 are structurally related but functionally distinct kinases that act at different points within the mammalian cell cycle. Inhibition of either or both CDKs could mediate the synthetic-lethality with MYC over-expression. Understanding which CDK is responsible for this effect may provide a greater understanding of the underlying mechanism and allow for the development of more selective inhibitors. Since CDK1 and CDK2 are structurally similar, no currently available small molecule inhibitor is entirely selective for either kinase. This limits our ability to study their individual functions *in vivo*. We will use an innovative chemical-genetic approach to generate analog-sensitive alleles called CDK1-AS and CDK2-AS, respectively. Endogenous CDK1 or CDK2 alleles will be replaced with the corresponding analog-sensitive alleles in mammalian cells. Using this approach, acute and reversible inhibition of each CDK can be achieved with unparalleled specificity. **A)** We will determine if inhibition of either or both CDKs can cause synthetic-lethality with MYC over-expression and at what points within the cell cycle the synthetic-lethality occurs. The chemical-genetic approach employed should allow us, for the first time, to determine which CDKs are required for the survival of cells that over-express MYC. **B)** We will also use small molecule CDK inhibitors that target both CDK1 and CDK2 to determine if combined inhibition can kill receptor triple-negative breast tumor cells that over-express *MYC*. Such tumors are amongst the most difficult breast cancers to treat.

Aim II: Validate that BIM is key to the synthetic lethal effect of CDK inhibition in cells that over-express MYC, and identify the mechanisms by which CDK inhibition cooperates with MYC to induce BIM.

We have identified a novel interaction between MYC over-expression and CDK inhibition resulting in coordinate up-regulation of the pro-apoptotic, BCL2 family member, *BIM*. **A)** We will test if *BIM* induction is critical for synthetic lethality of cells engineered to over-express MYC. **B)** Using transgenic mouse models, we will also ask if primary lymphomas driven by MYC but deficient for BIM are less sensitive to CDK inhibition.

Our studies seek to elucidate important biological relationships between CDKs, the apoptotic machinery, and regulation of cell survival via *BIM*. At least three different mechanisms have been implicated in regulating BIM expression in mammalian cells: **C)** alteration of *BIM* protein activity and stability by phosphorylation at regulatory sites, **D)** changes of BIM transcription, **E)** and attenuation of BIM translation by miRNAs. We will ask if CDK inhibition in the context of MYC over-expression employs one or more of these mechanisms to increase *BIM* expression. For example, in our exploratory studies we have found that CDK inhibition results in down-regulation of miR-19, a miRNA that negatively regulates BIM translation. Alteration of miRNA expression is a novel potential mechanism through which CDK inhibitors may act that has not been previously identified or explored.