Goal of the Research Proposal

Sharpen your skills in

a) Critical evaluation of the literature to identify and understand major unanswered questions in a field.

b) Formulate research hypotheses, which address one or more questions or gaps in knowledge in a field.

c) Devise experimental strategies to answer these questions, with controls and unbiased data analysis so that solid conclusions can be made.

And

d) Familiarize you with the practical use of the Research Proposal format

Research Proposals

Requires in depth knowledge of a research area.

Articulate major unanswered questions or gaps in knowledge in a research area.

Describe experimental approaches & data analysis that test specific hypotheses and/or address unanswered questions in a research area.

Proposal organizational format is directed at telling the reader what questions/hypotheses you are addressing, why the findings/answers are important and what experimental approaches will be employed to obtain the findings.

Three broad classes of research proposals

1. Hypothesis-driven
2. Hypothesis-generating
3. Methods development
Three broad classes of research proposals

1. Hypothesis-driven
2. Hypothesis-generating
3. Methods development

Three essential sections

Specific Aims
- A stand-alone description of the problems/hypotheses that will be examined, and usually includes a discussion of how the findings would advance the field.
- A listing of what lines of investigation will be used in the study and what will be learned.

Background and Significance

Experimental Design and Methods
Three essential sections

Specific Aims

Background and Significance
- Description of the current state of the field, critically evaluating existing knowledge and gaps that the proposed Aims will fill.
- Address the broader significance of the field and the findings that will arise from your proposed work.

Experimental Design and Methods
- Description of the experimental approaches that will be used to execute each Aim. The logic behind the experiments, controls and interpretations is more important than details.
Three essential sections

Specific Aims

Background and Significance

Experimental Design and Methods
- Description of the experimental approaches that will be used to execute each Aim.
- The logic behind the experiments, controls and interpretations is more important than details.
- Briefly describe, if relevant, alternative outcomes and/or approaches.
- At the end of each section, summarize the possible results in relation to advancing the Aim.

The Specific Aims has three components

1. Background narrative (like an abstract) that provides a context for the questions that will be addressed.
2. List of the questions /how the questions will be addressed.
3. Discussion of the significance of the results that will be obtained.

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Aggregation-induced cell death is a cell death phenomenon in which cell death occurs in a specific cell type in response to a specific stimulus. The mechanism underlying aggregation-induced cell death is not fully understood, but it is thought to involve the activation of certain pro-apoptotic enzymes, such as caspase-3, which lead to the degradation of cellular components and ultimately cell death.

General background

Specific background

Novel finding

Model/hypothesis to explain finding

Goal & what will be learned
**Listing of the Aims**

(Here as a list of declarative statements.)

Aim 1. In order to test the hypothesis that dpy-12 tonically represses lin-28, the temporal profile of endogenous lin-28 mRNA and protein levels in wildtype and dpy-12 mutant animals will be determined.

Aim 2. Determine if lin-4 independent repressor elements (LIREs) in the lin-28 3'UTR are necessary and sufficient for the normal regulation of lin-28 by the LIR pathway.

Aim 3. Determine if LIREs in the lin-28 3'UTR are required for developmental timing of the L2-L3 transition during larval development.

Aim 4. Perform genetic screen to identify genes that are required for LIR.

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**The listing of Aims or subAims can be either as declarative statements or as a question or a hypothesis.**

Aim 3. Determine if lin-4 independent repressor elements (LIREs) in the lin-28 3'UTR are required for developmental timing of the L2-L3 transition during larval development.

Are the LIREs in the lin-28 3'UTR required for developmental timing of the L2-L3 transition during larval development?

I hypothesize that the lin-28 3'UTR is required for developmental timing of the L2-L3 transition during larval development.

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**At least some Aims/subAims should have a logical progression**

Aim 1. In order to test the hypothesis that dpy-12 tonically represses lin-28, the temporal profile of endogenous lin-28 mRNA and protein levels in wildtype and dpy-12 mutant animals will be determined.

Aim 2. Determine if lin-4 independent repressor elements (LIREs) in the lin-28 3'UTR are necessary and sufficient for the normal regulation of lin-28 by the LIR pathway.

Aim 3. Determine if LIREs in the lin-28 3'UTR are required for developmental timing of the L2-L3 transition during larval development.

Aim 4. Perform genetic screen to identify genes that are required for LIR.
An Aim can also stand on its own, but should still be integrated/ highly related to the other Aims in the proposal.

Aim 1. In order to test the hypothesis that ada-21 transcriptionally represses lin-39, the temporal profile of endogenous lin-39 mRNA and protein levels in wildtype and ada-21 mutant animals will be determined.

Aim 2. Determine if the 4 independent expression elements (LEEDs) in the 3' UTRs of the 39 mRNA are necessary and sufficient for the derepression of the 39 by the LEED pathway.

Aim 3. Determine if LEEDs in the 39 3' UTR are required for the developmental timing of the L2-L3 transition during larval development.

Aim 4. Perform genetic screens to identify genes that are required for LEED.

Description of the significance of the findings, if the proposed studies are successfully completed.

(Need not be a separate section, can be embedded into the narrative.)

Overall Significance of Aims. First identified in the C. elegans heterochronic pathway, translational repression by mRNAs is emerging as a common mode of regulation in development. Regulation of gene expression by mRNAs may be shared from worms to mammals as indicated by the conservation across phyla of the 3-mRNA (Pasquinelli et al. 2000). Although, it has been demonstrated that the mRNA lin-4 regulates lin-25 in early larval stages, it remains to be tested whether a second mRNA pathway is involved in the LEED pathway.

This proposal will examine this hypothesis and has the potential to identify novel mRNA regulators.

Background and Significance

- Set reader up for the Aims and experiments in the Aims.
- Convince the reader why the results obtained will move a field forward --- why we should care?
- Move from general to specific.
- Use headings to divide sections.
- Employ figures & tables to facilitate explanation.
- Avoid presenting extraneous information.
- Length constrained.
Importance of lin-28 3'UTR regulation

What proposal will focus on

Another pathway that needs to be explored

Model to be tested in this proposal

Imported...
In the Experimental Section, each Aim or subAim should have four components

1. Rationale for the experiment
2. Experimental plan and controls
3. Interpretations
4. Alternative approaches and limitations

Ok to use these as heading. But more useful to have headings telling the reader what is the experiment, question or hypotheses that is being examined in the section.
Logic for what will be done

General outline of experiment (limit the detail)

Controls

Typos!!

Methods

The results, analyses, comments, and conclusions will not be subjected to any further

Expectations.

The results of the experiments are not interpreted and evaluated.

Approach if alternative result

Exploration

The approach for the experiments in Aim 2 will be as follows:

- The results will be analyzed and interpreted.
- The conclusions will be drawn.
- The experiments will be repeated to verify the results.

Expected finding leads to experiments in Aim 2.

Approach if alternative result

All the results will be analyzed and interpreted. The conclusions will be drawn from these analyses.

Methodology

The methodology will be as follows:

- The experiments will be performed in duplicate.
- The data will be analyzed using appropriate statistical methods.
- The results will be presented in a clear and concise manner.
- The conclusions will be drawn from these analyses.

All the results and conclusions will be subjected to further analysis.

The results will be analyzed and interpreted.
Relevant background

Question to be addressed

How, in general, will it be addressed

Relevant background

Question to be addressed

How, in general, will it be addressed

Relevant background

Question to be addressed

How, in general, will it be addressed

Relevant background

Question to be addressed

How, in general, will it be addressed
In hypotheses driven Aims

- Show that daf-12 & LIR translationally regulated lin-28 (Aim 1)
- Identify sequences in the lin-28 3’UTR responsible for the regulation (Aim 2)
- Show that 3’UTR sequences regulate lin-28 level and activity in vivo (Aim 3)

- The next logical question is what genes/gene products are acting in trans for LIR regulation of lin-28? (This is a discovery Aim)
Aim 4. Perform genetic screen to identify genes that are required for LIR.

Proposed experimental approach.

Types of genes that might be identified.
Should provide a rationale for the approach, even if it is obvious to you.

DAF-12 is a nuclear hormone receptor, therefore it is unlikely to be a direct translational regulator of lin-28. Thus, there must be other genes controlled by DAF-12 that act in trans to repress lin-28.

John A. Perdue generic screen to identify genes that are required for DAF-12.

Rationale: To identify genes involved in the DAF-12 pathway for lin-28 downregulation, I will perform a generic screen for bioenergetic mutants using the daf-12-1 strain. This strain has been used to identify genes involved in the regulation of aging. The daf-12-1 strain has a mutation in the daf-12 gene, which encodes a nuclear hormone receptor. This receptor plays a role in the regulation of metabolism and aging.

The screen will involve the use of a transgenic strain that expresses wild-type DAF-12 under the control of the daf-12 promoter. This strain will be crossed with a strain carrying a random insertional mutation to generate a large number of transgenic animals. The transgenic animals will then be screened for increased longevity and reduced fat content.

New technique, so explain in sufficient detail that the reviewer believes you can execute the experiment.

Note that would now simultaneously do polymorphism mapping and whole genome sequencing to identify the genes.

For a "Discovery" Aim, it is essential to provide possible genes that could be identified, and expectations for their behavior.
By describing experimental limitations you convey knowledge of the approach.

However, too many limitations will make the reviewer think it is a poor approach.

"Discovery" Aims are viewed as problematic for grants as they are risky - uncertain if they will be successfully completed.

Nevertheless, in a number of cases, what is identified in a discovery Aim will significantly advance a field.

For Advanced Genetics, a discovery Aim is possible. We want to expand your skill in coming up with a logical experimental approach to fill a gap in the field of interest. (Needs to be justified & well explained.)

Where do Aims come from?

Three essential elements in coming up with an Aim.

1. Information from the literature and/or preliminary experimental findings.

2. A hypothesis that derives from the literature findings and/or preliminary results.

3. Experiment or set of experiments that will test the hypothesis.
Example

Literature/experimental findings:
- Sugar transporter Glut8 co-localizes with the autophagy protein ATGx in mouse liver cells, based on immunofluorescence.
- Glut8 (-/-) knockout mouse displays an increased autophagy phenotype in liver cells (and also suppresses fatty liver disease in mice fed a high fructose diet).

Hypothesis:
- Glut8 is a negative regulator of ATGx activity in the autophagy pathway and acts as an inhibitor through binding ATGx and making it unavailable to stimulate the autophagy pathway.

Prediction: Blocking Glut8 - ATGx binding, but not other Glut8 protein functions, will lead to constitutive autophagy.

Example continued

An experimental approach for testing the hypothesis:

a) Determine if the cytoplasmic domain of Glut8 binds to ATGx, using the yeast two-hybrid screen.

b) Identify small regions of Glut8 and ATGx that are responsible for binding, using the reverse yeast two-hybrid system.

c) From the regions/amino acids of Glut8 and of ATGx responsible for binding, generate knock-ins (or transgenes) that contain alanine mutations in these sites, which should block binding but retain other functions (these are alanine-scanning, separation of function mutations).

Test each mutant in liver cells, under conditions where the corresponding endogenous gene product is absent, for constitutive autophagy.

While the aims should be hypothesis driven (not just data collection) don't be hypotheses limited or paradigm blinded.

The best experiment is if either outcome is informative in addressing the goal of the Aim.
For your class proposal

1) From the literature, find an area of interest where there are open questions or gaps in knowledge.

2) Derive one or more testable hypotheses related to the open questions/ gaps in knowledge.

3) Assemble an experimental approach that addresses the hypotheses and any predictions that might arise from the hypotheses.

Advanced Genetics students:
Approach should include at least some genetic analysis.

General Tips

1. Look at successful proposals.
2. Have a good idea.
3. Know the literature, issues, questions/controversies in the area.
4. Instead of just feedback, try feed forward, where you discuss your ideas with others before beginning the writing process.
5. Place the work in a broader perspective, indicating significance.
6. Use clear and concise writing style.
7. Proofread - zero tolerance for typos, formatting & citation errors
8. Critique your own proposal.
9. Have others critique your proposal.
Plagiarism

Two useful websites that define plagiarism and provide tips on how to avoid it in your writing.

https://wts.indiana.edu/writing-guides/plagiarism.html
http://writingcenter.unc.edu/handouts/plagiarism/

Week of Jan 27, Small Group Discussion Sections

Long Chain FA Proposal
ADAR Editing Proposal

- For each component of the proposals, what are the positives and negatives in the authors' execution of the section.
- Is the writing clear as to what the author is proposing?
- Are you convinced it is a significant problem?
- Do the experiments address the issues/questions?
- Are you convinced that the author can execute the proposed studies?