

CAMB 695: Scientific Writing

SYLLABUS for 2025

Five Principles of Clear Scientific Writing

When scientists use dense language to describe their work, the story gets murky and no clear message emerges. In this course, we will show you how to assemble the parts of a research proposal into a comprehensible story that has a clear message.

1. Get to the subject quickly and follow the subject as soon as possible with its verb.

Gopen and Swan pp 551-552 “Subject-Verb Separation”

(Additional material: Williams *Style: The Basics* Chapter 8 “Shape” pp 94-97)

○ **Get to the subject quickly**

- Avoid long, abstract subjects – noun clusters

In English, one noun is commonly used to modify another noun, e.g. blood flow, lung function, ion concentration. But adding more nouns to an existing noun pair (a noun cluster) creates long, abstract subjects and is confusing. To untangle a noun cluster, start at the end of the cluster and add words to indicate how the nouns relate to each other.

- Example: *Early childhood thought disorder misdiagnosis* often results from unfamiliarity with recent research describing such conditions.
Problem: Confusing noun cluster, abstract subject
Revision: *Physicians misdiagnose disordered thought in young children* because they are unfamiliar with recent research describing such conditions.

- Avoid lots of introductory words - “throat clearing”

Readers have a problem with sentences that open with long introductory phrases and clauses, because, as they read them, they have to keep in mind that the subject and verb of the main clause are still to come.

- Exercise: In most cases, because of the efficacy of the recently available treatment regimens, physicians are able to diagnose and successfully treat PCP.
Problem: Lots of introductory words
Revision: *Physicians are able to diagnose and successfully treat PCP* because good treatments are now available.

○ **Get past the subject and to its verb quickly** – avoid subject-verb separation

Readers expect the grammatical subject to be followed almost immediately by its verb. Anything of length that separates the subject and verb is read as an interruption. An interruption after the subject forces the reader to hold his mental breath until he

reaches the verb. Without the verb, the reader does not know what the subject is doing, or what the sentence is about.

- Example: The smallest of the URF's (URFA6L), a 207-nucleotide reading frame overlapping out of phase the NH₂-terminal portion of the adenosinetriphosphotase (ATPase) subunit 6 gene has been identified as the animal equivalent of the recently discovered yeast H⁺-ATPase subunit 8 gene. Problem: 23 words separate the subject "the smallest" and the verb "has been identified."
Revision A: The smallest of the URF's is URFA6L, a 207-nucleotide reading frame which overlaps, out of phase, the NH₂-terminal portion of the adenosinetriphosphotase (ATPase) subunit 6 gene. URFA6L is the animal equivalent of the recently discovered yeast H⁺-ATPase subunit 8 gene. This revision incorporates the interrupting material into the sentence structure.
Revision B: The smallest of the URF's (URFA6L) is the animal equivalent of the recently discovered yeast H⁺-ATPase subunit 8 gene. This revision deletes the interrupting material and also eliminates the unnecessary phrase "has been identified."
- Exercise: Some scientists, because they write in a style that is impersonal and objective, do not communicate easily with laypeople.
Problem: Subject-verb separation
Revision: Some scientists do not communicate easily with laypeople because they write in a style that is impersonal and objective.

2. Put the action in the verb.

Gopen & Swan pp 557 "Locating the Action"

(Additional material: Williams *Style: The Basics* Chapter 3 "Actions" pp 27-38)

- Readers expect that the action of a sentence will be articulated by its verb. If the action of the sentence is expressed by the verb, the sentence is direct and easy to understand. If the action is not expressed as a verb, it is usually lodged in a noun made out of a verb. We call such a noun a *nominalization*.
 - Examples:

| | |
|-------------|-----------------------|
| <u>verb</u> | <u>nominalization</u> |
| prolong | prolongation |
| inhibit | inhibition |
| measure | measurement |
| evaluate | evaluation |
| remove | removal |
| exist | existence |
- Nominalizations are not bad in and of themselves, only in the way they are (mis)used. The thing is to know which nominalizations to keep and which to turn into verbs.
- Turn a nominalization into a verb when it expresses the action of the sentence.

- Example: Removal of potassium perchlorate was achieved by centrifugation of the supernatant liquid at 1400xg for 10 min.
Revision A: Potassium perchlorate was removed by centrifugation of the supernatant liquid at 1400xg for 10 min.
Revision B: Centrifugation of the supernatant liquid at 1400xg for 10 min removed potassium perchlorate.
Revision C: We removed potassium perchlorate by centrifuging the supernatant liquid at 1400xg for 10 min.
- Exercise: Our lack of data prevented evaluation of the role of the D1 receptor in the locomotor stimulant effects of cocaine.
Revision: We could not evaluate the role of the D1 receptor in the locomotor stimulant effects of cocaine because we lacked data.
- Keep a nominalization when it does the following:
 - Refers to the previous sentence
 - Example: When added to the nuclear extract, the egg extract inhibited transcription generally. This inhibition could be alleviated in part by supplementing the mixture with RNA pol III.
 - Replaces an awkward “The fact that”
 - Example: The fact that the Zip2 protein localized to discrete foci on meiotic chromosomes suggested that...
 vs
 Localization of the Zip2 protein to discrete foci on meiotic chromosomes suggested that...
 (However, an alternative would be “Zip2 protein localized to discrete foci on meiotic chromosomes, suggesting that ...”)
 - Names what would be the object of the verb
 - Example: We accepted what they found.
 vs
 We accepted their findings.

3. Put information where the reader expects it.

- There are two moments in the reading process which occur over and over again and are very important to both writer and reader: They are the beginnings and the ends of sentences.
 - We’ll label the ends of sentences the “**Stress Position.**”
 - We’ll label the beginnings of sentences the “**Topic Position.**”
- **The Stress Position – save the best for last**
 Gopen and Swan pp 552-554 “The Stress Position”
 (Additional material: Williams *Style: The Basics* Chapter 6 “Emphasis” pp 66-71, 74-78)
 - Readers naturally assign emphasis to the words at the end of a sentence, the “Stress Position.”

- Use the stress position of a sentence to introduce long, complex, or otherwise difficult-to-process material, particularly unfamiliar technical terms and “NEW” information.
 - Example: The role of *calcium blocker drugs* in the control of *cardiac irregularity* can be seen through an understanding of the role of calcium in the activation of muscle groups.
Problem: New, complex terms are at the beginning of the sentence.
Revision: If we understand how calcium influences the contraction of muscles, we can see how *cardiac irregularity* is controlled by the family of drugs called “*calcium blockers*.”
 - Exercise: A determination of involvement of lipid-linked saccharides in the assembly of the oligosaccharide chains of ovalbumin *in vivo* was the principal aim of this study. *In vitro* and *in vivo* studies utilizing oviduct membrane preparations and oviduct slices and the antibiotic tunicamycin were undertaken to accomplish this.
Problem: New, complex terms are at the beginnings of the sentences.
Revision: The principal aim of this study was to determine how lipid-linked saccharides are involved in the assembly of the oligosaccharide chains of ovalbumin *in vivo*. To accomplish this aim, we conducted studies on preparations of oviduct membrane and on oviduct slices *in vitro* and *in vivo*, utilizing the antibiotic tunicamycin.
- Use the stress position to place emphasis on words that you feel deserve it.
 - Example: compare (a) and (b)
 - (a) Overall, although this proposal is scientifically sound, the preliminary results are not persuasive.
 - (b) Although the preliminary results are not persuasive, overall this proposal is scientifically sound.
 While neither of those statements would be considered a rave review, version (a) will probably make the scientist less happy than version (b). In (a), the bad news occupies the stress position; in (b), the good news occupies the stress position.
 - Exercise #1: The data offered to prove ESP are too weak for the most part.
Problem: The words at the end of the sentence are not emphasis-worthy.
Revision: The data offered to prove ESP are generally too weak.
 - Exercise #2: Mucosal and vascular permeability altered by a toxin elaborated by the vibrio is a current hypothesis to explain this kind of severe condition.
Problem: The words at the end of the sentence are not emphasis-worthy AND the long, complex material is at the beginning of the sentence.
Revision: One explanation for this kind of severe condition is that a vibrio toxin alters mucosal and vascular permeability.

○ **The Topic Position – first things first**

Gopen and Swan pp 554-556 “The Topic Position”

(Additional material: Williams *Style: The Basics* Chapter 4 “Characters” pp 39-45)

- The topic position extends through the first few words of a sentence up to and including the grammatical subject. It stops short of the verb.
- Use the topic position to introduce “whose story” a sentence is going to be. Readers expect a sentence to be a story about whoever shows up first.
 - Example: Compare (a) and (b) and (c)
 - (a) In the 1970s a few scientists in the United States and Europe began to find a way through disorder. They were mathematicians, physicists, biologists, chemists, all seeking connections between different kinds of irregularity.
 - (b) Finding a way through disorder was accomplished in the 1970s by American and European scientists. This goal was accomplished by a variety of scientists all seeking connections between different kinds of irregularity.
 - (c) The United States and Europe made it a priority in the 1970s that their scientists should find a way through disorder. These two countries funded mathematicians, physicists, biologists, and chemists, to find connections between different kinds of irregularity.

In each of these examples, by changing the occupant of the topic positions, we’ve changed the answer to “whose story is this?” In (a) the answer is “a few scientists,” in (b) it is “finding a way through disorder,” in (c) it is “the U.S. and Europe.”

- Exercise: Rewrite the following sentence. Make the story about “plants.”
Richly fertilized plains and river valley are places where plants grow most richly, but also at the edges of perpetual snow in high mountains.
Revision: Plants grow most richly in fertilized plains and river valleys, but plants also grow at the edges of perpetual snow in high mountains.

- Use the topic position to communicate “OLD” information that forges a logical backward link to the previous sentence. The term “old information” refers to any material that has already appeared in the particular piece of text. Often it will have appeared in the sentence immediately preceding; sometimes it will have appeared farther back within the paragraph. When a piece of old information appears at the beginning of a sentence it gives the reader context.
 - Example: In both (a) and (b), old information in the topic position of the second sentence provides a backward-link to the first sentence.
 - (a) Meteorologists look cheerful and confident when they report normal weather, but tense and crisis-ridden when they warn us about *hurricanes*. *These storms* cannot be predicted with any sense of surety, despite the great leaps forward we have made in meteorology.

(b) *Hurricanes* fascinate and haunt us, acting like irrational characters in a high-intensity, reality TV drama. *Hurricanes* cannot be predicted with any sense of surety, despite the great leaps forward we have made in meteorology.

- Exercise: A is a B-class GTPase. C-type kinases phosphorylate some B-class GTPases.
Problem: The old, backward-linking information in sentence 2 is at the end, not the beginning, of the sentence.
Revision: A is a B-class GTPase. Some B-class GTPases are phosphorylated by the C-type kinases.

○ **Two Ways to Depict Reader Expectations about Sentence Structure**

In terms of a diagrammed sentence

| | |
|--|---|
| Topic Position | Stress Position |
| Old, simple, backward-linking information | New, complex, emphasis-worthy information |
| Subject | Verb |
| Person, thing or concept whose story it is | Action, what is going on |

In terms of the progress readers expect as they travel through a sentence

| | |
|------------------------|---|
| <u>Time</u> | <u>Question a reader expects to have answered</u> |
| Right away | How does this link backward to what I've just read? |
| ASAP | Whose story is this? (= the grammatical subject) |
| Immediately thereafter | What's going on? (= the verb) |
| Then, at leisure | How will this thought develop? |
| At the end | What is the most important piece of information here? |

4. Write cohesive sentences and coherent paragraphs.

Gopen and Swan pp 556-557 "Perceiving Logical Gaps"
 (Additional material: Williams *Style: The Basics* Chapter 5 "Cohesion and Coherence" pp 59-60)

- **Cohesion: a sense of flow from sentence to sentence**
 - We judge sentences to be cohesive, or connected, when the first few words in a sentence lean backward (provide OLD information), and the last few words lean forward (provide NEW information).
 - This information flow in a sentence can be depicted as OLD→NEW.
 - Put old, familiar information in the topic position of a sentence.

- Put new or complex information in the stress position. The information in the stress position often becomes the “whose story,” or the old information in the topic position of the next sentence.
- Example: Some astonishing questions about the nature of the universe have been raised by scientists studying black holes in space. The collapse of a dead star into a point perhaps no larger than a marble creates a black hole. So much matter compressed into so little volume changes the fabric of space around it in puzzling ways.
Problem: The information flow from sentence to sentence is not old→new.
Revision: Some astonishing questions about the nature of the universe have been raised by scientists studying black holes in space. A black hole is created by the collapse of a dead star into a point perhaps no larger than a marble. So much matter compressed into so little volume changes the fabric of space around it in puzzling ways.
- **Coherence: a sense of the whole point of a paragraph**
 - We judge a passage to be coherent when three features are present:
 - The opening sentence prepares us for the themes of the passage by emphasizing them in its stress position.
 - Individual sentences follow the old → new principle, connecting to the sentence before and after (see Cohesion, above)
 - The words beginning each sentence in the passage cumulatively constitute a limited and related set of words (a “topic string”) that tell us what the passage is about.
 - Example: Compare (a) and (b).
 - (a) Since the discovery that one factor of its development might be genetic, great strides in the early and accurate diagnosis of Alzheimer’s disease have been made in recent years. Senility in an older patient who seemed to be losing touch with reality was often confused with Alzheimer’s. Genetic clues have become the basis of newer and more reliable tests in the last few years. The risk of human tragedy of another kind, though, has resulted from the increasing accuracy of these tests: predictions about susceptibility to Alzheimer’s have become possible, long before the appearance of any overt symptoms. An apparently healthy person could be devastated by such an early diagnosis.
 - (b) In recent years, researchers have made great strides in the early and accurate diagnosis of Alzheimer’s disease. However, these improved diagnoses have raised new concerns about how to inform those most at risk. Previously, when a physician examined an older patient who seemed out of touch with reality, she had to guess whether that person had Alzheimer’s or was senile. Now, physicians can utilize new and more reliable tests focusing on genetic clues. Yet, in the accuracy of these new tests lies the risk of another kind of human tragedy: while physicians may be able to predict Alzheimer’s long before its overt

appearance, such an early diagnosis could psychologically devastate an otherwise healthy person.

Version (a) feels unfocused and unorganized, while version (b) feels cohesive and coherent. Why?

In version (b) we revised the passage to make the topics more related – the topic string now focuses on researchers/physicians and testing. Also, in (b) we revised the first sentence so that its end stressed those words expressing the themes that the rest of passage develops.

Exercise: 1. EGFR forms dimers and higher order oligomers with itself and other members of the ErbB family (ErbB2/HER2, Erbb3/HER3, and ErbB4) via a primary dimerization domain, as well as several secondary receptor–receptor contact points. 2. The 170 kD Epidermal Growth Factor Receptor (EGFR, also known as ErbB1), is one of four members of the ErbB/HER family of transmembrane tyrosine kinase growth factor receptors. 3. Binding of naturally occurring extracellular ligands (e.g., amphiregulin, epiregulin, HB-EGF) to the extracellular ligand-binding domain (domain III) of EGFR induces conformational shifts that permit homo- and hetero-dimerization events between EGFR molecules and its family members. 4. EGFR autophosphorylation activates multiple key signal transduction cascades that are mitogenic, antiapoptotic, angiogenic and pro-invasive. 5. Multimer formation promotes tyrosine autophosphorylation of the EGFR intracellular domain; the resultant open configuration of the kinase domain enhances access by ATP and substrate and creates binding sites for signaling molecules. 6. The EGFR kinase is also active at a low level when the protein is in the unliganded state; the degree of activity varies by cell type, glycosylation state and environment.

Problem: The sequence of sentences does not develop coherently: there is not an old/new progression; no concluding “high note”. Solution requires reordering of the sentences to be 2-1-3-5-6-4.

Revision: The 170 kD Epidermal Growth Factor Receptor (EGFR, also known as ErbB1), is one of four members of the ErbB/HER family of transmembrane tyrosine kinase growth factor receptors. EGFR forms dimers and higher order oligomers with itself and other members of the ErbB family (ErbB2/HER2, Erbb3/HER3, and ErbB4) via a primary dimerization domain, as well as several secondary receptor–receptor contact points. Binding of naturally occurring extracellular ligands (e.g., amphiregulin, epiregulin, HB-EGF) to the extracellular ligand-binding domain (domain III) of EGFR induces conformational shifts that permit homo- and hetero-dimerization events between EGFR molecules and its family members. Multimer formation promotes tyrosine autophosphorylation of the EGFR intracellular domain; the resultant open configuration of the kinase domain enhances access by ATP and substrate and creates binding sites for signaling molecules. The EGFR kinase is also active at a low level when the protein is in the unliganded state; the degree of activity varies by cell type, glycosylation state and environment. EGFR autophosphorylation activates multiple key signal transduction cascades that are mitogenic, antiapoptotic, angiogenic and pro-invasive.

5. Make the words mean what you want them to say.

- Often, the words do not mean what you want them to say because they are not written in parallel form. If parallel ideas are not written in parallel form, the logical relation of the ideas (similarity, alternatives, contrast, comparison) is obscured.
 - Example #1: DNase I nicking interference patterns correspond precisely to methylation interference patterns with both 10 bp sequences.
Problem: Makes it sound like both patterns are 10 bp long.
Revision: Interference patterns induced by DNase I nicking correspond precisely to interference patterns induced by methylation for both of the 10 bp sequences.
 - Example #2: These results are similar to previous studies.
Problem: Comparison of unlike things
Revision A: These results are similar to the results of previous studies.
Revision B: These results are similar to those of previous studies.
 - Example #3: Like poliovirus, interaction of coxsackievirus with its receptor triggers release of its viral RNA.
Problem: Interaction is not like poliovirus
Revision A: Like the interaction of poliovirus with the poliovirus receptor, the interaction of coxsackievirus with its receptor triggers release of the viral RNA.
Revision B: Like poliovirus, coxsackievirus interacts with its receptor to trigger release of its viral RNA.
 - Exercise #1: Activation-controlled relaxation in these membrane-deprived cells resembled intact myocardium from frogs.
Problem: Comparison of unlike things
Revision: Activation-controlled relaxation in these membrane-deprived cells resembled relaxation in intact myocardium from frogs.
 - Exercise #2: In the transgenic animals, expression of beta-galactosidase was limited to pharyngeal muscle, a pattern identical to that observed in wild-type animals.
Problem: Makes it sound like pharyngeal muscle is the pattern.
Revision: In the transgenic animals, expression of beta-galactosidase was limited to pharyngeal muscle; this pattern was identical to that observed in wild-type animals.

A few comments on ACTIVE versus PASSIVE voice:

Active is preferred ...

For direct writing, we prefer "active" rather than "passive" verbs, especially if we can avoid nominalization. Here are three examples:

Passive-nominalized:

An investigation WAS CONDUCTED into why so few **interviews** of minority applicants WERE DONE.

Active-nominalized:

We CONDUCTED an **investigation** into why the employment office DID so few **interviews** of *minority applicants*.

Active-verbal:

We **INVESTIGATED** why the employment office **INTERVIEWED** so few *minority candidates*.

... but passive is OK

Passive can be useful!

Passive can help shift a long and complex bundle of information from the beginning to the end of a sentence. In the sciences, the passive contributes to an objective point of view.

We must decide whether to focus on A or B. The weight given to two factors, X and Y, will influence this decision.

We must decide whether to focus on A or B. This decision will be influenced by two factors, X and Y.

GENERAL GUIDELINES ABOUT VERB TENSE USAGE

Use present tense to describe established findings that have passed peer review and are regarded as fact.

“Resting CD4+T cells are the best-defined reservoir of HIV-1 infection.”

“Establishment of the intricate nervous system of vertebrate animals requires the specification of diverse neuronal cell types.”

“Translation initiation of some viral and cellular mRNAs occurs by ribosome binding to an internal ribosome entry site.”

Use present tense for the question.

“We hypothesized that cigarette smoking by young men causes abnormal metabolism of plasma cholesterol.”

“We asked whether these fragments arise from the same point of cleavage as the naturally occurring fragments of B-100 and B-74.”

“To determine whether four different asthma drugs inhibit the late asthmatic reaction, we...”

Use past tense for methods.

“We dehydrated the pellets and cleared them with propylene oxide.”

“After 30 sec, we centrifuged the samples.”

“To prepare surface layers for EM, we resuspended the pellets in...”

Use past tense to describe the experiments done.

“We assessed these variables in 24 sensitized subjects divided into 4 groups of 6 subjects each.”

“We used kallikrein to digest LDL from human plasma and compared the resulting fragments with B-74.”

“Subjects in each group received one drug for 7 days according to a double-blind, placebo-controlled study.”

Use past tense to describe the results obtained.

“Slow-release theophylline partially inhibited the increase in FEV₁ but had no effect on airway responsiveness to methacholine.”

“Sham nucleus tractus solitarius lesions and lesions lateral to the nucleus produced no changes.”

“Pulmonary lymph flow doubled within 2 hr.”

Use present tense for the answer.

“These results indicate that *ceh-22* and *nkx2.5* perform similar functions.”

“These experiments demonstrate that lesions of the NTS alter PA pressures.”

“Thus, only the high-dose inhaled steroid beclomethasone inhibits late asthmatic reactions.”

Use hypothetical verbs for implications or speculations.

“These results suggest that the identified EGases may facilitate intracellular migration through plant roots by partially degrading the cell wall.”

“We propose that Zip2 promotes the initiation of chromosome synapsis.”

“Our findings could partly explain the high incidence of coronary artery disease in older male smokers.”

Use future tense for future, planned, or proposed work.

“We will identify the steps involved in coxsackievirus B uncoating during entry into polarized epithelial cells.”

“The temporal production of SarA will be assessed by Western blot of *S. aureus* whole cell extracts with an affinity-purified anti-SarA antibody.”

“I will use heat shock-inducible transgenic zebrafish to determine when BMP signaling is required to specify *lim1+* INs.”

REWRITING EXERCISES

For each of the five “blinded” re-writing exercises below do the following:

- (1) Identify the writing principle that is ignored in the sentence(s)
- (2) Rewrite the sentence(s) so that it follows that principle

***Suggested revisions for these rewriting exercises appear at the end of the syllabus. Please rewrite the sentences yourself before turning to the suggested revisions.

Exercise #1: Prolongation of life for uremic patients has been made possible by improved conservative treatment and hemodialysis.

Exercise #2: A disease that progresses with few or no symptoms to indicate its gravity is an “insidious” disease, under this definition. Asbestosis, neoplasia, mesothelioma, and bronchogenic carcinoma are all examples of insidious diseases. Asbestos insulation installers who have inhaled asbestos fibers over a period of many years regularly contract these diseases.

Exercise #3: Laboratory animals are not susceptible to these diseases, so research on them is hampered.

Exercise #4: Propranolol had variable effects on the hypoxemia-induced changes in regional blood flow. In the cerebrum, the increase in blood flow caused by hypoxemia was not significantly altered by propranolol. However, in other organs and in the peripheral circulation, propranolol caused a more severe decrease in blood flow than did hypoxemia alone.

Exercise #5: The molecular events determining the developmental lineage of the gonadotrope in the anterior pituitary, utilizing approaches in transgenic mice including ectopic expression of regulatory proteins, will be investigated.

“PROBLEMATIC” ABSTRACT AND SPECIFIC AIMS PAGE

For the “problematic” abstract and specific aims page below do the following:

- (1) Identify the writing principles that are ignored
- (2) Re-write each so that it follows the 5 principles
- (3) Correct anything else that you consider problematic

***Comments about the “problematic” abstract and specific aims page appear at the end of the syllabus. Please use these examples to help apply them to your own writing and critiques.

“PROBLEMATIC” ABSTRACT

Third-stage larvae of parasitic nematodes, which, in most species are the infectious stages for the mammalian host, including humans, of whom more than 3.5 billion may be infected worldwide, share common behavioral, morphological and developmental characteristics with the developmentally arrested dauer larvae of the free-living nematode *Caenorhabditis elegans*. It is proposed that molecular regulation of the transition from free-living to parasitic forms of parasitic nematodes and *C. elegans* dauer larva development regulation are similar. Significantly for the present study, it has been shown that in *C. elegans*, one of the key factors regulating the dauer transition is the insulin-like receptor kinase DAF-2. The parasitic nematode *Haemonchus contortus* has an insulin-like receptor (*Hc-daf-2*), which displays significant homology to insulin receptors in both vertebrates and invertebrates and is predicted to contain conserved structural domains. Examination of the parasite by RT-PCR showed *Hc-daf-2* transcription in all life stages. An important proteolytic motif was identified in the predicted peptide sequence of *Hc-DAF-2* and is consistent with the HIR (human insulin receptor), suggesting that it could be involved in the formation of the insulin receptor complex. To test this, comparison of the patterns of expression between *Hc-daf-2* and *Ce-daf-2* was performed with reporter constructs fusing the *Ce-daf-2* or *Hc-daf-2* promoter to the coding sequence of *gfp*. These were microinjected into the N2 strain of *C. elegans*, and establishment and examination of transgenic lines were performed. These showed similar patterns of expression in amphidial/head neurons for both genes, which may be related to sensation and signal transduction, which are important processes in host finding by infective parasitic nematode larvae. For further functional analyses of *Hc-daf-2*, heterologous genetic complementation studies were attempted in the CB1370 *daf-2* mutant strain of *C. elegans*. These studies revealed that this mutation can be partially rescued by *Hc-daf-2*. Taken together, these data support the hypothesis that *Hc-DAF-2* plays a crucial role in the transition from the free-living state to parasitism.

“PROBLEMATIC” SPECIFIC AIMS

As part of the adult *Strongyloides stercoralis* (SS) life cycle (LC), female SS lay eggs in the intestinal mucosa that hatch into rhabditiform larvae, which are shed in the stool. Caused by the parasitic nematode (pn) SS, and being characterized by extreme hyperchronicity with infected individuals being diagnosed decades after leaving the endemic environment, human SS affects ~100 million people globally. It has been shown by Schad et al. (19) that maintenance of hyperchronic SS may be by a process unique to SS in which parasite larvae develop precociously to successive generations of parasitic females in the same host, which is called autoinfection (18). It has been shown that in most cases, senescent parasitic females are gradually replaced with new individuals through a continuous process of tightly regulated low-level autoinfection (9, 10). However, these chronic, clinically latent infections, in patients immunosuppressed by corticosteroid therapy (CT) or underlying HTLV-1 infection, become unregulated, resulting in a fulminant often-fatal hyperinfection (8). Clearly, a better understanding of mechanisms initiating and maintaining autoinfection by SS is critical for preventing disseminated strongyloidiasis in at-risk patients.

Hyperchronic strongyloidiasis can be modeled experimentally in infected dogs by administering low dose CT, and it is widely assumed that such autoinfection is driven primarily by steroid suppression of immune responses that would normally clear the parasite (10). However, our preliminary data are supportive of the fact that steroid-induced autoinfection results from direct action by the drug on a parasite-intrinsic steroid signaling pathway. First, we have shown that autoinfection by SS in immune-deficient NSG mice requires exogenous (hereafter ‘medicinal’) CT. Second, our multidisciplinary team, which includes the PI, a medicinal chemist, a statistician, and members of the PIs laboratory, including graduate students and post-docs, has amassed compelling evidence that endogenous steroid signaling regulates larval development in SS. Specifically, we have shown that DAF-12, a corticosteroid-class nuclear hormone receptor (NHR) signaling pathway that regulates larval development in *Caenorhabditis elegans* (CE), is conserved in SS. Moreover, dafachronic acids (DAs), natural ligands of the CE receptor DAF-12, regulate crucial developmental events when applied exogenously to SS (11).

Therefore, we will characterize the action of medicinal steroids or their host metabolites with the parasite homolog of DAF-12 during the process of autoinfection by SS. We will also evaluate how parasite-intrinsic NHR signaling relates to very low levels of autoinfection the relationship of this autoinfection to host immunity. We know that disseminated hyperinfection is observed in immunocompromised patients in the absence of CT. Therefore, in Aim 2, we will determine if the residual innate immune effectors of NSG mice prevents NHR-dependent autoinfection in the absence of medicinal steroids. Evidence that medicinal steroids or their metabolites function as ligands for endogenous NHR signaling in SS to promote autoinfection will constitute a milestone supporting translational studies where compounds identified as agonists or antagonists of SS NHR signaling in an existing high-throughput screen will be prioritized for in vivo testing. Prioritized compounds will be tested for efficacy in preventing autoinfection in gerbil and/or NSG mouse models of autoinfection. Milestones indicating success will be three or more lead compounds that clear hyperchronic SS infection. Our specific aims are to:

SPECIFIC AIM 1: Characterize the interaction of medicinal steroids or their host metabolites with SS during autoinfection. To this end, we will determine **a)** the effect of medicinal CT of young SS larvae on the frequency of autoinfection, **b)** whether medicinal steroids act as direct ligands for SS NHR signaling **c)** whether the DA-synthetic enzymes of CE

are conserved in SS, and **d**) whether bile acid precursors in steroid-treated hosts are substrates for nematode DA-synthetic enzymes.

SPECIFIC AIM 2: Investigate the roles of remaining immune functions in the NSG mouse in regulating autoinfection. We will assess remaining components of immune functionality in the NSG mouse (neutrophils, basophils, and eosinophils) for their role in autoinfection in non-steroid treated mice.

SPECIFIC AIM 3: Identify hits from an existing high throughput screen (HTS) for compounds that agonize or antagonize the SS NHR Ss-DAF-12. Using cell-based assays in a multi-well format we will screen small molecule libraries for hits that interfere with autoinfection.

SPECIFIC AIM 4: Advance HTS hits from Aim 3 as appropriate to testing in in vivo models of autoinfective strongyloidiasis. Hits from the HTS will be assayed for ability to prevent autoinfection in a well-characterized model of autoinfective strongyloidiasis in gerbils and in the NSG mouse model.

SPECIFIC AIM 5: Develop new in vivo models for testing. We will explore whether other animal models are also appropriate for testing hits from HTS.

HOW TO GIVE AND RECEIVE EFFECTIVE CRITIQUES

In addition to providing a principled framework for improving our scientific writing, the goal of this course is to help build skills needed for giving *and* receiving constructive criticism.

Below is excerpted from:

Science relies on constructive criticism. Here's how to keep it useful and respectful

William A. Cunningham, Jay J. Van Bavel, Neil A. Lewis, Jr., June Gruber
Science, 24 Mar 2021, doi: 10.1126/science.caredit.abi6902

“How to give criticism

Be humane (i.e., don't be a jerk). When handing out criticism, remember that you are talking to a human with real emotions. This is someone's work—perhaps even their life's work. They might be at a precarious stage in their career, and your criticism could mean the difference between getting a job or tenure. This does not mean you should shy away from criticism. It simply means that when you do criticize research, you should focus on being constructive and framing your critique in such a way that minimizes any personal attacks. Criticisms need not be so cruel that they drive people away from the community. We strongly recommend following philosopher Daniel Dennett's guide for criticizing with kindness.

Embrace intellectual humility. Even if you think you have found a potential shortcoming or flaw in someone else's work, bear in mind that you may be wrong. It is possible that you misread the paper, conducted the statistics incorrectly, or misunderstood what the authors were reporting. Or perhaps your critique does not undercut the claims of the original author, but merely adds a new perspective or nuance to the work. Even if you do find a clear error, it might not be large enough to undermine the claims of the original authors. Embrace a sense of humility when you criticize others' work.

Avoid straw men. Rigorous critiques are fundamental for scientific progress. But we have noticed too much criticism relies on straw man arguments where an idea or statement is taken badly out of context and criticized on flimsy grounds. We have been on both sides of this issue and, over time, have become more cautious in leveraging critiques. Before making a critique public, one strategy is to reach out to the other person privately. That way, you can make sure they actually disagree with you and you are not criticizing differences where none exist.

Assume the best. The most concerning criticisms are ones that suggest, explicitly or implicitly, some degree of unethical behavior. Unless you are absolutely certain academic misconduct has occurred, it is often best to assume the other researcher made an honest mistake. Remember that mistakes can and do happen to everyone. No one, including you, is perfect. But if you do find a significant ethical lapse, you will want to document and report it immediately through the appropriate professional channels.

How to receive criticism

Don't take it personally. By definition, science invites scrutiny and revision of any claims. Indeed, it is a compliment to have your work taken seriously enough that someone is willing to read it and

share their critiques. Whenever possible, try to look for the useful parts of the criticism. What can you learn from it to improve your work? Focus on the substance of the critique and not the fact that your own work is part of the criticism. For instance, Jay has had several of his papers come under criticism. In each case, it stung. But once that wore off, he was able to reflect more critically on the work and use the feedback to improve his thinking and to design superior studies for his future research. Over time, it made his work much better.

Be open to being wrong. It's hard to be on the wrong end of scientific criticism—especially if the criticism addresses a fatal flaw in your work—and it is only natural to feel defensive. But we think it is usually a better career move to be open to the possibility that the criticism has merit. Wil once criticized his own prior work in the introduction of one of his papers. If something similar happens to you and you realize your own work deserves to be criticized, it's OK to admit that publicly. In our experience, such a mindset is key to moving on with a more advanced understanding of your science.

Take the high ground. Regardless of the tone of the criticism, it is always best to respond politely. Thank the person for taking the time they took to engage with your work, and think carefully about whether the issues they raise are worth addressing. Focusing on a constructive exchange of ideas, rather than a person's tone, will set a good example and may open the door to a productive collaboration. For instance, Jay is currently engaged in a collaboration with researchers who criticized one of his prior papers. The joint effort is designed to help determine which side is right by using a method that everyone on the collaboration finds convincing. The joint effort will help get to the bottom of a hot debate in the field and help push the science forward.

Ignore the trolls. Up to now, our advice has focused on engaging with your critics and striving to learn from their critiques. Unfortunately, there are some critiques that are not constructive because they aim to agitate rather than inform. As scientific dialogue has moved to the internet, and now social media, you are likely to attract criticism from trolls—especially if you work on a politicized topic, such as climate change or racism. If it becomes clear that someone is criticizing you in bad faith, we recommend disengaging from the conversation.

Science is a process, but it's also a community. Strive to be a constructive critic and a scientist who invites helpful feedback on your own work. Adding toxicity into the mix creates a culture where people feel the need to be defensive and closed off to valuable critiques. Remember that, more often than not, science involves people who are trying their best. We all deserve to be treated with respect—and you can start by modeling the type of behaviors you hope to encounter in others.”

See also:

Universal Principled Review: A Community-Driven Method to Improve Peer Review

<https://doi.org/10.1016/j.cell.2019.11.029>

Sample Critique

****keep in mind that your writing samples for this class are limited to 1 page**

****some of the terminology has been changed here so don't focus on the science**

☺**Metabolic and effector properties of invariant natural killer T cells in the neuroblastoma tumor microenvironment**

SIGNIFICANCE

Neuroblastoma is the most common extracranial solid tumor in children, comprising 15% of pediatric cancer-related deaths (1). Despite intensive multimodal therapies, long-term survival rates of children with high-risk neuroblastoma are only 40-50%, underscoring the urgency to develop more effective therapeutic strategies. Recent advances in cellular immunotherapy have exploited the cytotoxic capacity of immune cells present in the tumor microenvironment (TME) to specifically target tumors for elimination (2). While these therapies have shown some promise in hematopoietic malignancies, the TME presents many barriers to their success in solid tumors like neuroblastoma. Immune populations in the neuroblastoma TME are poorly understood relative to those in other malignancies. A better understanding of immune effectors in the neuroblastoma TME will enable us to harness their antitumor potential. In doing so, we can more effectively overcome these challenges and ultimately achieve better tumor control and patient survival.

Our laboratory uses a preclinical murine model of high-risk neuroblastoma (*TH-MYCN* transgenic mice) (3). Preliminary immunophenotyping of tumors from various stages of disease progression in this model suggests a significant intratumoral population of invariant natural killer T (iNKT) cells. These iNKT cells are a subset of lymphocytes with anti-tumor features of both innate and adaptive immune cells. iNKT cells are activated by glycolipid antigens presented by ABCD1, an MHC class I-like molecule (4). Once activated in this manner, iNKT cells exert cytotoxicity by directly killing ABCD1-expressing tumors (5). iNKT cells also act indirectly by activating natural killer cells (6), inhibiting tumor-suppressive macrophages (7), and converting myeloid-derived suppressor cells into functional antigen-presenting cells that activate T cells (8). Several tumors have low iNKT cell frequencies or downregulated ABCD1 expression (9), and for these, therapeutic strategies engaging iNKT cells effectively limit tumor growth and improve survival in several tumor models (10, 11). ☺☺Collectively, these studies support the importance of iNKT cells for anti-tumor immunity and suggest that enhancing activated iNKT cells in the TME may be clinically beneficial.

To activate iNKT cells in tumors with low ABCD1 expression, like neuroblastoma, our lab has developed chimeric molecules composed of ABCD1 loaded with antigen (Ag) biochemically conjugated to an antibody directed towards a tumor-specific antigen (TSA). We term these chimeric molecules "AAb" (ABCD1-Ag-anti-TSA mAb). Our initial studies suggest that exposure of iNKT to AAb directed against neuroblastoma-specific tumor antigens induces robust anti-tumoral responses in *TH-MYCN* mice. However, it is not clear whether iNKT cells maintain their ability to produce cytokines and kill tumors upon sustained or repeated exposure.

It is essential to delineate the metabolic properties and effector functions of AAb-activated iNKT cells to fully understand the mechanisms and clinical efficacy of these cells in the neuroblastoma TME. A major challenge limiting the efficacy of T cell immunotherapies in solid tumors is the metabolic stress – namely low glucose and hypoxia – within(?) the TME, which hampers the long-term persistence of active, effector cells (12). Recent evidence has tightly coupled T cell metabolism with effector capacity. In resting and memory states, T cells preferentially utilize mitochondrial fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) to produce energy (13). By contrast, effector T cells rely on glycolysis to fuel rapid proliferation, cytokine secretion, and cytotoxicity (14). Nonetheless, chronic stimulation in cancer causes T cells to become "exhausted". In this state, the T cells revert to a quiescent metabolic phenotype and lose the ability to respond to antigenic stimulation and exert effector function (15). It is not known how iNKT cells metabolize nutrients, proliferate, and sustain effector capacity in the TME. Unlike conventional T cells, which have phenotypically and functionally distinct effector and memory states, iNKT cells have phenotypic features of memory cells and yet very rapidly produce cytokines and exert cytotoxicity in an innate-like manner upon activation (16). Given their unique

activation kinetics and multiple effector mechanisms, 😊😊we hypothesize that iNKT cells have a unique metabolic profile from T cells in the TME that may impact their effector capacity and potential use in cellular immunotherapy.

INNOVATION

This proposal will provide the first characterization of the metabolic profile of iNKT cells upon acute and repeated stimulation, and importantly, link this metabolic profile to effector capacity using both *in vitro* and *in vivo* approaches. The utility of iNKT cells for cellular immunotherapy may offer several advantages over conventional T cells. The innate-like activation kinetics and multitude of effector mechanisms exerted by iNKT cells may provide more potent anti-tumor activity. Given their hybrid effector and memory states, iNKT cells may be able to maintain a glycolytic state even upon repeated antigenic stimulation, avoiding the exhausted state that T cells succumb to and conferring longer persistence in the TME. Our new AAb molecule allows for rapid iNKT cell activation and proliferation at the tumor and can be modified to accommodate any tumor-specific antigen, thus expanding their utility beyond neuroblastoma. The proposed research strategy will help determine how to modulate iNKT cell metabolism to enhance their cytotoxic capacity and surmount challenges facing current cellular immunotherapies to inhibit or block tumor progression and improve and patient outcomes.

Bullet Points

Positives

😊 Great that you've got a working title. Consider my small edit.

😊😊 You state an explicit, testable hypothesis. Maybe consider trying to do this earlier in the write-up. I suggested one spot in paragraph 2 that seemed on the verge of being a hypothesis.

😊😊😊 Your background information on tumor immunology is pitched well for a non-specialist scientist. You give a convincing statement of the overall health significance of the work at the beginning, and then come back to patient outcomes at the very end. Well done.

Points for improvement

👉👉 You tend to write very long sentences, which can hinder the logical flow of the narrative. I suggested breaking several of these into two with the second sentence linking backward to the first to improve the flow.

👉👉 There were some lengthy introductory clauses that prevented "getting to the subject quickly".

👉👉👉 Don't forget to strive for sentences in which the info progresses OLD→NEW. I flipped the order of one to achieve this.

SPECIFIC AIMS

The Specific Aims page is the most important page of the grant. The Specific Aims should clearly lay out the problem you are addressing, the hypotheses you are testing, and the experimental steps you will take. The Research Design and Methods section of the proposal will expand on how you propose to accomplish the Aims. Reviewers will read the Specific Aims quite carefully; if they don't like what you propose, don't understand it, or think your goals are unrealistic, they may be prejudiced against you as they read on. The Specific Aims page also serves as a summary page of your grant for the reviewer; they'll return to that page often to remind them of the major goals of your proposal.

The first 1-2 paragraphs of the Specific Aims page should explain the scientific problem and its importance. The Aims follow and are usually listed as hypotheses that you will test or positive statements of what you intend to do. Preferably every Aim you propose will provide important information whether or not the experiments turn out the way you expect. That is not always possible. However, no Aim should depend on another Aim. The proposal should be structured so that a negative result in Aim I doesn't make it impossible to proceed to Aim II or III.

When writing the Specific Aims for your preliminary exam, keep in mind that the work should be able to be accomplished in 2 years. For people who are new to grant writing or are applying for their first R01, a common mistake is to outline a career rather than a piece of work that can reasonably be accomplished in 3-5 years. This is often criticized by reviewers as overly ambitious. If you have too many great ideas to fit in one grant, mention them as something you want to do in the future, once you've accomplished the Aims of the present proposal. Examples of successful Specific Aims pages are provided on the following pages (note 1 page limit!):

Sample Specific Aims Page #1 (Arial 11 font, one of the NIH preferred fonts)

****The names have been changed to protect the innocent**

The esophageal lining is regularly exposed to irritants such as alcohol, cigarette smoke, hot beverages, dietary nitroso-compounds, and refluxate of gastro-duodenal contents, and the cells lining the esophagus respond to these stressors to maintain normal homeostasis. Nonetheless, disorders of the esophagus are significant health problems in the U.S. and throughout the world. For example, gastroesophageal reflux disease (GERD) leads to 8.9 million clinic visits in the U.S. annually, and esophageal cancer is the 6th most common cause of cancer death worldwide. In esophageal epithelia, the key transcriptional regulator Esophageal Factor 5 (EF5) promotes normal proliferation and migration, as we have shown, and we recently identified a novel relationship between EF5 and Factor X (FX) in esophageal epithelial cells, whereby FX acts as a “molecular switch” for EF5. FX mutation in primary human esophageal keratinocytes (HEK) converts EF5 from pro-proliferative to anti-proliferative, an effect mediated predominantly by *Factor X Target Gene1 (FXTG1)*, which is differentially bound and regulated by EF5 in the presence or absence of mutant FX. In HEK harboring mutant FX, EF5 loss alone is sufficient for transformation, epithelial-mesenchymal transition (EMT), and the development of invasive squamous cell cancer. As such, FX and EF5 coordinately regulate target genes, leading to biologically-relevant, functional responses. In *Preliminary Data*, we demonstrate that EF5 suppresses FX in HEK and provide evidence for genome-wide coordinate regulation by EF5 and FX. Our overarching hypothesis is that EF5 and FX orchestrate a broad transcriptional program in esophageal epithelial cells that controls proliferation, growth arrest, apoptosis, and transformation. To test this hypothesis, we will pursue the following interrelated **Specific Aims**:

Aim 1. To delineate the mechanisms through which EF5 regulates FX levels and function

When cells are stressed, FX protein increases rapidly, correlating with decreased activity of the factors that degrade FX protein and an increase in a variety of post-translational modifications that regulate FX levels and function. In unstressed normal cells, even with increased FX transcription, FX protein is rapidly degraded and levels remain low. Surprisingly, in unstressed HEK, EF5 knockdown markedly increases FX mRNA and protein. Here, we will examine the mechanisms of FX regulation by EF5 in HEK and the effects on FX function.

Aim 2. To define the mechanism for EF5 functional switching on FXTG1

FX is a nodal point, directing cells towards arrest via FXTG1 or apoptosis via Pro-Apoptotic Protein X (PAPX). EF5 transcriptionally regulates *FXTG1* in HEK, and FX mutation converts EF5 from a transcriptional repressor of *FXTG1* to an activator. Here, we will explore how EF5 and FX coordinately regulate cell proliferation and growth arrest in esophageal keratinocytes through mechanistic studies of EF5 and FX on *FXTG1*.

Aim 3. To identify common and exclusive targets of EF5 in the context of wild-type and mutant FX

EF5 binding and function vary depending upon FX status, as we have shown for FXTG1. However, coordinate regulation by EF5 and FX has not been examined genome-wide. Here, we will employ ChIP-seq with validation of functional targets to identify the EF5 *cis*-acting targets (cistrome) on a genome-wide scale in HEK with wild-type FX and in HEK with FX mutation.

In sum, the work proposed in this application will define the functional interplay between EF5 and FX, two critical regulators of normal epithelial homeostasis, as well as delineate their downstream molecular targets. These studies will provide key insights into the transcriptional regulation of esophageal epithelial homeostasis and the molecular pathways that underlie esophageal diseases. Overall, through this work, we expect to identify new diagnostic and therapeutic targets for esophageal diseases, both benign and malignant.

Sample Specific Aims Page #2 (Arial 11 font, one of the NIH preferred fonts)

Parasitic nematodes infect over one billion people and cause morbidity, disfigurement and retarded physical and cognitive development in hundreds of millions (1-4). Lack of practical vaccines against these parasitisms, and a small armamentarium of drugs that is threatened by resistance make it imperative to discover new drug and vaccine targets in nematode parasites. Molecules regulating crucial steps in parasite development, such as developmental arrest of infective larvae in the extrinsic environment and resumption of development upon host invasion, are logical points of attack in such interventions. Because of their intractability to laboratory culture and the consequent difficulty in developing modern molecular methodologies for them, these processes have not yet been well characterized in parasitic nematodes. Notably, our laboratory has recently advanced this field by generating the first transgenic *Strongyloides stercoralis*, a medically important obligate parasitic nematode in humans and dogs that is also capable of undergoing one or more generations of free-living development. We have utilized this system to directly determine if mechanisms involved in the arrest of third-stage or “dauer” larvae under conditions of stress in the free-living nematode *Caenorhabditis elegans* (5) are functionally conserved in parasitic nematodes. Importantly, our data reveals that the insulin-like (6) signal (ILS) transduction pathway, regulated in part by a nuclear hormone receptor (NHR) and its steroid ligands(7, 8), that regulate dauer arrest in *C. elegans* are conserved in parasitic nematodes, leading us to hypothesize that these pathways are adapted to control larval development during the infective process. In support, in the previous funding period of this grant, we discovered orthologs of nine key insulin signaling molecules in *S. stercoralis*: seven insulin-like peptides (ILPs), the insulin-like receptor kinase Ss-DAF-2, the insulin-regulated PI3 kinase Ss-AGE-1 and the insulin-regulated fork head transcription factor Ss-DAF-16 (9). Moreover, using a novel strategy to generate transgenic *S. stercoralis* L3i (10, 11), we provided the first direct evidence that Ss-DAF-16 is essential for normal development of infective third-stage larvae (L3i) (12). Finally, we demonstrated that regulation of the Ss-DAF-12 NHR by its steroid ligands blocks formation of *S. stercoralis* L3i and promotes their development within the host. As the identification of novel endogenous small-molecule regulators of parasitic nematode development has strong implications for future drug development of clinically relevant chemotherapeutics, studies proposed in our competing renewal will continue to delineate ILS in *S. stercoralis* and identify new links to NHR signaling. Specifically, we will ask

1. How does ILS regulate formation and maintenance of infective *S. stercoralis* third-stage larvae (L3i)? We hypothesize that downregulated signaling through the insulin receptor Ss-DAF-2 and the PI3 kinase Ss-AGE-1 is required for normal development of *S. stercoralis* L3i and that its resumption is required for developmental reactivation upon host invasion. We will test this hypothesis in Aim 1A by evaluating phenotypes in transgenic *S. stercoralis* expressing Ss-DAF-2 and Ss-AGE-1 with putative dominant gain- and loss-of-function mutations. In Aim 1B, we will evaluate the function of two known ILPs in *S. stercoralis* by mis-expressing them in the parasite and assessing their effects on resumption of development by L3i in culture and their effects on developmental switching by post parasitic L1.

2. Does Ss-DAF-12 NHR signaling augment developmental regulatory effects of ILS in *S. stercoralis*? We will test the hypothesis that endogenous steroid-NHR signaling suppresses formation of L3i and autoinfective L3 (L3a) in *S. stercoralis* and promotes their developmental reactivation in the host. We will first identify the natural ligands of Ss-DAF-12 and assess their ability to suppress formation of L3i and L3a and accelerate clearance of adult worms from the host gut. We will also ascertain whether chemical inhibitors of steroidogenesis block resumption of L3i development in culture. Finally, we will ascertain links between Ss-DAF-12 signaling and ILS by assessing phenotypic effects of steroidogenic inhibitors in transgenic *S. stercoralis* expressing an activated mutant form of Ss-DAF-16.

Completing these aims will provide unequivocal evidence that ILS modulates formation of *S. stercoralis* L3i and, more importantly, promotes their development upon entering the host. Furthermore, new research on the interface between ILS and steroid/NHR signaling will identify the first endogenous small-molecule regulators of the infective process. In doing so, it will open a much needed new approach to drug development for a large group of neglected tropical diseases that affect some 20% of the world's population.

Strong points of sample Specific Aims:

- Start with background for the informed non-expert, writing at about the level of *Scientific American*.
- Put less technical information first.
- Point out a gap in knowledge that will be addressed in the proposal.
- Indicate the biologic question and describe how this will be tested
- Scope of research is limited to (typically) no more than three specific aims listed in bold and followed by a brief description of how each aim will be accomplished.
- The aims are the steps designed to prove the hypothesis.
- Spaces and bold type add readability.
- Reiterate the importance of the proposed research near the page bottom.

Specific Aims Checklist

- Introductory Paragraphs
 - Scientific problem
 - Its importance
 - What is known specific to the problem
 - Gap in our knowledge
 - Hypotheses you are testing (emphasis on the biologic question)
 - Steps by which you plan to go about testing your hypothesis
- Aims don't depend on each other
- Writing
 - Subject early
 - Verb nearby
 - Good use of topic and stress positions
 - Nominalizations --> verbs
 - Concise, cohesive sentences
 - Coherent paragraphs
- White space
- "Big finish"

Reading for Week 3 - HOW TO WRITE THE APPROACH (grants); HOW TO WRITE THE DISCUSSION (Papers)

HOW TO WRITE THE APPROACH for grants.

In this section, explain how you will accomplish your Specific Aims and convince the reviewers that you will be able to do what you propose. It is not enough just to describe the experiments--you need to state why they are being performed, why you have chosen the specific approach, which methods will be employed, what results are possible, how the data will be interpreted, what problems you anticipate, and how you will deal with them.

Many people write the Approach section for each Aim in a standard format with the following subheadings:

Specific Aim #1. Restate the Aim exactly as written on the Specific Aims page.

Rationale. Explain why your proposed studies are important and how they will advance the field.

Research Design and Methods. Describe the specific experiments, including methods, that you will perform in this Aim. Subdivide this section to clearly communicate the order of the experiments and how they relate to the Aim.

Possible Results and Interpretations. Discuss the different results that are possible and how you would interpret their significance. For example: If we find X, then this supports our hypothesis that ... If we find Y, then this does not support our hypothesis, but would be a very exciting result because...

Potential Problems and Alternative Approaches. Are there potential pitfalls to the experiments you proposed? If a pitfall arises, what alternative method could you use? It is important to convince the reviewer that you have thought deeply about your experiments and have already considered and addressed alternatives for the obvious pitfalls.

If your proposed experiments involve techniques that are new to your lab, your proposal will be strengthened by letters from consultants or collaborators who will provide any help you need. If specific reagents are needed, be sure to document how you will obtain them.

HOW TO WRITE THE DISCUSSION (Papers)

A well-written Discussion puts the findings of the paper in the proper context, directing and guiding the reader through the implications of the study. The Discussion should not merely recapitulate or summarize the results but should extend the findings, making conclusions and examining the significance of these results to the field. Do not attempt to hide obvious limitations of the study but address these in the text; smart reviewers will have picked up on these already. The results and conclusions should also be compared and contrasted with previously published work; if these differ from published work, speculate on the reasons for these differences.

One should be bold in stating the importance of the conclusions but must also be careful not to overstate the significance or to misinterpret the meaning of the results. An example of

this comes from the story of the biologist who trained a flea, from “How to Write and Publish a Scientific Paper” by Robert Day and Barbara Gastel:

“After training the flea for many months, the biologist was able to get a response to certain commands. The most gratifying of the experiments was the one in which the professor would shout the command “Jump,” and the flea would leap into the air each time the command was given. The professor was about to submit this remarkable feat to posterity via a scientific journal but he - in the manner of the true scientist - decided to take his experiments one step further. He sought to determine to location of the receptor organ involved. In one experiment, he removed the legs of the flea, one at a time. The flea obligingly continued to jump upon command, but as each successive leg was removed, its jumps became less spectacular. Finally, with the removal of its last leg, the flea remained motionless. Time after time the command failed to get the usual response. The professor decided that at last he could publish his findings. He set pen to paper and described in meticulous detail the experiments executed over the preceding months. His conclusion was one intended to startle the scientific world: *When the legs of a flea are removed, the flea can no longer hear.*”

Day and Gastel also point out, “Much as the Methods and Results should correspond to each other, the Introduction and Discussion should function as a pair...Be sure the Discussion answers what the Introduction asked.” Finally, the Discussion should end with a brief but strong summary of the overall significance of the paper. In other words, as Anderson and Thistle said in 1947, “good writing, like good music, has a fitting climax.”

PARTS OF A GRANT PROPOSAL

The preliminary exam tests your ability to formulate a research proposal and to construct a sound experimental approach to accomplish the proposal's specific aims. Your preliminary exam proposal should be scholarly and original. Your prelim proposal may not mirror exactly the current NIH grant format, though recent revisions to the prelim proposal format bring the prelim proposal more in line with what research scientists confront in communicating their work. The NIH format typically includes five scored criteria. Three of these are Significance, Innovation, and Approach, included within a broader Research Strategy section. The other two scored criteria are Investigator and Environment.

- **Project Summary/Abstract (No longer than 30 lines of text using the required font and margin specifications):** Give a clear, succinct summary of the proposed work that can stand alone when separated from the rest of the application.
- **Specific Aims (1 page):** State the specific purposes of the research proposal and the hypothesis to be tested.
- **Research Strategy (12 pages)**
 - **Significance and Innovation:** Give a brief overview of the significant background to the proposal. Convey the importance of your proposed research by relating the specific aims to broader, long term objectives.
 - **Approach:** Describe the research design and methods that will be used to accomplish the specific aims. Discuss the expected outcome and interpretation of results. Explore potential experimental difficulties together with alternative approaches that could achieve the desired aims.
 - **Preliminary Studies (for New Applications) or Progress Report (for Renewal Applications) are included within the above sections (these are required in NIH grants, but NOT in your preliminary exam):** Present the observations that led you to the hypothesis for your proposal and explain any significant changes and new directions if this is a renewal application.

For both your preliminary exam and an NIH grant, attention to organization and clarity, in addition to the science, is essential. The proposal cannot be just a list of experiments-- the underlying scientific thought must be evident. The proposal must be written so that it is clear and convincing even to someone who is not familiar with the field. Use the page limitations wisely and present only important and relevant information.

To learn more about the preliminary exam requirements:
<https://www.med.upenn.edu/camb/prelim-exam.html>

You can also read annotated examples of well-written NIH grant applications:
<https://www.niaid.nih.gov/grants-contracts/sample-applications>

How to write SIGNIFICANCE AND INNOVATION: Selling your proposal

Formats for research proposals are as varied as the many granting agencies that solicit them. Also, proposal formats for a given donor organization can be expected to evolve over time. An example of this is the recent change in the Research Strategy format of individual research grant applications to the National Institutes of Health (NIH "R01s"). This new format

emphasizes significance and biomedical impact over methodological or technical detail and mandates clarity, conciseness and brevity by decreasing the page limitation for this section from 24 to 12. Such variety notwithstanding, virtually all scientific proposal formats call on you to make a case for the work you propose by placing it in the context of general biomedical needs and/or specific gaps in scientific knowledge that you propose to fill.

The current format for this section of an NIH grant proposal calls for dividing the narrative into two sub-sections: Significance and Innovation. Please note that while Innovation is a required heading for an independent NIH grant application, this heading is not required for your prelim proposal and will not be required for your homework assignment for this class. However, it may be beneficial to incorporate some of the elements of Innovation into your proposal and/or assignment, to highlight the novelty of your work.

The instructions for Significance and Innovation from NIH Form PHS 398 are quoted verbatim below. **Please use this format for your Week 3 writing assignment**

“(a) Significance

- Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.
- Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
- Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

(b) Innovation

- Explain how the application challenges and seeks to shift current research or clinical practice paradigms.
- Describe any novel theoretical concepts, approaches or methodologies, instrumentation or intervention(s) to be developed or used, and any advantage over existing methodologies, instrumentation or intervention(s).
- Explain any refinements, improvements, or new applications of theoretical concepts, approaches or methodologies, instrumentation or interventions.”

How to write **INTRODUCTION: Putting your paper in context**

The essential elements of a good Introduction to a paper, as presented in Chapter 10 of Day and Gastell’s *How to Write and Publish a Scientific Paper*, overlap to a degree with the Background or Significance/Innovation section of a grant application in that they include

- 1) a clear and concise presentation of the character and extent of the problem under investigation,
- 2) a brief, focused and, above all, relevant literature review, sufficient to orient the reader to the current state of the art in the area under investigation as well as the open questions addressed in the paper and

3) a brief statement of the major methodology used in the paper and, if appropriate, the rationale for its selection.

Because a paper reports results and interpretations from experiments already conducted, two additional elements of a well-written Introduction depart from the Background or Significance/Innovation of a proposal. These are:

- 4) the principal results of the study and
- 5) the principal conclusions based on the results.

While elements 1-3 should be included in any Introduction, journals differ in their requirement results and conclusions in the Introduction section and some prohibit their inclusion. It is wise to consult the instructions for authors in the target journal on this point prior to writing the Introduction to your paper.

Of all the five essential elements discussed by Day and Gastell, the first, a statement of the problem with a level of clarity and directness that captures the reader's attention, is the most crucial. At its best, this statement is said to provide the "hook" that stimulates the reader to want to read further. Wordiness or ambiguity in this statement serves to discourage readers, making it a chore to finish reading the paper, and potentially causing them to lose interest altogether. The other feature of a well-written Introduction is that the problems and questions it articulates will ultimately be resolved in the Discussion.

PRELIMINARY DATA/RESULTS

The information below is applicable to the preliminary data included in scientific grants and to the Results sections of scientific papers.

Preliminary Data (grants)

NIH grants, in general, require preliminary data, although there is no longer a specific “Preliminary Data” section; instead, preliminary data are incorporated into the Research Strategy section (remaining within the page limits) and may lie within the Significance, Innovation, and/or Approach. For example, you might provide such data as part of the rationale or justification for a particular approach. Generally, these preliminary data use the module format for results detailed below. For the preliminary exam proposal, there is no expectation that extensive preliminary data should exist.

In a grant proposal, you may use Preliminary Data to discuss the observations that led you to the hypotheses for your proposal, to demonstrate the feasibility of the proposed approaches, and/or to convince the reviewers that you are capable of conducting the proposed experiments. As such, it is important to present data that are relevant to each of the Aims. Experiments presented as preliminary data within a grant often do not meet all of the standards used in evaluating data for publication.

Results Section (papers)

Within a scientific paper, the results are the real substance – without good results, the rest of the paper is rendered essentially meaningless - and your job is to describe your findings in the clearest possible way. Within the broader context of the paper, the Introduction will set-up the Results by conveying why these findings would be important and the Discussion will put the results into context. In some formats, the Results and Discussion may be combined.

The results text narrates the story that the figures tell visually. Ideally, you should start planning your paper as soon as you get your first results. Remember that results are different from data. Data are facts, often numbers, obtained from experiments and observations. Results are statements that interpret the data. Keep data to a minimum in the results text. Data can rarely stand alone; the results (=the meaning of the data) must be stated.

Organization of the results text. A helpful way to organize the results text is as a repeating four-part pattern. The four-part pattern is **QERA**: (**Q**) Question, overview of the (**E**) Experiments, (**R**) Results, (**A**) Answer to the question. Ideally, each repeat of the pattern is a separate paragraph.

The results text should state the meaning of the data. The purpose of the results section is to make the point clear. To make the point clear, state the result first, and then present the data, or, better, cite a figure or table that contains the data in () at the end of the sentence.

Example: In the 20 control subjects, the mean resting blood pressure was 85 ± 5 (SD) mmHg. In comparison, in the 30 tennis players, the mean resting blood pressure was 94 ± 3 mmHg.

Problem: Data but no Result. Are the data similar? Different? What is the point?

Revision: The mean resting blood pressure was 10% higher in the 30 tennis players than in the 20 control subjects (Fig. 3).

In the revision, the statement “was 10% higher” states the result and gives a clearer idea of the magnitude of the difference than do the data alone. The figure containing the supporting data is cited in () at the end of the sentence.

Subordinate figure legends and table titles. Do not repeat a figure legend or a table title as a topic sentence. Do not direct traffic “Figure 1 shows...” Cite figures and tables in () after results statements.

Example: A summary of renal function data is presented in Fig 2. Continuous positive airway pressure (7.5 cm H₂O) in newborn goats decreased urine flow, sodium excretion, and the glomerular filtration rate.

Problem: The topic sentence is essentially a figure legend. For a more powerful topic sentence, omit the figure legend, state the results, and cite the figure in () at the end of the sentence.

Revision: Continuous positive airway pressure (7.5 cm H₂O) in newborn goats decreased urine flow, sodium excretion, and the glomerular filtration rate (Fig. 2).

Subordinate methods. Do not use a methods statement as a topic sentence.

Example: We administered propranolol during normal ventilation. This beta-blocker decreased phospholipid (Fig. 1).

Problem: The topic sentence is a methods statement. The next sentence is the results statement.

Revision: When propranolol was administered during normal ventilation, phospholipid decreased (Fig. 1).

Figures and Tables

A figure or table, like text, needs careful editing and revision. Present the most important data in figures and tables where the data are highly visible and easy to read. The point should be evident independent of the results text or the legend. Show the figure to a colleague (preferably someone in another lab) to see if she can explain it without the legend. If not, revise.

In the legend, the first sentence/phrase should summarize the findings shown by the entire figure or table, providing a *de facto* title. Additional description (e.g. for each panel of a figure) typically follows.

When Writing/Revising Results Sections

- Look closely at the figures.
 - What is each figure about? What is the figure’s point or message?
 - Is it easy to discern? Is it heavily dependent on the figure legend, or does the figure tell a story that the legend identifies more specifically?
- Look over the sequence of the figures.
 - Is there a story to the data?
 - How is the story of the figures structured?
- Read the text of the results section.
 - What is its story? How is it structured and organized?
 - How is it related to the story told by the figures?
- Evaluate the story.

- Is this a good piece of science? Is it interesting, significant, logical?
- What aspects are compelling? Strongest?
- Where did you hesitate? Find problems? Disagree?

Sample Results Text

In this paper, investigators asked whether the nematode gene *ceh-22* and the zebrafish gene *nkx2.5* perform similar functions. They examined the ability of the zebrafish gene *nkx2.5* to substitute for the nematode gene *ceh-22* in transgenic *C. elegans*.

Zebrafish Nkx2.5 Can Activate Myo-2 Expression When Expressed In C. Elegans Body Wall Muscle.

QTo determine whether *nkx2.5* can function similarly to *ceh-22*, we expressed n in *C. elegans* body wall muscle and examined expression of the endogenous *myo-2* gene by antibody staining. The rationale for this approach was as follows. In wild-type *C. elegans*, *ceh-22* is expressed exclusively in pharyngeal muscle, where it activates expression of the pharyngeal muscle-specific myosin heavy chain gene *myo-2* (14). However, ectopic expression of *ceh-22* in body wall muscle can activate expression of *myo-2* (15). Because *myo-2* is normally never expressed in body wall muscle, this ectopic expression assay provides a sensitive test for *ceh-22* function. **E**We generated two transgenic lines expressing an *nkx2.5* cDNA under the control of the *unc-4* body wall muscle-specific promoter. **R**In both lines, we detected *myo-2* expression in the body wall muscles (Fig. 1A and B). **A**These results show that *nkx2.5* can function like *ceh-22* to induce *myo-2* expression.

QWe next asked whether Nkx2.5 directly interacts with the same sequences recognized by CEH-22. **E**To answer this question, we examined expression of a reporter gene under the control of multimerized CEH-22 binding sites. CEH-22 binds a region within the *myo-2* enhancer termed the *B* sub-element (14). In wild-type animals, a *lacZ* reporter under control of a synthetic enhancer consisting of four copies of a 28-bp *B* sub-element oligonucleotide is expressed specifically in pharyngeal muscle; only occasional expression is observed outside the pharynx (Table 1; 14). **E**In a transgenic strain bearing the *unc-54::nkx2.5* expression construct, **R**we found a significant increase in the number of animals expressing beta-galactosidase in body wall muscle (from 2.5 to 16.5%) (Table 1; Fig. 1C). To rule out the possibility that Nkx2.5 was indirectly increasing expression of *myo-2* or the *B* sub-element reporter by activating ectopic expression of the *ceh-22* gene, we examined expression of a *ceh-22::lacZ* fusion in animals bearing the *unc-54::nkx2.5* transgene. Expression of beta-galactosidase was limited to pharyngeal muscle in the transgenic animals (Table 1); this pattern was identical to that observed in wild-type animals (14). **A**Thus, Nkx2.5, like CEH-22, activates transcription by interacting directly with the *B* sub-element of the *myo-2* enhancer.

Nkx2.5 Can Substitute for ceh-22 During Normal Pharyngeal Development.

BIn addition to its role in *myo-2* activation, CEH-22 likely regulates other genes required for pharyngeal development. **B**Indeed, a *ceh-22* mutant exhibits profound contractile and morphological defects in the pharynx, despite expressing *myo-2* nearly as well as wild-type (15). **Q**To examine the extent to which Nkx2.5 and CEH-22 are functionally equivalent, we asked if expression of *nkx2.5* in pharyngeal muscle can rescue a *ceh-22* mutant....

Strong points of this sample results text:

- Follows the organizational pattern **QERA**
 - Each repeat of the pattern (each paragraph) moves the story line forward by adding more evidence that *nkx2.5* functions like *ceh-22*.
 - Within each paragraph, additional information makes the story line clearer.
 - in paragraphs 1 and 2, rationale for the design of the experiment
 - in paragraph 2, the purpose and result of the control experiment
 - in paragraph 3, background leading to the next question
- States the result first and cites the figure/table that contains the supporting data in () at the end of the sentence.

HOW TO WRITE AN ABSTRACT

Abstract for a Grant Proposal

The abstract should explain your proposal - what you want to do, why it is important, and how you plan to do it. In your abstract, state your hypothesis, specific aims, objectives, and why they are important and innovative. Make your abstract a clear, succinct summary of your project. It also must have two to three sentences written in lay language that describe your project's potential contribution to public health.

When writing the abstract, ask yourself

- Is it a succinct description of all major aspects of the proposed project?
- Did I state my hypothesis?
- Does my abstract describe my objectives and specific aims?
- Does it state the importance of the research and how it is innovative?
- Does it outline the methods I will use to accomplish my goals?
- Did I keep the language easy to understand for a broad audience?

Sample Grant Abstract

(from <http://funding.niaid.nih.gov/researchfunding/grant/pages/titleabs.aspx>)

[*Significance*] The integrin alpha 4 beta 1 (VLA-4) contributes to the etiology of common autoimmune disorders, including multiple sclerosis, inflammatory bowel disease, and systemic lupus erythematosus. Although VLA-4 is widely viewed as contributing to T cell function by directing cell trafficking and by enhancing cell adhesion, VLA-4 potentially costimulates T cell activation. The mechanisms underlying this costimulation are not well understood and may play a significant role in the etiology of human immune disorders. Our long-range goal is to understand how to manipulate the costimulatory functions of VLA-4 in order to regulate T cell activation in vivo. Our immediate objective is to determine how VLA-4 modulates T cell responses to antigen. Here, we present preliminary data characterizing a previously unknown effect of VLA-4 ligation on the movement of signaling complexes induced by the TCR. Our specific hypothesis is that structures containing SLP-76 and ADAP are required for the transmission of tension-dependent costimulatory signals initiated upon VLA-4 ligation.

[*Innovation*] The rationale for the proposed work is that it will provide an enhanced understanding of the fundamental mechanisms that enable the integration of the signaling pathways downstream of the TCR and VLA-4.

[*Specific Aims*] Three aims will examine how ADAP contributes to T cell costimulation and how cytoskeletal tension contributes to VLA-4 dependent costimulatory signals:

- 1) How does ADAP contribute to the assembly and translocation of SLP-76 microclusters?
- 2) How does costimulation depend on the VLA-4-dependent immobilization of microclusters?
- 3) How does cytoskeletal tension contribute to T cell costimulation by VLA-4?

[*Reemphasis of the proposal's innovation*] These studies explore a novel effect of VLA-4 ligation, the lateral immobilization of TCR-induced complexes, and use it as a tool to dissect the pathways involved in costimulation by VLA-4. We expect these studies to define the mechanisms by which VLA-4 ligation costimulates T cell activation. This will have a positive

impact on our understanding of autoimmune disease and will assist in the identification of unique intracellular targets for drug development. This work will also generate insights into the systems linking cell shape to cell growth and proliferation, providing useful insights into cancer.

This grant abstract:

- Describes the protein to be studied
- States the significance (including with regard to health) early in the abstract.
- Addresses the long-term goal.
- Addresses the innovation of this proposal.
- States hypothesis and specific reasons for hypothesis.
- Describes three specific aims, using numbers to guide the reader.
- Includes additional information after the specific aims, reemphasizing the proposal's significance and innovation.
- Is a succinct summary of the project.

Abstract for a Scientific Paper

The abstract should provide an overview of the paper by presenting highlights from each section. The abstract should concisely state the question (**Q**) that is being asked, the experiments (**E**) that were done, the results(**R**) that were obtained, and the answer (**A**) to the question. In addition to these 4 basic parts, **QERA**, the abstract may begin with 1-2 sentences of background (**B**) information to orient the reader, and may end with a sentence stating an implication (**I**) based on the answer, giving the abstract the form **BQERAI**.

Sample Paper Abstracts

#1)

B Development of pharyngeal muscle in nematodes and heart muscle in vertebrates and insects involves the related homeobox genes *ceh-22*, *nkx2.5*, and *tinman*, respectively. **Q** To determine whether the nematode gene *ceh-22* and the vertebrate gene *nkx2.5* perform similar functions, **E** we examined the activity of the zebrafish *nkx2.5* gene in transgenic *Caenorhabditis elegans*. **R1** We found that ectopic expression of *nkx2.5* in *C. elegans* body wall muscle directly activated expression both of the endogenous *myo-2* gene, a *ceh-22* target normally expressed only in pharyngeal muscle, and of a synthetic reporter construct controlled by a multimerized CEH-22 binding site. **R2** *nkx2.5* also efficiently prevented *ceh-22* growth defects when expressed in pharyngeal muscle. **A** These results indicate that *ceh-22* and *nkx2.5* perform similar functions. **I** Further, these results suggest that an evolutionary conserved mechanism underlies pharyngeal development in nematodes and heart development in vertebrates and insects.

This abstract has all of the standard elements in the right order:

- **B** Background
- **Q** Question
- **E** Experiments
- **R** Results
- **A** Answer
- **I** Implication

#2)

During mitosis, the mitotic spindle, a bipolar structure composed of microtubules (MTs) and associated motor proteins, segregates sister chromatids to daughter cells. Initially some MTs emanating from one centrosome attach to the kinetochore at the centromere of one of the duplicated chromosomes. This attachment allows rapid poleward movement of the bound chromosome. Subsequent attachment of the sister kinetochore to MTs growing from the other centrosome results in the bi-orientation of the chromosome, in which interactions between kinetochores and the plus ends of MTs are formed and stabilized. These processes ensure alignment of chromosomes during metaphase and their correct segregation during anaphase. Although many proteins constituting the kinetochore have been identified and extensively studied, the signaling responsible for MT capture and stabilization is unclear. Small GTPases of the Rho family regulate cell morphogenesis by organizing the actin cytoskeleton and regulating MT alignment and stabilization. We now show that one member of this family, Cdc42, and its effector, mDia3, regulate MT attachment to kinetochores.

This abstract deviates from the standard form of an abstract:

- Too much background
- Buried question
- Background again after the buried question
- No description of the experiments that were performed
- No statement of the results
- The answer is not parallel to the (buried) question

PUTTING IT ALL TOGETHER (Grants)

While this course takes a deconstructionist view of scientific writing, the whole of the grant must be greater than the sum of its parts. Be mindful of the order in which the various grant sections we have studied will occur in the finished proposal. This information is presented on page 19 of this syllabus. A good grant writer weaves specific themes through all of the sections of the grant, making a strong case throughout for the merits of the application. Repetition is also essential, as reviewers will rarely read your grant in its entirety in one sitting. However, one should avoid repeating entire phrases, sentences, or paragraphs verbatim. If you are including published figures (even your own), be sure to cite them.

Imagine that your grant is a journey and that you are taking the hand of the reviewer to guide him or her along the path. What do you think is important to point out? What are the concepts that are absolutely essential for the reviewer to understand? Do you explain these concepts in language that is clear and understandable for a knowledgeable scientist not in your field?

Consider using figures and models to guide the reviewer and provide a roadmap. Use foreshadowing (i.e. early in the proposal, set-up the questions you will address and the techniques that you will employ in the "Approach" section). Make strong declarative statements but do not overstate the implications of your results. As with Specific Aims, close the whole Research Strategy with a "big finish" to indicate how your work, if successful, will advance the field.

Also keep in mind that most reviewers will likely not have read your grant in its entirety. A typical study section may have more than 30 members, of whom only three or so will be assigned to read your entire grant. For the others, the abstract and specific aims pages may hold particular importance, as these may be the only sections that they read.

TIMELINE

It is helpful to the reviewers to provide a timeline for your experiments. A timeline entails listing each grant year and what you expect to accomplish in that year. While not required for your preliminary exam, a timeline is a useful exercise, because it makes you think realistically about the time needed to perform and analyze your experiments. In the current NIH grant format, such timelines generally take the form of a table or chart and appear at the very end of the research strategy. An example from a recently funded R01 application is below:

REFERENCES

You should be familiar with the references that you cite and the references should indeed

TIMELINE OF WORK

| Activity | Year-1 | Year-2 | Year-3 | Year-4 | Year-5 |
|------------------------------------|--------|--------|--------|--------|--------|
| AIM-1 | | | | | |
| Ss-DAF-2 homologous studies | | | | | |
| Ss-AGE-1 homologous studies | | | | | |
| Ss-ILP-1, 6, 7 homologous studies | | | | | |
| AIM-2 | | | | | |
| Isolate natural Ss-DAF-12 ligands | | | | | |
| Ss-DAF-12 ligand activity studies | | | | | |
| Ketokonazole / Etomoxir studies | | | | | |
| Ss-DAF-12 / Ss-DAF-16 interactions | | | | | |
| Ss-DAF-12 based therapy in vivo. | | | | | |

contain the information that you reference. In NIH grants, references are not subject to the page limit. It is best not to be stingy with references.

BOTTOM LINE

Dr. William Dauer, Chair of the Chronic Dysfunction and Integrative Neurodegeneration Study Section and Professor in the Departments of Neurology & Cell and Developmental Biology of the University of Michigan notes that, "Obviously, the underlying science must be of high quality, and no amount of terrific writing will succeed in winning funding for work that is not well conceived or wanting in any other number of ways. But among much strong science, those applicants who can most clearly and compellingly communicate their message will be the ones that rise to the top of the pile."

Plenty of tips are available for successful grant writing, including through the *Insider's Guide to Peer Review for Applicants* on the website of the NIH Center for Scientific Review (CSR).

<http://public.csr.nih.gov/aboutcsr/NewsAndPublications/Publications/Pages/InsidersGuide.aspx>

Please keep in mind that there is no secret formula for writing a successful grant – many different approaches can work. As you formulate your approach, consider incorporating multiple viewpoints. Remember that all of the faculty in this course have had success in securing grants and in publishing their work in peer-reviewed journals. Their thoughts, advice, and suggestions,

along with those of colleagues, instructors, and other advisors (e.g. research mentors) may form the basis for your own approach.

CREDITS

This syllabus is based substantially upon the syllabus for 2007 CAMB 695, which was directed by Erica Golemis.

Here are her acknowledgements:

“In preparing the 2007 CAMB 695 syllabus, we drew principles and examples of clear and unclear writing from several excellent sources of information. Those sources are: Style: The Basics of Clarity and Grace and Style: Ten Lessons in Clarity and Grace by Joseph Williams, “*The Science of Scientific Writing*” by George Gopen and Judith Swan, *American Scientist* (1990) vol 78: 550-558, Essentials of Writing Biomedical Research Papers by Mimi Zeiger, The Sense of Structure: Writing from the Reader’s Perspective and Expectations: Teaching Writing from the Reader’s Perspective by George Gopen, The Elements of Style by William Strunk, Jr. and E.B. White, The Craft of Research by Wayne Booth, Gregory Colomb and Joseph Williams, the 2005 CAMB 695 course materials, the CAMB website, the NIH and NIAID websites, and past and current CAMB 695 instructors. “

In addition, Drs. Katz and Wells acknowledge *How to Write and Publish a Scientific Paper* by Robert A. Day and Barbara Gastel. The passages on active and passive voice were prepared by Hillary Nelson.

SUGGESTED REVISIONS FOR REWRITING EXERCISES

***Note that some revisions may seem better than others, although all generally follow the 5 principles.**

Exercise #1: Prolongation of life for uremic patients has been made possible by improved conservative treatment and hemodialysis.

Principle : Put the action in the verb.

Problem: The action of the sentence is expressed by a nominalization.

Revision A: The lives of uremic patients have been prolonged by improved conservative treatment and hemodialysis.

Revision B: Uremic patients live longer because of improved conservative treatment and hemodialysis.

Revision C: Improved conservative treatment and hemodialysis allow uremic patients to live longer.

Exercise #2: A disease that progresses with few or no symptoms to indicate its gravity is an “insidious” disease, under this definition. Asbestosis, neoplasia, mesothelioma, and bronchogenic carcinoma are all examples of insidious diseases. Asbestos insulation installers who have inhaled asbestos fibers over a period of many years regularly contract these diseases.

Principle: Put information where the reader expects it.

Problem: The information flow is NEW→OLD, NEW→OLD, NEW→OLD.

Revision: Under this definition, a disease that progresses with few or no symptoms to indicate its gravity is an “insidious” disease. Examples of insidious diseases are asbestosis, neoplasia, mesothelioma, and bronchogenic carcinoma. These diseases are regularly contracted by asbestos insulation installers who have inhaled asbestos fibers over a period of many years.

Exercise #3: Laboratory animals are not susceptible to these diseases, so research on them is hampered.

Principle: Make the words mean what you want them to say.

Problem: Unclear that “them” refers to “these diseases.”

Revision A: Laboratory animals are not susceptible to these diseases, so research on these diseases is hampered.

Revision B: Research on these diseases is hampered because laboratory animals are not susceptible to them.

Exercise #4: Propranolol had variable effects on the hypoxemia-induced changes in regional blood flow. In the cerebrum, the increase in blood flow caused by hypoxemia was not significantly altered by propranolol. However, in other organs and in the peripheral circulation, propranolol caused a more severe decrease in blood flow than did hypoxemia alone.

Principle: Write cohesive sentences and coherent paragraphs.

Problem: The information flow in sentence 2 is NEW→OLD. The subject of sentence 2 disrupts the topic string in the paragraph.

Revision: Propranolol had variable effects on the hypoxemia-induced changes in regional blood flow. In the cerebrum, propranolol did not significantly alter the increase in blood flow caused by hypoxemia. However, in other organs and in the peripheral circulation, propranolol caused a more severe decrease in blood flow than did hypoxemia alone.

Exercise #5: The molecular events determining the developmental lineage of the gonadotrope in the anterior pituitary, utilizing approaches in transgenic mice including ectopic expression of regulatory proteins, will be investigated.

Principle: Get to the subject quickly and follow the subject as soon as possible with its verb.

Problem: Subject-verb interruption.

Revision A: We will investigate the molecular events that determine the developmental lineage of the gonadotrope in the anterior pituitary by studying ectopic expression of regulatory proteins in transgenic mice.

Revision B: We will investigate the molecular events that determine the developmental lineage of the gonadotrope in the anterior pituitary. To do this, we will study ectopic expression of regulatory proteins in transgenic mice.

COMMENTS ON THE “PROBLEMATIC” ABSTRACT AND SPECIFIC AIMS

***This is by no means complete, and there may be other issues that are not specifically addressed by these comments.**

“PROBLEMATIC” ABSTRACT

Third-stage larvae of parasitic nematodes, which, in most species are the infectious stages for the mammalian host, including humans, of whom more than 3.5 billion may be infected worldwide, share common behavioral, morphological and developmental characteristics with the developmentally arrested dauer larvae of the free-living nematode *Caenorhabditis elegans*. It is proposed that molecular regulation of the transition from free-living to parasitic forms of parasitic nematodes and *C. elegans* dauer larva development regulation are similar. Significantly for the present study, it has been shown that in *C. elegans*, one of the key factors regulating the dauer transition is the insulin-like receptor kinase DAF-2. The parasitic nematode *Haemonchus contortus* has an insulin-like receptor (*Hc-daf-2*), which displays significant homology to insulin receptors in both vertebrates and invertebrates and is predicted to contain conserved structural domains. Examination of the parasite by RT-PCR showed *Hc-daf-2* transcription in all life stages. An important proteolytic motif was identified in the predicted peptide sequence of *Hc*-DAF-2 and is consistent with the HIR (human insulin receptor), suggesting that it could be involved in the formation of the insulin receptor complex. To test this, comparison of the patterns of expression between *Hc-daf-2* and *Ce-daf-2* was performed with reporter constructs fusing the *Ce-daf-2* or *Hc-daf-2* promoter to the coding sequence of *gfp*. These were microinjected into the N2 strain of *C. elegans*, and establishment and examination of transgenic lines were performed. These showed similar patterns of expression in amphidial/head neurons for both genes, which may be related to sensation and signal transduction, which are important processes in host finding by infective parasitic nematode larvae. For further functional analyses of *Hc-daf-2*, heterologous genetic complementation studies were attempted in the CB1370 *daf-2* mutant strain of *C. elegans*. These studies revealed that this mutation can be partially rescued by *Hc-daf-2*. Taken together, these data support the hypothesis that *Hc*-DAF-2 plays a crucial role in the transition from the free-living state to parasitism.

“PROBLEMATIC” SPECIFIC AIMS

As part of the adult *Strongyloides stercoralis* (SS) life cycle (LC), female SS lay eggs in the intestinal mucosa that hatch into rhabditiform larvae, which are shed in the stool. Caused by the parasitic nematode (pn) SS, and being characterized by extreme hyperchronicity with infected individuals being diagnosed decades after leaving the endemic environment, human SS affects ~100 million people globally. It has been shown by Schad et al. (19) that maintenance of hyperchronic SS may be by a process unique to SS in which parasite larvae develop precociously to successive generations of parasitic females in the same host, which is called autoinfection (18). It has been shown that in most cases, senescent parasitic females are gradually replaced with new individuals through a continuous process of tightly regulated low-level autoinfection (9, 10). However, these chronic, clinically latent infections, in patients immunosuppressed by corticosteroid therapy (CT) or underlying HTLV-1 infection, become unregulated, resulting in a fulminant often-fatal hyperinfection (8). Clearly, a better understanding of mechanisms initiating and maintaining autoinfection by SS is critical for preventing disseminated strongyloidiasis in at-risk patients.

Hyperchronic strongyloidiasis can be modeled experimentally in infected dogs by administering low dose CT, and it is widely assumed that such autoinfection is driven primarily by steroid suppression of immune responses that would normally clear the parasite (10). However, our preliminary data are supportive of the fact that steroid-induced autoinfection results from direct action by the drug on a parasite-intrinsic steroid signaling pathway. First, we have shown that autoinfection by SS in immune-deficient NSG mice requires exogenous (hereafter ‘medicinal’) CT. Second, our multidisciplinary team, which includes the PI, a medicinal chemist, a statistician, and members of the PIs laboratory, including graduate students and post-docs, has amassed compelling evidence that endogenous steroid signaling regulates larval development in SS. Specifically, we have shown that DAF-12, a corticosteroid-class nuclear hormone receptor (NHR) signaling pathway that regulates larval development in *Caenorhabditis elegans* (CE), is conserved in SS. Moreover, dafachronic acids (DAs), natural ligands of the CE receptor DAF-12, regulate crucial developmental events when applied exogenously to SS (11).

Therefore, we will characterize the action of medicinal steroids or their host metabolites with the parasite homolog of DAF-12 during the process of autoinfection by SS. We will also evaluate how parasite-intrinsic NHR signaling relates to very low levels of autoinfection the relationship of this autoinfection to host immunity. We know that disseminated hyperinfection is observed in immunocompromised patients in the absence of CT. Therefore, in Aim 2, we will determine if the residual innate immune effectors of NSG mice prevents NHR-dependent autoinfection in the absence of medicinal steroids. Evidence that medicinal steroids or their metabolites function as ligands for endogenous NHR signaling in SS to promote autoinfection will constitute a milestone supporting translational studies where compounds identified as agonists or antagonists of SS NHR signaling in an existing high-throughput screen will be prioritized for in vivo testing. Prioritized compounds will be tested for efficacy in preventing autoinfection in gerbil and/or NSG mouse models of autoinfection. Milestones indicating success will be three or more lead compounds that clear hyperchronic SS infection. Our specific aims are to:

SPECIFIC AIM 1: Characterize the interaction of medicinal steroids or their host metabolites with SS during autoinfection. To this end, we will determine **a)** the effect of medicinal CT of young SS larvae on the frequency of autoinfection, **b)** whether medicinal steroids act as direct ligands for SS NHR signaling **c)** whether the DA-synthetic enzymes of CE

are conserved in SS, and **d**) whether bile acid precursors in steroid-treated hosts are substrates for nematode DA-synthetic enzymes.

SPECIFIC AIM 2: Investigate the roles of remaining immune functions in the NSG mouse in regulating autoinfection. We will assess remaining components of immune functionality in the NSG mouse (neutrophils, basophils, and eosinophils) for their role in autoinfection in non-steroid treated mice.

SPECIFIC AIM 3: Identify hits from an existing high throughput screen (HTS) for compounds that agonize or antagonize the SS NHR Ss-DAF-12. Using cell-based assays in a multi-well format we will screen small molecule libraries for hits that interfere with autoinfection.

SPECIFIC AIM 4: Advance HTS hits from Aim 3 as appropriate to testing in in vivo models of autoinfective strongyloidiasis. Hits from the HTS will be assayed for ability to prevent autoinfection in a well-characterized model of autoinfective strongyloidiasis in gerbils and in the NSG mouse model.

SPECIFIC AIM 5: Develop new in vivo models for testing. We will explore whether other animal models are also appropriate for testing hits from HTS.