The Writing Process

- When to start?
- *At least* three months in advance.
  - Longer for new project.
  - Longer for complex project.
- Don't assume that a renewal will be automatic or easy.
  - Competitive renewals are as hard to get as new grants.
  - Sometimes, they are harder, e.g., if "new investigator" advantage is lost.

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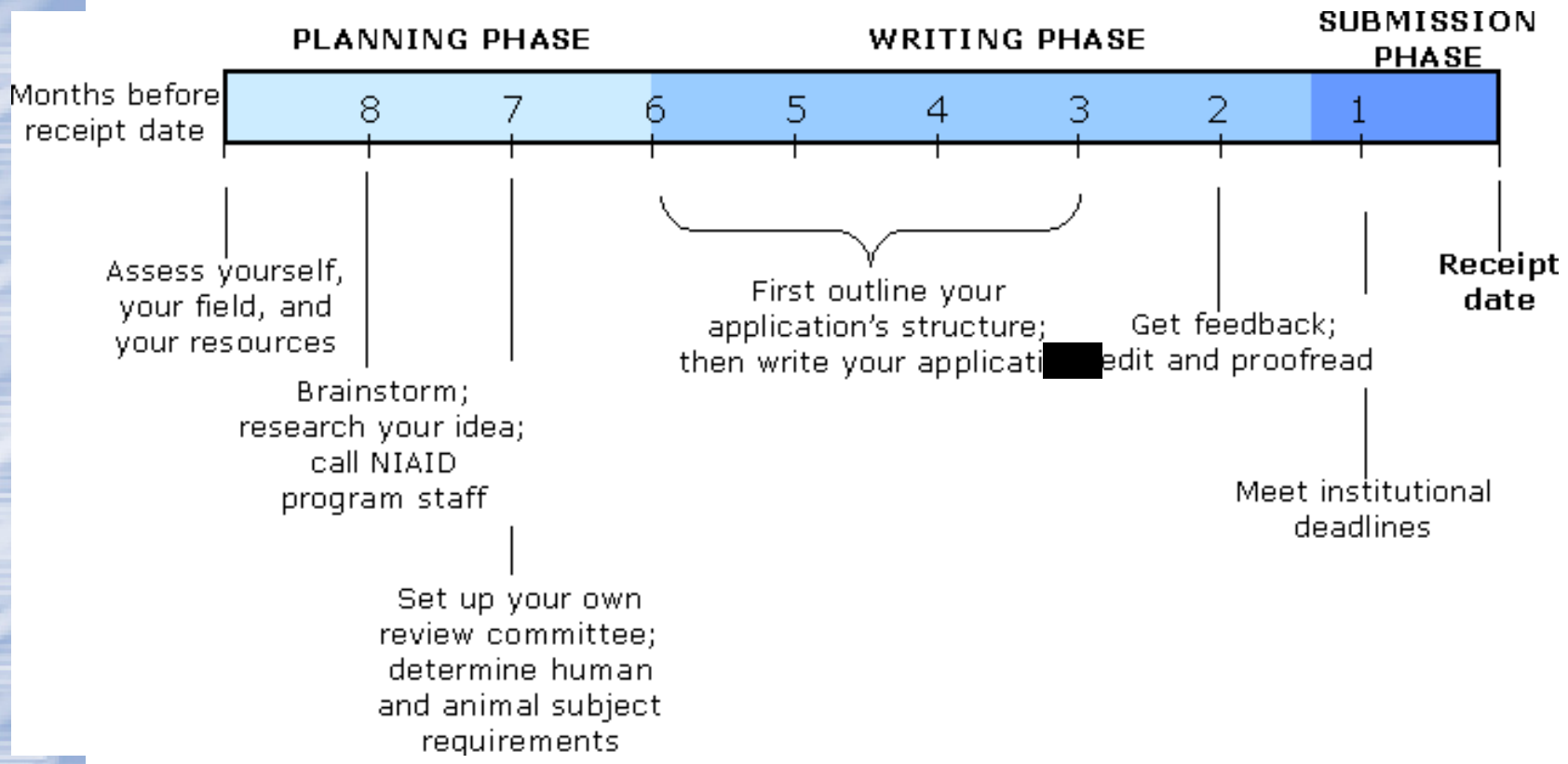
Timeline for Proposal Preparation



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Writing the application

- Format and content vary dramatically for different agencies.
  - Read the instructions.
  - Follow them ***to the letter***.
  - May need to alter focus.
  - May need to alter scope to match money and time available.

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Proposal Sections

- Format varies with sponsor.
- Follow instructions exactly.
- Conform to required length.
  - Can always be shorter.
  - Can never be longer.
- Don't try to get around length limits by using tiny fonts, small margins or appendices.
  - Many agencies reject such grants without review.
  - Even if they don't, the reviewers will be extraordinarily unpleased.

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Scientific sections of an NIH application (revised in January 2010)

- Specific Aims
- Research Strategy
  - Significance
  - Innovation
  - Research Design and Methods
- Literature Cited
- Human Subject/Vertebrate Animals/ Etc.
- Appendices

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Former scientific sections of NIH proposals (prior to January 2010)

- Specific Aims
- Background and Significance
- Preliminary Studies/Progress Report
- Research Design and Methods
- Literature Cited
- Human Subject/Vertebrate Animals/Etc.
- Appendices

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In Your Proposal...

- Assume you are not writing for an expert.
- Emphasize general medical importance and then specific importance of your topic.
- Avoid jargon.
- Discuss controversies in the area.
- Avoid selective citation of the literature.
- Make your story interesting- make the reviewer want to read more!
- Correct English, grammar, and attention to typographical errors is important.
- Reviewers like a "pretty" application.

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Background and Significance

- Give scientific background and context for project.
- Establish importance and novelty of proposed project.
- Review prior work in area of project and literature related to the project.
- Goals of this section:
  - Orient reader to subject and importance of project.
  - Show your knowledge of the area through a solid review and objective citation of prior related work.

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Background and Significance

- This is likely to be read by all the reviewers, so write this section in **nontechnical terms** for the broader audience.

- Convey the significance of your research. Reveal you are aware of opportunities, gaps, and roadblocks in your field.

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Background and Significance

- Show reviewers your intimate familiarity with the field, and refer to all relevant scientific literature, positive and negative.
- Show the breadth of your knowledge of your field and highlight why you are uniquely qualified to do the research.
- Illustrate complicated mechanisms with figures.

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Background and Significance

B. BACKGROUND AND SIGNIFICANCE

B.1. Histone modifications represent a key layer of epigenetic information.

DNA in the eukaryotic nucleus is wrapped tightly around nucleosomes, the fundamental unit of chromatin (1). Each nucleosome is composed of two subunits of each of the four core histone proteins (H2A, H2B, H3 and H4), which are evolutionarily well-conserved basic proteins …..

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B. Background and Significance (from Huston grant (AI072021):

Amebiasis: *Entamoeba histolytica, the intestinal protist that causes amebic colitis and liver abscess, causes* an estimated 50 million symptomatic infections annually, and is the second leading protozoan cause of death worldwide [1, 2]. Approximately 50% of children in Dhaka, Bangladesh are infected with *E. histolytica by age* five, highlighting the seriousness of amebiasis as a health problem in the developing world [3]. In the United States, water-borne pathogens are of growing concern because of the risk of bioterrorism (CDC Category B) [4]. A recent outbreak of amebiasis due to disruption of the city water supply in Tblisi, Republic of Georgia, demonstrates the susceptibility of more developed nations to re-emergence of *E. histolytica [5].*

Entamoeba histolytica is spread by fecal-oral transmission (reviewed in [6]). After ingestion of the infectious cyst form of the organism, excystation occurs in the terminal ileum or colon. The motile trophozoites then colonize the colon by adhering to intestinal mucous glycoproteins, in large part via an amebic Dgalactose/N-acetyl-D-galactosamine (Gal/GalNAc) specific adherence lectin [7, 8]. In a minority of infections, trophozoites penetrate the intestinal epithelium causing amebic colitis, and extra-colonic spread may result in amebic liver abscess. Acute inflammation, tissue destruction (probably due directly to amebic proteinases and cytotoxic ability, and to the host's acute inflammatory response), and amebic phagocytosis of host erythrocytes and immune cells are the major pathologic features of early invasive disease [9-15]. Interestingly, inflammation is often minimal despite ongoing tissue destruction in well established disease [9, 10, 16, 17].

Background and Significance

Innovation:

- If your proposal is highly innovative, make a very strong case for why you are challenging the existing paradigm and have data to support your approach.

- Innovation does not necessarily mean a new paradigm:
  - If the result of the research is critical, your means may not need to be innovative and vice versa.

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Preliminary Results/Studies/Data

The Preliminary Results section builds reviewer confidence that you can handle the **technologies**, understand the **methods**, and interpret **results**.

- Interpret your preliminary results critically.
- Include enough information to show you know what you are talking about.

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Preliminary Results

- While you may include related studies by others, this is the place to highlight your own preliminary data:
  - Present your data carefully and clearly.
  - Use high quality graphs, photos, and tables.
  - Show, discuss appropriate controls.
  - Analyze appropriately.
  - Use appropriate statistics.
  - Interpret your findings carefully and critically; acknowledge limitations of techniques and data.

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Preliminary Data will be reviewed to:

- Evaluate the basis of the project.
- Predict the chance of success.
- Evaluate your:
  - Ability to develop and test hypotheses.
  - Ability to design rigorous experiments.
  - Expertise with experimental techniques.
  - Expertise in analysis of data.
  - Rigor in interpreting the data.
  - Ability to present findings clearly and effectively.
- Sloppiness here is absolutely fatal.

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Preliminary Results

- Include any previous experience that shows you can direct the proposed research and achieve its aims.
- Illustrate relevance of preliminary data to your Specific Aims.

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Preliminary Results

C. PRELIMINARY STUDIES

C.1. The RNA Toxicity Model and the Feasibility of *FMR1* mRNA Knock-Down (toward Aims 1 and 2)

C.1.a. Elevated levels of expanded-CGG-repeat *FMR1* mRNA are due to increased transcriptional activity.

In previously published work, our group was the first to demonstrate that premutation CGG-repeat expansions (55-200 CGG repeats) in the *FMR1* gene led to substantially (2 to 10-fold) elevated levels of *FMR1* mRNA (Tassone and Hagerman, 2003; Tassone et al., 2000a; Tassone et al., 2000c)

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Preliminary Results

* from Huston grant (AI072021)

2. Preliminary studies in support of specific aim 1: Collectin-dependent phagocytosis: The collectins and C1q bind to apoptotic cells and bacteria, and facilitate macrophage phagocytosis [47, 50-52]. C1q is restricted to serum, but the collectins are present in serum and in mucosal secretions [55-60, 62-64]. Consistent with the possibility that the collectins or C1q facilitate *E. histolytica phagocytosis, we found that* 20% more *E. histolytica trophozoites engulfed serum-treated apoptotic lymphocytes than lymphocytes treated* with bovine serum albumin (BSA) (negative control) (n=3, p=0.0006 (flow cytometry data, not shown)). We compared phagocytosis of apoptotic lymphocytes that had been pre-treated with either purified human C1q (Quidel Corp.), or with bovine serum albumin (BSA) (negative control) to determine if opsonization of apoptotic cells with C1q facilitates amebic phagocytosis. Significantly more trophozoites engulfed cells pretreated with C1q than control cells, and the effect was dose dependent (Figure 5A and B). *C1q-biotin bound to both apoptotic and healthy cells as determined by flow cytometry using a streptavidin-Alexa 488 conjugate for detection, but C1q had no effect on phagocytosis of healthy cells (data not shown). Confocal microscopy showed that C1q-biotin bound diffusely to healthy cells, but localized to blebs on apoptotic cells (Figure 5C).*

Preliminary Results

* from Huston grant (AI072021)

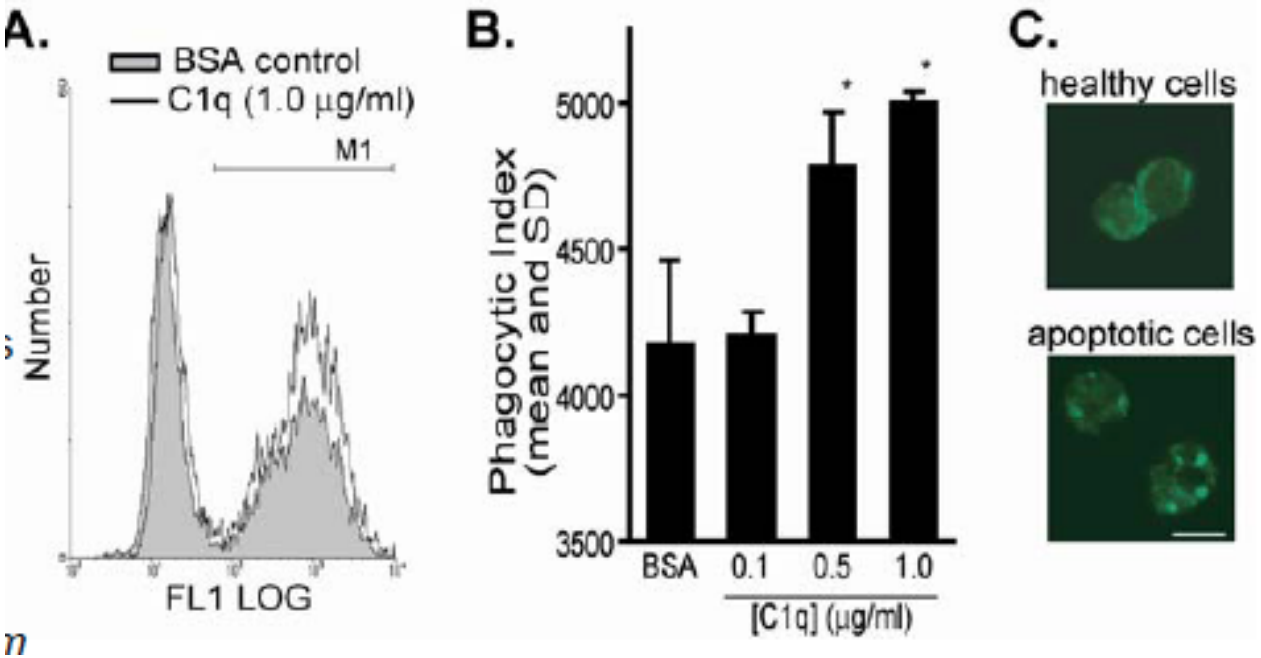

Figure 5: C1q facilitates clearance of apoptotic lymphocytes by HM-1:IMSS *E. histolytica trophozoites.* Apoptotic (UV-treated) lymphocytes were CFSE-labeled, treated with purified human C1q or BSA (negative control)(30 min, 37ºC), and incubated with amebic trophozoites (30 min, 37ºC, ameba:host cell=1:4) in the presence of 100 mM D-galactose to inhibit the amebic Gal/GalNAc lectin. (A) Representative FACS histograms showing amebic fluorescence. M1 gate indicates phagocytic amebae. (B) Dose effect of C1q on amebic phagocytosis (mean and SD of phagocytic index, n=3, *indicates $p < 0.02$ vs. BSA control). *(C) Confocal microscopy showing different patterns of C1q-biotin binding to healthy and apoptotic cells. Scale bar is 10 µm.*

Research Design and Methods

- Generally the longest section of the research strategy.
- This is where you develop the details of your project.

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Research Design and Methods

- This section is not just methods.
- Outline your experimental plan.
  - Base on specific aims - restate aims and describe flow of experiments under each aim.
  - Develop logic of project.
  - Describe timeline, sequence of experiments.
  - Describe potential pitfalls and what you will do if they occur.
  - Talk about relevance (clinical relevance for those grantmakers with a clinical mission).

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Research Design and Methods

- Describe methodology.
- Cite appropriate references.
- Establish your expertise with the techniques to be used.
  - Give citations to your work using the techniques.
  - Provide accurate discussion of techniques and of their strength and limitations.
  - Give methodology details where critical (especially if unpublished or unique).
  - Describe alternatives you will use if a technique is inadequate or the results are inconclusive.

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Research Design and Methods

- When reviewers judge your application, your Research Design and Methods section has the **most weight**. It describes the **experimental design** and **procedures**—how you will perform the research.

- Give details: specify animal models, exposure times, reagents and how you will get them, statistical analysis methods, etc.

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Research Design and Methods

Specific Aims:

- Link your experiments to your Specific Aims, so reviewers can see how you will achieve them.
- Put experiments in a logical sequence. They should flow from one to the next with clear starting and finishing points.

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Research Design and Methods

D. RESEARCH DESIGN AND METHODS

Introductory paragraph to orient reviewer as to what you plan to do.

D.1. Aim 1. Repeat Specific Aim from page 1.

D.1.a. Hypothesis and Rationale.

D.1.b. Experimental Plan.

D.1.c. Expected Outcomes/Potential Pitfalls and Alternative Approaches

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D. Research Design and Methods (from Huston grant (AI072021):

General methods to be used throughout these studies: *Unless otherwise indicated,* experiments will be conducted using HM-1:IMSS strain *E. histolytica* trophozoites (American Type Culture Collection (ATCC)) grown in TYI-S-33 medium (trypticase yeast extract, iron, and serum) supplemented with 100 U of penicillin/ml and 100 μg/ml of streptomycin [117]….

Aim 1: Test the hypothesis that *E. histolytica* has a phagocytosis receptor specific for the collagenous collectin tail.

Rationale: C1q and the collectins both facilitate macrophage phagocytosis by binding to apoptotic blebs with their globular head domains, and then to macrophage calreticulin via their conserved collagenous tail [50-52]....

Method:

1.1: Determine if C1q tails specifically stimulate amebic phagocytosis: *This subaim is now partially complete*. Our collaborator, Dr. Henson, has provided us with purified SP-A collagenous tails that partially inhibited binding of amebic calreticulin to human C1q in our hands (see Figure 14). *Furthermore, we have purified the collagenous C1q tail using a published protocol [50, 101, 119]*....

Interpretation, potential pitfalls, and alternative approaches: *We anticipate that soluble collectins, SP-A tails, and C1q tails present in excess will inhibit phagocytosis of C1q tail-coated single-ligand particles and apoptotic cells coated with intact C1q*....

Research Design and Methods

Potential Pitfalls and Alternative Approaches:

- Discuss the limitations of each approach you are proposing and how they may affect your results and data. Call attention to potential difficulties and propose alternatives.

- State what you'll do if results are negative, how negative findings will also advance the field, and what you'll do next.

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Research Design and Methods

Timeline:

- Propose an appropriate amount of work for the funding period. Reviewers are sensitive to proposals that seem "overly ambitious."

- Provide a diagram of your timeline over the funding period, showing when you will hit certain milestones, or provide a list of milestones you will achieve in each year.

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Research Design and Methods

Timeline:

	Year 1		Year 2		Year 3		Year 4		Year 5	
Months	1-6	7-12	1-6	7-12	1-6	7-12	1-6	7-12	1-6	7-12
Activity										
Activity #1 (Aim 1)	→									
Activity #2 (Aim 2)		→								
Activity #3 (Aims 2 & 3)		→								
Activity #4 (Aim 1)			→	→	→	→				
Activity #5 (Aims 2 & 3)			→	→	→	→				
Activity #6 (Aim 3)			→	→	→	→				
Activity #7 (Aim 1)							→	→		
Data analysis (Aims 1-3)			→	→	→	→	→	→	→	
Preparation of report and publications (Aims 1-3)								→	→	→

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Research Design and Methods

Timeline (from Huston grant (AI072021):

Specific Aim	Year 1	Year 2	Year 3	Year 4	Year 5
Specific Aim 1-Collectin-dependent phagocytosis					
1.1-C1q tail expts.					
1.2-C1q/collectin binding expts.					
1.3-N-glycanase treatment of collectins					
1.4-collectin-dependent phagocytosis of bacteria					
Specific Aim 2-Functional studies of calreticulin					
2.1-calreticulin silencing					
2.2-recombinant calreticulin expts.					
2.3-expts. to test if *E. his.* calreticulin is a collectin receptor					
2.4-calreticulin mapping expts.					
Specific Aim 3-Collectin or calreticulin receptor identification					
3.1-candidate identification					
3.2-confirmation of candidate-bait interaction					
3.3-functional evaluation of candidates					

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Research Design and Methods

D.4. Milestones and Timeline

Year 1

- Synthesize 20-25 histone peptide arrays.
- Clone, express, purify 8 targets including full-length and multiple domains.
- Screen 8 targets against peptide arrays.
- Validate candidate peptides thereby establishing candidate marks.
- Raise and validate monoclonal antibodies against confirmed novel mark candidates.

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For techniques that are new for you

Tell us how you will obtain expertise.

- Collaborator?
  - at Berkeley
    Include as investigator.
  - from Outside
    - Consultant: biosketch, letter.
    - Subcontract: agreement between institutions.
- Someone who will teach you?
  - Letter
  - Biosketch
- Use a core facility.
- Take a course.

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Literature Cited

- Follow required format exactly.
- Be complete, but not excessive.
- Be accurate:
  - Read entire article carefully.
  - Cite accurately.
- Include your own work but also cite others, including competitors.
- Be objective.
- Don't ignore literature you don't like, instead cite and discuss it.

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Appendices

- Follow instructions carefully.
  - Some applications have mandatory appendices.
  - Some do not allow appendices.
  - Some limit appendices (standard NIH limit is 10, of which 3 can be certain types of papers; NIH no longer accepts color photographs or images of gels, micrographs, etc.).
- Possible appendices.
  - Letters of collaboration.
  - Letters of recommendation.
  - Papers not available online.
  - Manuscripts in press.

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Appendices

- Only the primary reviewers may have them, and they are not required to look at them.
- Most reviewers will never see them.
- Do not try to use them to circumvent page limitations.
- Do not use them for critical information; put everything critical in the body of the application.
- They may be separate from the main application - label them clearly.

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