

Papers vs. proposals

Key points:

- Papers and proposals make the same two key arguments
- Key arguments are
 - Research is exciting—important and innovative
 - Science is sound/ feasible; results are/ will be reliable
- Difference between future and past research require different ways of making these arguments
 - **Papers: make outcome seem uncertain → research seems more exciting**
 - Proposals: make outcome seem certain → experiments seem more feasible
- Leads to differences in two parts:
 - Research question
 - Background

Most writing that you will do as a researcher can be classified in one of two ways: writing about research that's already complete or writing about research that's yet to be done. Writing about work that's already complete includes primary papers, conference abstracts, and dissertations. The other type includes grant and fellowship proposals and thesis proposals. Some lessons in this course, on the writing process and general principles like organization and coherence, are applicable to any scientific writing, including writing about others' work, or even to non-scientific writing. In contrast, the lessons at the beginning focus on specific parts of papers and proposals. To reduce overlap between lessons, each lesson on a section covers both, e.g. the abstract of a paper and the specific aims page of a proposal.

Similarities between papers and proposals

- Divided into sections
- Tell stories
- Argue that work is exciting and valid

This organization of the writing course is possible because papers and proposals share some features in common. Both are separated into distinct sections: summary, explanation of why you did or will do the work, description of the work that was done or will be done. Also, both types of writing are narrative in nature—they tell a story, though papers may not necessarily tell a completely historically accurate one. Telling a story requires explaining the logic connecting one section or experiment to the others, for example, how the first result relates to your overall question. Most importantly, they both make the same arguments about the research they explain: that it's exciting and scientifically sound. Further, editors, reviewers, and study section members must first be persuaded that the work is significant and novel before they will care about soundness or feasibility.

Key difference: construction of argument

- Papers' known outcome works against excitement argument
 - Explain how outcome couldn't have been predicted prior to experiments to minimize this
 - Question instead of hypothesis
 - Introduction should justify the *question*, not the results
 - Include evidence that suggests other outcomes were possible
- Proposals' unknown outcome helps make excitement argument
 - Should argue against uncertainty to convince reviewers that you will get results
 - State hypothesis
 - All background supports hypothesis
 - Make excitement argument on other bases: unknown and innovation

Despite the similarity of the arguments in the two types of documents, the most convincing way to make these cases for work that has already been done differs from that for work yet to be done. The key (and perhaps obvious) difference between papers and proposals is that papers explain a finished project, while proposals justify experiments that haven't yet been completed. If the work has been done, the outcome is now known, which is inherently less exciting than an outcome that is not yet known. Thus, making the results of a paper seem as exciting as those in a proposal requires explaining why the experiments had to be done to determine the outcome—why it couldn't have been predicted from prior observations. In other words, papers should make the case that the results were *uncertain* before you did the work. Uncertainty comes across more clearly when the introduction doesn't state a hypothesis and gives evidence that suggests possible outcomes other than the paper's actual conclusion.

Defense of no-hypothesis guideline

Our recommendations for how to set up the argument of a paper may not fit with your perception of what constitutes a good paper. Omitting your hypothesis might seem to hide your reasoning, which you may fear would lead readers to wonder whether you had specific expectations for what your research would yield. However, following these guidelines will actually more accurately represent your logic by keeping you from overstating how certain you were of what you would find before you began your work. Another reason our no-hypothesis suggestion may be unexpected is that the introductions of many papers do only give evidence that supports the paper's conclusion and state hypotheses. Nonetheless, that this way of setting up the context for research doesn't prevent it from being published doesn't mean that it's a terribly effective approach. Since giving only background that supports your conclusion makes it seem as though you predicted your result, editors may think that others in the field could have predicted them as well. This is the reason the hypothesis-based introduction appears less often in high-impact journals; the research itself or the editor's knowledge of the field would have to convince her of how exciting the results are since the introduction wouldn't.

Whether you state your motivation for the work in a paper as a question or as a hypothesis might seem like a minor issue, since you can easily rephrase a hypothesis as a question by changing “we hypothesized that...” to “we tested whether...”. What really matters is the content of the introduction (and the summary of the introduction in the abstract), and that content affects which structure of that sentence makes the most sense. The introduction should explain why you did the work, that is, why the experiments were necessary to lead to the understanding your paper provides. If your results could have been predicted from what was already known, then the experiments have less value to the field. To convince your reader that your experiments were worth doing, you must convince them that outcomes other than your results were likely. This requires either including evidence that supports a model other than the one your results do or, as in the example at the end of this lesson, identifying what evidence would make your results predictable and pointing out that it doesn’t yet exist. Including this sort of argument naturally leads to a question; a hypothesis would seem unjustified following evidence that undermines it.

In contrast, the results of a proposal are inherently uncertain, so this case does not have to be made. Further, not only the results are uncertain—whether your experiments will yield conclusive findings is also uncertain. Since reviewers will only fund proposals that they are convinced will lead to publications, you must convince them that your project will get clearly interpretable results. One very effective way to do this is to say that you’re already fairly certain of what the conclusions will be: if you already know the likely outcome, then you can design your experiments in a way that will let you determine whether they confirm or refute it. Thus, proposals should state a hypothesis (or hypotheses, if you give one for each aim). This hypothesis should be logically supported, so all the background you give should lead to it.

Since you can’t use the uncertainty of your results to argue for excitement, you must make that argument on different grounds. Some parts of that argument will be based on similar premises as that in a paper—how your work addresses an important problem and how it will reveal something previously unknown. Both of these are crucial; to get funding or approval to do your research, those evaluating your proposal must be convinced that it’s worthwhile. Since these lines of reasoning are so standard, you’re probably familiar with the sorts of evidence you would use to support them (if not, see lesson 4, “Explaining rationale”).

Innovation in proposals

The remaining portion would not necessarily be included in a paper: how the research proposed is innovative. (The importance of this to evaluating a proposal even led the NIH to add a required Innovation section to the new R01 format.) This doesn’t mean that your proposed research must use an unpublished method or invent a totally new model for the process you study (doing such a thing would be risky, since reviewers would likely doubt your chances of success; for suggestions on what level of innovation is safe, see <http://funding.niaid.nih.gov/ncn/grants/cycle/part04.htm>). Instead, explaining innovation may be about a smaller degree of novelty in your concepts, methods, instruments, or interventions—as the NIH defines it (http://enhancing-peer-review.nih.gov/docs/application_changes.pdf, see page 4), it may involve refining or

improving something that currently exists, or applying an existing idea or approach to a new problem. Another way to approach the innovation argument is to explain how your research will lead to change—how will your findings alter the way future research in your field will be done? If your research is clinical, how will your work change the future of patient treatment?

| | Excitement | Soundness/feasibility |
|-----------|--|--|
| Papers | Significance of problem Uncertainty of outcome | Controls, statistics Consistency of results within paper and with others' |
| Proposals | Significance of problem Innovation—ideas or approach | Certainty of outcome Controls, statistics |

Another minor difference is organizational—the specific aims serves as both summary and introduction, since it must convince reviewers to fund a proposal independently of the rest of the document, and so must make the case that the project will have significant impact and is innovative. Thus, the lesson on “Explaining rationale” applies to both the significance and innovation parts of the research strategy section and to the specific aims.

Compare and contrast the following examples, which provide rationale for the same project in different ways:

Example 1 Proposal: *background supports hypothesis*

[Note: this example precedes the current NIH format.]

Abridged Specific Aims:

Eukaryotic innate immune systems act as effective barriers to infection by microorganisms. Understanding the mechanisms that bacterial pathogens employ to circumvent innate immune systems will improve our ability to control disease. Plants and animals use specific pattern recognition receptors (PRRs) to recognize conserved molecules of microorganisms (known as PAMPs). Plants have numerous PRRs that can recognize specific virulence proteins specifically present in pathogens (known as Avr proteins). Many Gram-negative bacteria use type III protein secretion systems to inject effector proteins into host eukaryotic cells. We have shown that a primary role for many *Pseudomonas syringae* type III effectors is to suppress innate immunity.

However, the enzymatic activities and the mechanisms that type III effectors use to suppress innate immunity are not well understood. Identifying the enzymatic activities of type III effectors and their substrates is essential to identify important components of innate immunity and to improve strategies to control bacterial diseases.

Our *long-term goal* is to elucidate the molecular basis for suppression of innate immunity by type III effectors. The objective of this application is to identify targets of the *P. syringae* type III effector HopU1, a mono-ADP-ribosyltransferases (ADP-RTs), and to determine its roles in bacterial pathogenesis. *The central hypothesis of the proposed experiments is that the targets of the HopU1 ADP-RT type III effector will be components of innate immunity.* We formulated this hypothesis based on the literature and on our research on other type III effectors as well as our preliminary data showing that HopU1 suppresses outputs of innate immunity. Recently, we have shown that HopU1 can use several *Arabidopsis* RNA-binding proteins as high affinity substrates in *in vitro* ADP-RT assays. Based on our preliminary data, one of these proteins, AtGRP7, plays a role in innate immunity.

Example 1 cont.

The Specific Aims of this application include:

2. Identify additional substrates of HopU1 and verify their involvement in innate immunity. Our *working hypothesis* is that the plant targets for the HopU1 ADP-RTs will be important components of plant innate immunity.

Selected parts of Background and Significance:

3.f. Type III effectors of plant pathogens can act as suppressors of innate immunity.

TTSSs of pathogens may be capable of suppressing plant innate immunity. For example, Jakobek *et al.* (98) showed that a virulent *P. syringae* pathovar, *P. s. pv. phaseolicola*, suppressed induction of defense-related mRNA and phytoalexins in bean that were separately induced by an avirulent (i.e., nonpathogenic due to triggering innate immunity) *P. syringae* pathovar and nonpathogenic *E. coli*. These early studies suggested the TTSS of bacterial pathogens was involved in defense suppression. More recent studies suggested that specific type III effectors altered innate immunity (97, 176). Recently, several *P. syringae* effectors have been identified as suppressors of the HR and other responses associated with defense (1, 14, 24, 52, 99, 100, 108, 124, 127, 145). A subset of these also has been shown to suppress other hallmarks of innate immunity (24, 33, 99). Some type III effectors are able to suppress basal defenses triggered by pathogen-associated molecular patterns. For example, Hauck *et al.* (82) found that transgenically expressed AvrPto in *Arabidopsis* suppressed the expression of a set of genes predicted to encode proteins that are secreted cell wall and defense proteins. Moreover, when *P. syringae* TTSS defective mutants were infected into transgenic *Arabidopsis* plants expressing specific type III effectors, these mutants grew to significantly higher levels than control strains suggesting that plant defenses induced by these mutants were suppressed by these type III effectors (33, 82).

3.h. Chloroplast RNA-binding proteins, glycine-rich RNA-binding proteins, and their possible involvement in innate immunity. As shown in the Preliminary Studies section, the *P. syringae* type III effector HopU1 ADP-ribosylates *in vitro* a subset of *Arabidopsis* CP-RBPs and GR-RBPs. GR-RBPs have long been known to be induced by abiotic and biotic stresses in several different plant species. These stresses include drought (20, 72), salinity (11), cold temperatures (50, 110, 130), circadian rhythm (28, 86), wounding (171), and pathogenesis (139). Relevant to this application is that a soybean GR-RBP has been shown to be phosphorylated in response to a bacterial Avr protein (168), which suggests that phosphorylation may functionally activate this protein to participate in an Avr-triggered innate immune response. Taken together, these reports suggest that GR-RBPs are involved in RNA metabolism and that they could affect these processes during abiotic and biotic stresses. We found that the type III effector HopU1 modifies the *Arabidopsis* GR-RBPs *AtGRP7* and *AtGRP8* (177) and recently we determined that an *Arabidopsis* T-DNA insertion mutant lacking *AtGRP7* is more susceptible to *P. syringae*. This suggests that *AtGRP7* functions in plant innate immunity and experiments described in this application will address this hypothesis.

Like GR-RBPs, CP-RBPs are not well understood. While there is not extensive evidence that abiotic and biotic stresses induce these proteins, CP-RBP mRNAs have been found to be elevated by salt stress (23). As shown in the Preliminary Data section of this application, several *Arabidopsis* CP-RBPs are ADP-ribosylated by HopU1. Interestingly, IpaH9.8, a type III effector from the animal pathogen *Shigella flexneri* that contains leucine-rich repeats, was recently shown to bind to a mammalian splicing factor resulting in the suppression of pro-inflammatory cytokines (150). This splicing factor, U2AF35, is an RBP with an RRM (186). The substrates of the HopU1 ADP-RT suggest a novel strategy utilized by bacterial pathogens to modulate plant innate immunity by indirectly affecting host RNA status.

From the RO1 application titled “Suppression of innate immunity by an ADP-ribosyltransferase type III effector” by Alfano JR, available at <http://funding.niaid.nih.gov/ncn/grants/app/default.htm>

Example 2 Paper: background leads to question

Introduction of paper published from above grant:

Many Gram-negative pathogens of plants and animals and other eukaryotic-associated bacteria use type III protein secretion systems^{1,2}. Type III protein secretion systems are molecular syringes that inject bacterial proteins called effectors into eukaryotic host cells to modulate host physiology. In animal cells, their activities alter specific host cell functions, including phagocytosis, proinflammatory responses, apoptosis and intracellular trafficking³. Much less is understood about the activities and targets of type III effectors from plant pathogens. The emerging picture is that many type III effectors from plant pathogens suppress host immune responses^{4,5,6}. Thus far, effectors that possess cysteine protease, tyrosine phosphatase and E3 ubiquitin ligase activities have been implicated in suppression of plant innate immunity^{7,8,9,10,11,12,13}; however, the enzymatic activities for most plant pathogen type III effectors that suppress innate immunity remain unknown.

Genomic investigations of *Pseudomonas syringae* pv. *tomato* DC3000, a pathogen of *Arabidopsis thaliana* and tomato, have identified greater than 30 effector genes^{14,15}. Among these, hopO1-1, hopU1 and hopO1-2 (formerly hopPtoS1, hopPtoS2, and hopPtoS3, respectively) are predicted to encode proteins that contain potential active sites of mono-ADP ribosyltransferases (ADP-RTs)^{16,17} (Fig. 1a). ADP-RTs are well-characterized toxins in animal pathogens, including two that are type-III-injected^{18,19}, but they have not been demonstrated to be important in plant pathogenicity. Furthermore, genes that encode ADP-RTs have been found in eukaryotes, but have not been identified in plants²⁰.

From Fu ZQ et al., A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity. *Nature* 447, 284-288 (17 May 2007)

Analysis of examples

Differences in the research question

The research questions posed differ greatly. The proposal takes as given that HopU1 is an ADP-ribosyltransferase and asks whether its targets are involved in innate immunity, while the paper assumes that type III effectors suppress immune responses and asks whether HopO1 and HopU1 are ADP-RTs. Why might the authors have portrayed what is known and unknown so differently? In the proposal (as in any proposal), they had broad goals: to identify HopU1's targets and to determine its role in pathogenesis. In order to convince the reviewers that they would be able to reach these, they had to know something about HopU1, and they had evidence that it acts as an ADP-RT.

However, in the paper they needed to address a narrower question—something that they could conclusively answer. They couldn't conclusively show that HopU1 suppresses innate immunity—they only show that plants deficient in one of its targets is deficient in innate immune responses, which doesn't directly address what HopU1 does. Therefore, here they use the ADP-RT finding as the endpoint rather than the starting point. This example illustrates how changing the research question between the proposal and the paper serves the goals of each: the research question of the proposal allows them a broad enough goal to encompass three independent aims, while that of the paper leads to a conclusive answer.

Not only do the questions themselves differ, they're phrased in nearly opposite tones. In the proposal, the authors state a hypothesis based on their own preliminary data.

Framing the purpose of the work this way projects confidence in their early results and in the chances that their proposed experiments will lead to firm conclusions. This confidence may lead reviewers to invest confidence in the researchers' ability to complete their planned experiments. In contrast, in the paper, they simply state that the enzymatic activities for type III effectors are unknown, then suggest that they may be ADP-RTs by mentioning that they have potential active sites. They go on to add that ADP-RTs are not known to be involved in pathogenicity of microbes that infect plants. This second piece of information suggests that these effectors might not be ADP-RTs, which shows why the research in the paper is necessary to determine their function. If the authors had stopped at the potential active sites, the reader might wonder why they bothered to do the experiments since ADP ribosyl transfer is their most likely function. Similarly, if they had hypothesized that function, that could suggest that they could have predicted their conclusion.

Differences in background

A final difference may seem to result from the difference in space allowed for the two types of documents, but because it contributes to the discrepancy in perceived certainty, it's worth discussing. The background and significance section includes several pieces of evidence supporting the hypothesis, while the introduction of the paper only has one. Obviously, an intro to a *Nature* paper has to be short, and perhaps the authors would have built a stronger case for type III effectors as ADP-RTs if they'd had more space. However, if one suggestion of the possible result ("predicted to encode" and "potential active sites" sound fairly uncertain) is sufficient to provide a direction, there's no need for more evidence. A longer introduction could instead explain more thoroughly why the problem is important and give more detail about how other effectors of plant pathogens suppress immune responses. Again, we can draw conclusions about how to use background information to support different arguments in each document. Using more background in favor of the hypothesis in a proposal will better convince the reviewers that your work will lead to a definite conclusion, and including a minimum of evidence for your conclusion in a paper will make the research seem more exciting.