How to Write a Research Manuscript

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This article provides a step-by-step guide to help you turn your high-quality data into a high-quality manuscript for publication in a scientific journal. It covers all aspects of the writing process, including: choosing a journal to which to submit your paper, writing each section, formulating your "story," making figures, soliciting constructive criticism, and navigating the review process. © 2018 by John Wiley & Sons, Inc.

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INTRODUCTION

You've spent days, weeks, months, years in the lab, toiling away at a project. You did experiments, repeated experiments, chased down dead ends, struggled with difficult techniques, developed new methods, remade all your solutions after that contamination incident, tested hypotheses, proved them wrong, developed new ideas, produced data supporting a hypothesis, and now feel that you have learned something about biology that no one knew before you entered the lab. As fantastic and intellectually fulfilling as this is, if you don't publish your work, it simply doesn't exist. You've likely heard the phrase "publish or perish" applied to a researcher's career, but it applies equally well to the work itself. To have an impact on the knowledge base of your field, your work must be published in a peer-reviewed scientific journal.

So, how do you go about the monumental-sounding task of writing a scientific paper to raise your work from the morass of—hopefully well organized and detailed—laboratory notebooks? More importantly, how do you do this job well enough that your work can be appreciated by current and future scientists? This chapter aims to guide you through this process.

Let us first consider the qualities of a good paper. Obviously, the science must be sound (i.e., the evidence convincing, all the proper controls performed, etc.) and the work must be novel—science is, after all, about discovery. I will assume that you, your coworkers, and your mentor have already seen to this, and proceed from that foundation. A high-quality manuscript is:

1. *Clear:* As an author of a scientific paper, you should not aim to emulate the beautiful language of Marcel Proust or Maya Angelou. As art, literature aims to stir the soul of the reader and allow the reader to come to her own conclusions about what the work means. In contrast, scientific manuscripts aim to simply tell the reader the question that was asked, why it was interesting, the approach taken, the results, the conclusions, and how the author interprets those conclusions. To write clearly,

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you must always focus on the reader, who does not share your intimate knowledge of the facts. She may work in an unrelated field and be unfamiliar with the fundamentals of your field—knowledge so ingrained in you that you can't remember a time when you didn't know it. She may not know the big questions in your field or the jargon you use on a daily basis. But even if she is an expert in your field, she does not do the exact same work as you, nor is she in your lab at the next bench. You must carefully lead your reader through your work so that she understands its broader context (what was known already), the research question (why you did this work), the rationale (why your approach to answering the question is reasonable), the methods, the results, the interpretation of the results, and how this new knowledge affects our understanding of the field.

2. *Concise*. Readers of scientific papers are busy scientists who, generally, lack the time and inclination to read excessively long papers. Thus, you must convey your points as succinctly as possible.
3. *Compelling*. You must convince your reader that your paper is worth the time he will spend reading it by convincing him that your research question is interesting, your approach is appropriate, your data are convincing, and your conclusions and interpretations are reasonable. Additionally, your paper should be presented logically so that, together, the experiments tell a compelling story.
4. *Complete*. Your paper should include all of the information the reader needs to understand your experiments and your conclusions.
5. *Consistent*. A scientific paper is not the place to show off your exceptional vocabulary. If you use multiple terms to describe the same thing, your reader will think you meant multiple things. Choose simple terms and use them throughout your paper. Likewise, choose a logical style of presenting your data and stick with it throughout your paper.

WHEN TO START WRITING THE MANUSCRIPT

Although many scientists do not start writing their manuscript until they have completed all the experiments, I argue that you should start much earlier. A good time to start is when you have the data for two or three figures and a good sense of the direction the paper will go. This approach has several advantages. First, if you have the data for two or three figures, you have all you need to start writing! As I describe below, you can start by making a figure and then writing the accompanying figure legend and methods and results sections. As you accumulate more data and make more figures, you will slowly build up the main components of your paper. Second, you will often have "down" time in the lab—waiting for cultures to grow, gels to run, or seeds to germinate. During this time, you can start working on your paper. Third, starting to write your paper while doing experiments will allow you to maintain some productivity at the bench. Fourth, while doing experiments, those you are writing about will be fresh in your mind, so interpreting your notebook will be much easier than if you wait until all of your experiments are done, which could take several months to a year. Fifth, as you start writing, you may identify breaks in the logical flow of your paper that can only be filled by doing another experiment or control. If you have completely stopped doing experiments, it may take some time to get the appropriate cells or organisms growing, make the necessary solutions, purchase and receive a reagent that you thought you would never need again and thus didn't re-order, etc. Finally, the writing process will force you to read or re-read the literature and think about your work in a different, and more comprehensive, way than you do while conducting experiments. Thus, you may come up with a new experiment or perhaps even a completely new direction for the paper. It will be much easier to conduct these experiments if you have continued working at the bench.

AS YOU START WRITING

When you and the Principal Investigator (PI) of your lab are reasonably confident that you have obtained sufficient data for a paper, begin to think about and discuss authorship, where to submit your paper, and how to frame your story.

Authorship

Discuss with the PI of your lab who will be authors on the paper and the order in which their names will appear. Of course, the number or order of authors may change if you add or remove experiments from the paper. Nonetheless, scientists are people with feelings, and hurt feelings (yours or others') can be minimized if you discuss authorship early, when the stakes are low, instead of waiting until a week or two before submitting the paper. In the simplest and rarest case, a single person (1) thought of the question, (2) designed the experiments, (3) carried out the work, (4) interpreted the data, (5) generated new reagents, (6) considered how the work fits into the field, and (7) wrote the paper. In most cases, these duties have been shared by multiple people and possibly two or more labs. Although the question of authorship can raise many thorny ethical issues beyond the scope of this chapter, here are a few guidelines.

1. Authors should include anyone who participated significantly in any of the seven activities listed above.
2. The first author usually contributed the most to these activities. If two people equally deserve to be first authors, their names can be listed with a footnote stating, "these authors contributed equally."
3. The senior author—generally the PI of the lab—is usually listed last. In some cases, the senior author contributed so much more than anyone else that he or she is listed first.
4. Middle authors are often listed in descending order by the size of their contributions. If many people made small but equal contributions, their names can be listed alphabetically.
5. The corresponding author usually handles the submission process, and once the paper is published, serves as a contact to address questions from readers about the data or methods or to fill requests for reagents generated by the study. The corresponding author should be the person most likely to possess those reagents in ten years—usually the PI.
6. Someone who provided a published reagent (e.g., antibody, cell line, mutant organism, etc.) should generally not be an author, but should be mentioned in the Acknowledgements section.
7. If two or more labs collaborate on the paper, the PIs should decide as early as possible how authorship will be designated.
8. Many journals require that you specify each author's contribution, so read the instructions to authors.
9. Finally, the International Congress of Medical Journal Editors recommends that each author on a paper should know who was responsible for which aspects of the paper and be confident in the integrity of the work done by their co-authors, should be willing and able to be accountable for the integrity of their own work, and should be willing to resolve questions of integrity. See *https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html* for details.

Where You Will Submit Your Paper

Selecting a target journal early can help with the writing process, but need not be set in stone. The act of choosing a journal will force you to think carefully about the story you are planning to tell and how to make sure that your work gets the widest possible audience. Moreover, knowing your target journal will give you a format to follow and papers on which to model your own. As you consider where to submit your paper, ask yourself the following questions:

1. *What is your topic?* Choose journals that publish work similar to yours. Most journal websites include a description of the scope of work they publish. You can also search PubMed (*https://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed*) using keywords from your paper to identify journals that have published work related to yours in the last five years. If a particular journal rarely publishes papers on your topic, don't send your paper to that journal. Because the editors probably lack expertise in your field, they won't understand why your work is interesting or know how to critically evaluate your work, and they may do a poor job of choosing appropriate experts to review your paper. Although it is possible to get your paper published in a journal that doesn't regularly publish on your topic, it will probably be an uphill battle, and if you do succeed, your paper will be in a journal that may not be regularly read by the people you most want to see your paper—i.e., those who work on your topic.
2. *What are the main methods used in your work?* For example, if your work consists of protein purification and measurement of binding kinetics, it will be most appreciated in a journal that focuses on biochemical analysis and will likely not be well received in a more cell biologically oriented journal. Conversely, if you have a lot of images of immunofluorescent labeling of cells, your work is better suited to the *Journal of Cell Biology* than the *Journal of Biological Chemistry*.
3. *How interesting will your work be to those outside your field?* If you're working on a "hot" topic or you've uncovered something novel that you think will change how the scientific community views your topic, then you will probably want to submit your paper to a general-interest journal (e.g., *Science*, *Nature*, *Cell*, *Current Biology*, *eLife*). Keep in mind, however, that many of the "top" journals make editorial decisions before review and turn away many excellent papers without review. Some journals allow you to request editorial feedback—called a "pre-submission inquiry"—which can save time and heartache. Generally, your paper should be near ready to submit before you ask for editorial feedback.
   If your work is high quality and likely to be of interest to most scientists in your broadly defined field (e.g., evolutionary biology, epigenetics, cell biology, plant ecology), then you may want to consider a slightly more specialized journal (e.g., *Genes and Development, Plant Cell, Nature Cell Biology*, *PLOS Genetics*).
   Finally, if your work is likely to be of interest mainly to researchers in your specific field, you should choose a field-specific journal (e.g., *Molecular Human Reproduction, Journal of Neuroscience, Endocrinology, RNA*).
   You also need to critically ask yourself how mechanistic your results are. The "top" journals tend to have a—some might argue maddening—disregard for work they deem "too descriptive." Such work might include describing a new phenomenon, careful analysis of a particular process in normal development, or identification of proteins that localize to a specific subcellular structure. Despite adding valuable scientific knowledge, the work might be deemed "descriptive" if it doesn't provide some mechanism for the phenomenon, experimentally perturb the developmental process to test a hypothesis about how it works, or demonstrate something about the functions of proteins identified in a subcellular structure.
   A researcher's view of the impact of his or her work is often more positive than the opinion of the general scientific public. Thus, you may find it hard to critically consider which journal your work belongs in. A common method, therefore, is to aim one level higher than you feel you would be satisfied with. If the editor chooses not to review your paper, you will be able to quickly reformat it and submit it to another

journal. Some publishers will offer to automatically transfer your paper to another, lower-tier, journal within their "family" of journals, which can save you some time. If your paper is reviewed by the first journal to which you submit it but is rejected, you can use the reviewers' feedback to help you improve the paper before submitting to a more specialized journal. This takes a lot of extra time, so consider this option carefully. You or your PI may want the paper published in time to be listed as "in press" on a curriculum vitae for a fellowship or grant application. Once you finish writing your paper, ask colleagues to read it and give you an honest assessment of where you should submit your paper.

4. *Short or long format?* Look at the instructions to authors of the journals you are considering and pay particular attention to the page and figure limits. Many journals, such as *Science, Nature*, and *Proceedings of the National Academy of Sciences,* place severe restrictions on the length of articles. Many journals publish both long and short formats, often called "article" and "report," respectively. If you are considering submitting a short format, make sure your work can be sufficiently condensed. For example, if you are planning seven figures, you might need to submit in a long format, whereas three or four figures might fit nicely into a short format. Some researchers first write and submit a long-format paper, realizing that the editor may ask them to condense it into a short format after it has been reviewed. This is often accomplished by putting some of the figures into supplemental material maintained on the journal's website.
5. *Do you and your PI respect the journal?* Unfortunately, the rise of open access publishing has led to a proliferation of "predatory" journals that charge for "services" but have poor practices—e.g., provide extremely cursory reviews of papers, or list faculty members as editors who have not agreed to serve in such roles. These journals often have legitimate-sounding names and professional-looking websites. When in doubt, consult your university library or "white lists" such as the Directory of Open Access Journals (*https://doaj.org*), which lists journals who adhere to "principles of transparency and best practices in scholarly publishing." Your hard work deserves to be published in a reputable journal!

Once you choose a journal, carefully read the "Instructions to Authors" on the journal's website, where you will learn essential information including the following:

1. *Structure:* Journals have varying organizational styles for their papers. Some want a separate discussion section, whereas others require that it be integrated with the results section. The organization of long- and short-format papers might even differ in the same journal. Some journals have specific requirements for the abstract and introduction, such as a strict word limit or a structured abstract. Also, pay attention to the order in which the sections of the manuscript should be presented.
2. *Figures:* Pay careful attention to the file format required for figures and the computer software that is acceptable for figure construction. If you will be using color, check whether the journal uses RGB (red, green, blue) or CMYK (cyan, magenta, yellow, key [black]). If you submit in RGB and the journal uses CMYK, the resulting colors may not match what you had intended. Note that journals have size limits for figure panels and font size within figures.
3. *Reporting requirements:* To improve rigor and reproducibility of scientific work, many journals have specific requirements or checklists for reporting how experiments were conducted. Reading these early may help you design better experiments and keep complete records on your experiments.

What Your "Story" Will Be

A good paper does not chronologically recount the experiments you did, but, rather, it tells a story. An enthralling piece of fiction makes the reader care about or be interested in the characters, presents the motivations of the characters, develops the plot, and leaves the reader with a sense that she has learned something deeper about humanity. Similarly, an effective scientific manuscript convinces the reader that the broad biological question addressed is interesting, defines the specific question being addressed, describes the experiments and data that lead to the authors' answer to that question, and explains to the reader how this work has helped us to understand something new about the broader biological question. Deciding what your story will be is thus the most important, and often hardest, part of writing a paper. To convert your notebook full of data into results that can be easily understood by someone who is completely unfamiliar with your work, you should make notes or an outline to accomplish the following.

1. *Identify the specific question your paper addresses.* This question motivates the whole paper. If you don't clearly define the question, neither will your reader. He will be confused, try to guess what the question was, and judge your paper according to whether or not your findings satisfactorily answer the question as he sees it. Keep in mind that the question motivating your paper may not be the question you set out to address. Your understanding of the biology will mature during the course of your research, and your results may turn out differently than you anticipated. Additionally, the exact question may change over time as you write your paper.
2. *Determine the "punch line" for your paper.* What main conclusions from your data do you want to get across? How do your data and conclusions answer the question you posed? Write down your main conclusions, and then determine the figures you want to show to illustrate these conclusions. These will form the main plot of your story.
3. *Think about the order in which you want to show the figures.* This will likely NOT be the order in which you did the experiments. Don't get too hung up on this, as you may find during the writing process that it is helpful to change the order of figures so that your story maintains a logical flow. One logical flow that works well for many papers is to start with the big picture, such as the phenotype seen in your organism (e.g., the mutant mice have heart defects), then move to the more specific (e.g., experiments addressing the mechanisms by which the gene in question functions in heart development). This method hooks the reader with something they can easily relate to before diving into the details of genes and pathways they may be unfamiliar with. Another approach is to systematically describe how you tested and ruled out each hypothesis to describe a phenomenon before getting to the hypothesis best supported by your data. Finally, you may describe a new method you developed, show the experiments validating the method, and then describe your use of the new method to test a hypothesis.

WRITING YOUR PAPER

Writing a paper is hard work, but it can also be fun, educational, inspirational, thought-provoking, and rewarding. Through the writing process, you will likely gain a much deeper understanding of your research and how it fits into the current body of knowledge. Getting there can be tough, however, so take to heart two tricks to make the process less daunting. First, don't obsess about making the first draft perfect. Focus on writing a complete draft—you can do all the hard revising, rearranging, and editing later. Second, don't think of the paper as an enormous obstacle looming before you. Break it down into small bits and do the easiest and most fun parts first, saving the more difficult parts for the end. In this way, you will fool yourself into starting the paper, and the process will be easier and take less time than if you start with the more challenging parts. The order suggested below starts with the parts of the paper that should be easiest to prepare, and will help you to assemble a skeleton of your manuscript (each part will be discussed at greater length later in the next section).

1. *Figures.* Making the figures is often the most enjoyable part of writing a paper and is essential to framing the manuscript. Making figures may also be the easiest way to get started, as you will likely already have parts of figures on your computer from presentations you have given. As you are making each figure, think about how it fits into the story you are trying to tell in your paper.
2. *Figure legends.* Writing the legends forces you to articulate why the figure exists. As you initially describe each figure, you will likely find yourself writing text that you will later realize belongs in the results section, so you'll already have something to work with when you get to that section.
3. *References and Acknowledgements.* Fill these in as you proceed through steps 4 through 8.
4. *Materials and Methods.* Write this section while making each figure. If you wait until later in the writing process to write this section, you will likely dig into your notebook twice, once to find the data to put in the figure, and again to write the methods. It's most efficient to do it all at the same time.
5. *Results.* Again, write this section while making each figure. After having written figure legends and methods, you should have a good idea about what you want to say about each figure.
6. *Introduction*. This is one of the first sections of your paper that a reader will encounter, but I suggest saving it until nearly the end of the writing process for two reasons. First, as discussed in "Putting it All Together," your idea of the specific question addressed by your data may change as you write the paper, requiring you to refocus the Introduction. Second, thinking about and describing all of your data in the Results section will help you identify the background information the reader needs to know to understand your data and its significance.
7. *Discussion.* While writing your Introduction, you will read or reread many papers that relate to the question your work addresses. As you do, your understanding of your field will deepen, and you will then be prepared to write a Discussion explaining how your new work fits into, clarifies, expands upon, or challenges the current thinking.
8. *Abstract and Title.* As these provide brief synopses of your entire paper, any time spent worrying about them before you have your story completely worked out will likely be wasted.

Figures

A picture is worth a thousand words, right? Thus, well-made figures should allow a knowledgeable reader to understand the main points of your paper without reading a single word of text. Toward this end, design each figure so that it obviously reveals the essential points; the reader should not have to work to understand what she is looking at. When you finish making your figures, ask a colleague to look at them and tell you what she thinks each demonstrates. If she is unsure or comes to an incorrect conclusion, your figure obviously needs more work. The tips below should help you make sure that each of your figures is worth not just a thousand words, but the *intended* thousand words.

Before you start making your figures, reread the instructions to authors on the journal's website thoroughly, paying attention to size limits, required font sizes, acceptable programs and file formats (TIFF or PDF are the most common formats for submission), and color format.

Computer program

Choose a program that you will use to make your figures. Good choices are Adobe Photoshop, Adobe Illustrator, or ACD Canvas. All of these programs handle images well without losing information. Each program has its own advantages and disadvantages—it really comes down to which one you are most comfortable with. In general, avoid making

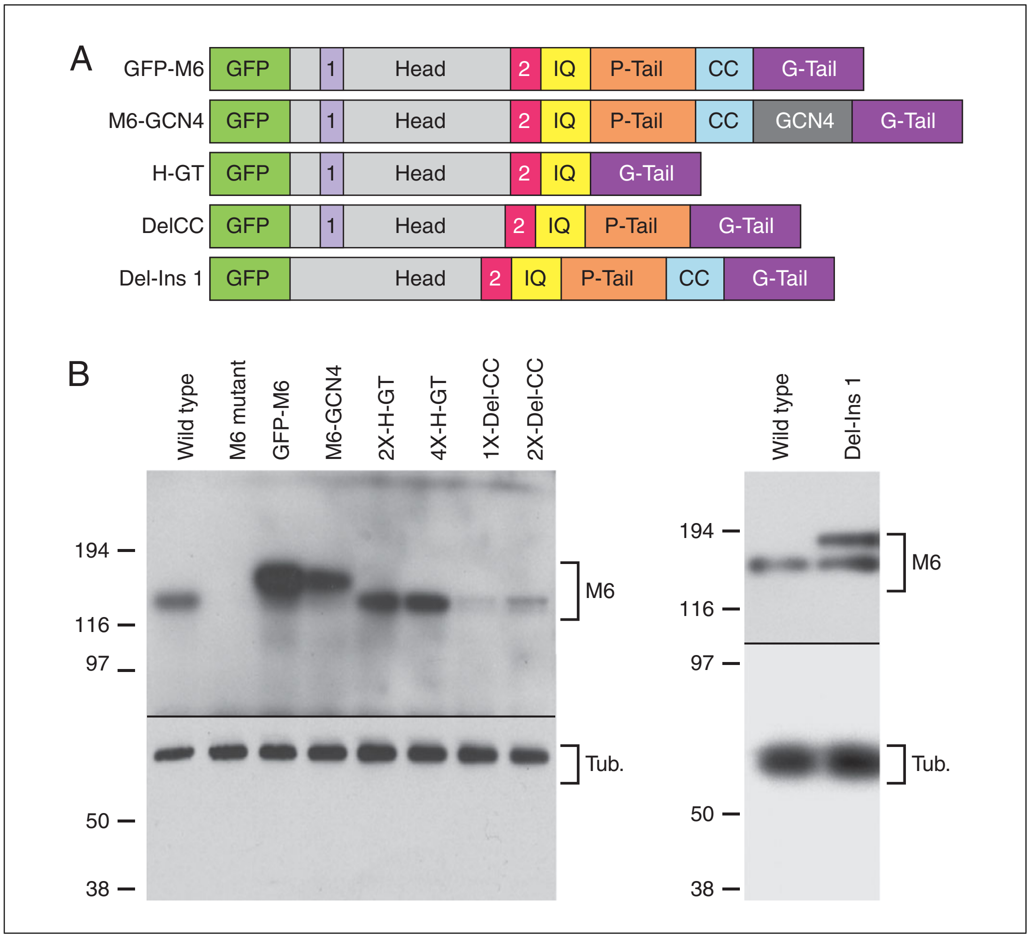

Figure 1 Myosin VI molecules used in this study. (**A**) Schematic diagram of constructs. GFP, green fluorescent protein; Head, motor domain; 1, 2, inserts found uniquely in class VI myosins; IQ, IQ motif; P-Tail, proximal tail; CC, core coiled coil; G-Tail, globular tail; GCN4, leucine zipper dimerization domain. Drawings are not to scale. (**B**) Western blots of testis extracts from flies expressing the indicated myosin VI molecules. The top halves of the blots were probed with polyclonal anti-myosin VI antibody and the bottom halves with anti-tubulin (Tub.) antibody. 1×, 2×, and 4× indicate the number of copies of the indicated transgene. Except for wild type, all on the blot on the left are in a myosin VI mutant background. Those on the blot on the right are in wild-type background, so both endogenous and exogenous myosin VI are evident. Sizes are indicated in kDa.

your figures in PowerPoint, as information will be lost in the figures if they are converted into TIFFs, and many journals will not accept PowerPoint files. Gallagher & Newman (2015) discuss important considerations when modifying figures, and Sedgewick (2017) describes actual manipulations.

Scope of a figure

In general, each figure should address one question in your paper and only contain information that is discussed in one section of the results. This way, your reader can read about the data in Figure 2, look at Figure 2, then move on without ever needing to return to Figure 2. In some cases, this may not be practical. For example, suppose you are describing western blot, immunofluorescence, and electron microscopy analyses of wild type and two mutants. Your text may describe the data from all three types of analyses for wild-type, then describe all three for mutant 1, followed by mutant 2. However, it may make the most sense to combine all the western blot data in one figure, the immunofluorescence images in a second, and the electron micrographs in a third.

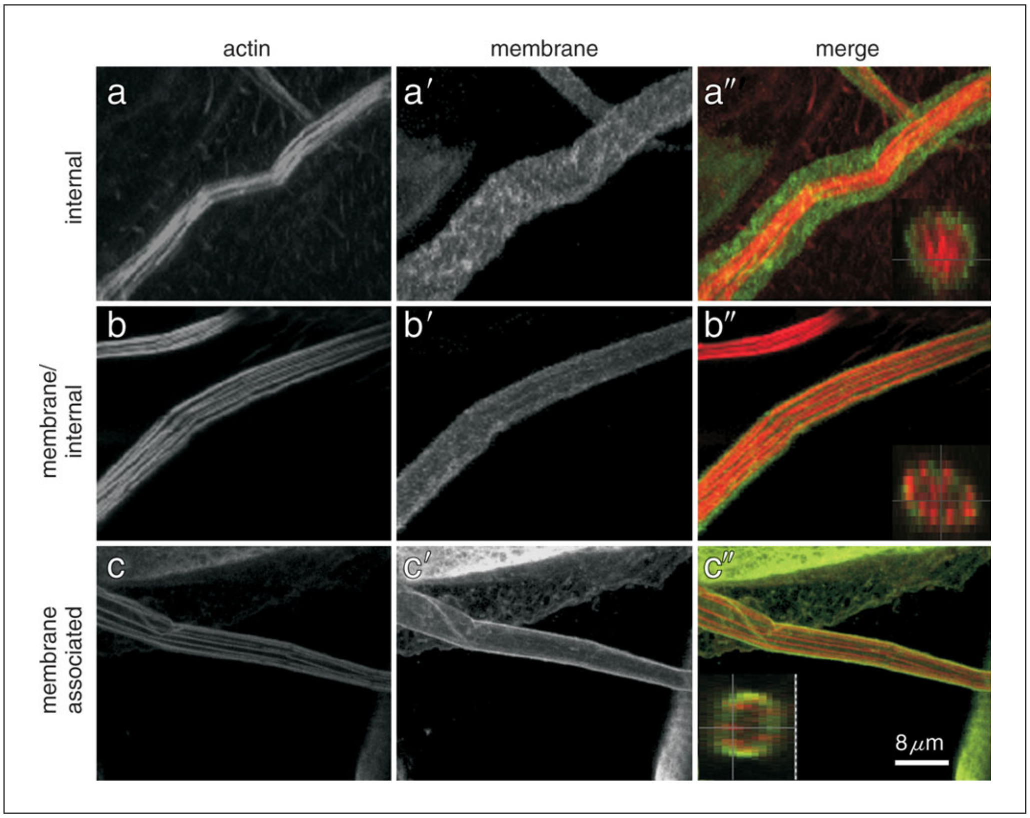

Figure 2 Range of actin displacement phenotypes observed in the *capping protein, arpc1* double mutant bristles. Each image is a projection of optical sections. Insets are computed cross-sections. Scale bar does not apply to insets.

Labels

Figure labels should be comprehensive enough that the reader does not need to read the figure legend to understand the figure. For example, a western blot with eight lanes should have words above or below each lane indicating the lane's content, as opposed to each lane having a number at the bottom referring the reader to the figure legend (see Fig. 1 for an example). Similarly, micrograph panels should be labeled with the genotype, type of labeling, etc. For example, if you show DNA staining and two different antibody labelings for each of three genotypes, arrange the panels in a three-by-three grid with each genotype in one column and each type of labeling in one row. You then only need to place a label at the top of each column and to the left of each row (see Fig. 2 for an example). If your figure has several panels, you might include a letter in each panel that can be referred to directly in the text. Consult the journal's instructions to see if they have specific requirements for font and type size. Make sure that every figure has identical font and type size.

Sizing

Make your panels big enough to see easily, but small enough that the figure as a whole will be an appropriate size for the journal. Make the figures the size you want them to appear in the article, rather than making them big and relying on the journal to resize them for publication. The journal's instructions to authors should give you guidance on figure size.

Cropping

Crop out extraneous parts of images, but don't crop too much. If you are showing a western blot, show at least a half-inch above and below the band(s) in which you are interested. If you crop too close to the band, a skeptical reader will wonder what else was in the lane that you are trying to hide. For micrographs, you may want to show one image that encompasses a large region of the sample (for example, an entire cell or field of cells) with an inset that shows the portion of interest (e.g., the nucleus) at higher magnification.

Image choice

You should have collected tens or even hundreds of pictures documenting your results. But in your paper, you will only show one or a few of them. Although it may be tempting to show the most extreme examples of a phenotype you observed, aim for showing one that is representative of the group as a whole, i.e., choose an "average" image. If you found a wide range of phenotypes, you could show three images arranged from least to most severely affected.

Image manipulation

We all know that currently available imaging software can be used to manipulate images in almost any way, but only some are ethically acceptable. In general, adjustments applied equally over the entire image are OK. For example, changing the brightness and contrast of the entire image is acceptable; changing the brightness and contrast of only a particular region of the image is not. The "smudge," "eraser," and "paintbrush" tools are completely off limits! Cropping an ugly black spot out of your northern blot is completely unacceptable, even if you are convinced it is an artifact, unless it is outside the area you are using for the figure (see "Cropping," above). Either leave the blemish in, or repeat the blot. For gels, it is best to run all the samples you want to show on one gel. However, if you need to combine three lanes from one gel with two lanes from another, make sure that the break point is clearly visible so the reader knows this is what you did. If scientific integrity alone fails to motivate you, note that some journals use software that can detect inappropriate image manipulation. Furthermore, some journals will require you to submit raw images along with those included in the figures. See Gallagher & Newman (2015) for further discussion of this topic.

Self-plagiarism of images

In general, you should not publish an image used in a previous paper, not even a control or wild type. A careful and skeptical reader who has read all of the work from your lab may notice that you reused an image and wonder why you were unable to obtain another one. To avoid such a credibility gap, the best option is to take the time to acquire another image. However, if doing so would be an exceptional burden (for example, if it requires booking time on a specific machine months in advance or spending a large amount of money in preparing a sample), you can request permission from the previous journal to use an image again. A publisher is unlikely to deny such a request, though processing may take a few weeks. If you use a previously published image, make sure you indicate this fact and cite the original paper.

Color usage

Read the journal's guidelines about the use of color in figures. In addition to learning whether the journal uses RGB or CMYK, you will find that some journals charge extra for color, which might cause you to avoid color if possible. However, color can be enormously helpful to the reader, especially when used consistently between figures. For example, if several figures or panels within one figure contain graphs comparing four experimental conditions, use black for condition A, grey for condition B, magenta for condition C, and blue for condition D in every graph. When presenting fluorescence micrographs, keep in mind that up to 8% of men of Northern European ancestry are red-green colorblind. If you present red, green, and merged images, consider presenting the red and green images in grayscale, with only the merged image in color. In general, the contrast of grayscale images is better than color images, so you will improve the quality of your images for non-colorblind readers as well.

Scale bars, arrows, and other inserted elements

Micrographs and photos should have scale bars. If all images within one figure panel are the same size, you can include a scale bar in only one of the images (for example, see Figure 2). Make sure that the scale bar is in a color that is easy to see against the image. Try both black and white to see which works better. If its not too intrusive or prohibited by the journal, include the size of the scale bar (e.g., 2 μm) so the reader can find the information without reading the figure legend. If you refer to particular aspects of an image in the Results, use arrows and arrowheads. The arrow point should be close to—but not quite touching—the object it's aimed at. If you need more than two types, you can use open and closed arrows and arrowheads. Again, try black and white to see which stands out best against the image. Size markers for gels should be indicated with the appropriate number. If you wish to call the reader's attention to a particular band, place an arrow (and accompanying label) to the side of the gel pointing at that band. Electron micrographs commonly include one- to two-letter abbreviations denoting each part of the cell (e.g., mi for mitochondria).

Graphs

Although bar graphs are still commonly used, many journals request that authors instead use dot plots or box-and-whisker plots because they more clearly indicate the number of data points or samples and the range of values. If you don't show a dot plot, indicate the sample sizes in the figure or figure legend.

Figure Legends

As you make your figures, write the figure legends. As noted above, if your figures are properly labeled, the legends will be fairly short. The legend should start with a title that states the result (conclusion) demonstrated in that figure. The figure legend titles in your paper should all be either sentences or phrases, not a mix of the two. If the figure has multiple panels, at least one sentence should describe each panel. Your goal is to simply describe what the reader is seeing (e.g., "The top half of the blot was probed with anti-myosin II antibody"), not provide details of the method (e.g., do not include antibody dilutions or amount of extract loaded) or your conclusion from the experiment. If you included arrows, the legend should state what the arrows indicate. State the size of the scale bar if it is not indicated in the figure. If you label parts of an image with abbreviations, define them in the legend alphabetically (e.g., er, endoplasmic reticulum; gm, golgi membrane; mt, microtubule). If you include any summary data, indicate the number of replicates. If your graphs include symbols such as asterisks to denote P-values, define them in the legend (e.g., "*$P<0.05$,**$P<0.01$").

Tables

Tables may seem like figures, but to most journals, they are part of the text. Tables should not be made in a graphics program, but rather should be part of the text document. It may be easiest to make tables in a spreadsheet program, then transfer them into the word processing document you are using for the text. Pay attention to the journal requirements for table sizes and formats. Use footnotes to define abbreviations in the table.

References and Acknowledgements

Software

If you don't currently possess reference managing software such as EndNote, Mendeley, or Zotero, check with your PI to see which one he uses and then buy or download a copy immediately! Each journal has specific reference formatting requirements, and these programs will automatically format your references according to the journal's style. Moreover, the software can quickly reformat your references to another style if you must resubmit your manuscript to a different journal. Reference libraries can easily be shared by authors co-writing the paper. Finally, if you get used to using the software, it's a great way to organize all the papers in your reference collection. The most recent versions of reference managers allow you to include attached PDFs, selected images, or your own notes. If you prefer to read articles printed on actual paper, you can organize your hard-copy collection by senior author or the unique number given to the paper by the program. Then, if you are trying to find a particular paper but do not remember the author, you can search your virtual library with information you recall (e.g., keywords "actin" and "*Drosophila*") to find the reference. Once you know the senior author or unique number, you can quickly pull the hard copy out of your file drawer. Trust me, once you start using one of these programs, you'll never regret it.

When to insert references

Although it is a matter of preference, I suggest you insert references as you write, rather than wait until you are done writing the manuscript, for the following reasons: First, while you are thinking about a certain issue that you want to write about, you can find the appropriate reference, reread it, and make sure it is the correct reference to use. Second, if you wait until the end, you may forget which reference you were thinking of, in which case you will need to spend time looking for it. Third, if you cite the reference incorrectly because it turns out the paper doesn't say quite what you thought it did, you can fix the logic of the text right away. Fourth, if you simply use a placeholder such as "ref," your co-authors and others who read the paper for you won't know which reference you intend to use. The downside to inserting references as you go is that it can break up your writing flow.

What types of references to use

Never cite a paper that you have not read. Make sure the paper really shows what you claim it shows. Cite original sources of information as much as possible. You owe it to the researcher who did the work to cite his or her paper, rather than a later paper. Imagine how you would feel if, ten years from now, you read a paper that describes work published in your paper, but cites a paper that references a paper that references your work! You will not be getting the credit you deserve. It's also extremely frustrating for a reader to look up a reference only to find that the information cited is actually in an even earlier paper. Although citing review articles instead of the original work has become common, try to cite original work as much as possible and only cite reviews when necessary. If you write a statement that refers to a huge body of work, then cite a recent review. For example, if you state, "Chromatin marks are often used for epigenetic regulation of gene expression," cite a review. But if instead you comment on a particular methyl transferase that modifies a certain amino acid, cite the original paper showing those data.

Politics

You know your field and who is likely to read—and perhaps even review—your paper. Although you cannot and should not cite every paper these people have written, a good way to anger a petty competitor is by failing to cite her paper. Your mentor has likely received at least one e-mail saying, "I enjoyed your recent paper. However, I wonder if you'd seen our paper . . . " Your job is certainly not to mollify all your competitors, but do be mindful to honor their contributions where appropriate.

Acknowledgements

It is best to write this section as you are writing the rest of the paper to avoid forgetting someone who should be acknowledged. You need to thank people who performed purely technical work but did not receive authorship, those who sent you reagents, anyone who gave you a pivotal suggestion, and the people who spent time critically reading your manuscript before submission. Finally, you must cite the source(s) of your research funding.

Materials and Methods

While you are making your figures and writing your legends, you will be actively digging through your old notebooks, making this the perfect time to write the materials and methods section. The goal of the methods section is to present what you did in enough detail that a knowledgeable reader could repeat your experiments. At the same time, brevity is an asset.

What can be omitted

Many protocols are quite standard, so you can often refer to protocol collections, such as this one. If your lab has previously published the use of a particular method, you can refer to that paper. For example, you can state, "Cell lysates were prepared as previously described (ref), with the following exceptions: . . . " However, make sure that the paper you are referring to does not itself refer to an even earlier paper for the method. Additionally, some journals require all methods to be described, even if they are identical to previously published methods. If you used a kit, you can state that you followed the manufacturer's instructions.

What must be included

Include a description for each type of experiment presented in your paper. As much as possible, these should be listed in the same order in which they are shown in the paper.

Western blots and immunohistochemistry experiments: Include the name, source, and concentration of the antibodies used. For protein gels, indicate how you generated the extract and how much was loaded in each lane (e.g., equivalent numbers of cells, nanograms of tissue, or numbers of fly heads). Describe the method (or kit) used to detect signal and the image capture and quantitation methods.

Plasmid constructs: Cloning methods for generating constructs are fairly general, so you need not go into extreme detail. Make sure, however, that you indicate the precise regions of any gene that you include, referring to a sequence published in a paper or in GenBank (*https://www.ncbi.nlm.nih.gov/Genbank/index.html*). Give a general sense of how you generated the construct—e.g., PCR followed by sequencing, or standard molecular biology techniques using restriction enzymes. PCR primers should be listed, and they should be depicted in a 5′-to-3′ direction. Provide names of all constructs generated, including any intermediate vectors, so that a reader will be able to request them in the future. If you have cloned a gene whose sequence is not already available in public databases, you should deposit the sequence with GenBank and provide the accession number in your paper (also see Chang, Zaila, & Coppola, 2016).

Mutant organisms: Provide the community-accepted allele nomenclature for each mutant used. If your paper includes numerous mutants, a table can be a useful way of displaying the data. If the paper introduces new mutants, describe how they were generated.

Northern and Southern blots: Indicate how RNA or DNA was isolated, only providing details if you did not use a standard method. Describe any hybridization probes used by providing nucleotide numbers relative to a published sequence. If oligonucleotide probes were used, include the sequence.

Microscopy: Indicate the make and model of the microscope used as well as the lens power, numerical aperture, and image-acquisition software. Describe how images were manipulated post-capture.

Materials: If a reagent is mentioned, indicate the company from which it was purchased as well as the city in which that company resides. For example, “Extracts were applied to a 0.5-ml anti-Flag resin (Sigma, St. Louis, MO) column.” If a reagent was provided by another researcher, include their name and an appropriate reference (and thank them in the acknowledgements). Some journals require catalog numbers for reagents, and some require lot numbers for antibodies. You do not need to list suppliers for common chemicals such as NaCl.

Animals: Some journals require checklists where you indicate on which page of the paper you addressed issues such as animal sample size, randomization, and blinding. For example, see the Animal Research: Reporting of In Vivo Experiments guidelines and checklist (*https://www.nc3rs.org.uk/arrive-guidelines*). Even if your target journal does not require it, following this checklist will help you ensure that you report your work in a rigorous and reproducible manner.

Results

This is the most crucial part of your paper, as here you will attempt to convince your reader that you have learned something new, interesting, and true. Most journals will allow you to denote subsections of the Results with headings, which are helpful to readers. Each subsection of the Results should include the following:

Question

Each subsection of the results should start with a question—what was it you wanted to know that led you to do the experiment you are about to describe? Although this may seem obvious to you, remember that your reader may not know very much about your experimental problem and system. Thus, you need to lead the reader through the process. A paragraph may begin “To determine the spatio-temporal expression pattern of gene X . . . ” or “Because we found that gene X was essential for male fertility, we wished to determine the step in spermatogenesis that was affected in the gene X mutant.” Sometimes, this part will only be one sentence, but in other cases, you may need several sentences of background to help the reader understand the context of your question.

It is important to remember that you are telling a story, not the chronological history detailed in your lab notebooks. Your original reason for doing an experiment may have been based on an idea that later turned out to be incorrect. Furthermore, you very likely did not do the experiments in the order in which it makes the most sense to write about them. So, when you write, “We next wondered . . . ,” you may not be telling the truth. But that is OK! If your paper was written based on the order you did the experiments—and the original rationale for doing them—it would likely be difficult for your reader to follow.

Rationale

After posing a question, explain why you set up the experiment in the way that you did. For example, “To determine whether phosphorylation of protein X is regulated during development, we made extracts from staged populations of embryos. If protein X is phosphorylated, it should migrate slower on a gel than if it is dephosphorylated. Extracts were thus treated with or without phosphatase and analyzed by western blot.” A statement like this will allow your reader to more easily interpret your data than will "We found that protein X was phosphorylated during development".

Method and result

Briefly describe what was done in each experiment with enough detail that a reader can get a picture of the experiment in his mind, but nowhere near as much detail as in the Materials and Methods section. Then, describe the results of the experiment, using the appropriate figure(s) to illustrate your point. Don't worry if you find yourself going into too much detail—that can be edited out later.

Conclusion

This is the last part of each subsection. You want to avoid speculation here (save it for the discussion), but you do need to sum up for the reader what you just demonstrated with the preceding data. For example, "Taken together, these results indicate that . . . " or "We conclude that . . . "

Introduction

The introduction is a difficult part of a paper to write, for it is here that you will set the stage for the entire manuscript. A good introduction will act like a funnel: start with a wide focus to interest the reader in the topic and the broad biological question addressed by the work, narrow a bit to familiarize the reader with the field, introduce key facts the reader needs to know to understand the work, narrow further to explain the specific question that the authors aim to answer in the paper, and lastly describe the rationale behind the choice of experimental system and plan.

The broad biological question

Your goal with the opening of your introduction is to hook your reader—i.e., make her want to invest time in your paper. So you need to help her understand why she should be interested in your topic. Start your introduction with a broad question that any life sciences researcher will be familiar with. For example, if your paper is about post-translational regulation of a particular ion channel that regulates membrane potential in uterine smooth muscle, you may start with the a general question about how the uterus transitions from being predominantly non-contractile for nine months of pregnancy to producing forceful, rhythmic contractions at term.

Familiarizing the reader

You need to provide the reader with enough information to understand the work done before this paper. Remember, however, that you are writing an introduction, not a review article. Thus, you need to focus the introduction on only the information the reader actually needs to know to understand your study and its relevance. Continually ask yourself, "Does the reader really need to know this? Do any of my experiments relate to this issue?"

Key facts

Explain the system that you are using to address the question you are asking. Again, be careful not to go into more detail than necessary, but make sure that you sufficiently describe the biological process you are studying to create an image in the reader's mind. Be careful with organism and gene names. Although it may seem obvious to you what *Aspergillus* is, it doesn't hurt to remind your reader that it is a mold. Similarly, many genes and proteins have different names depending on the organism being discussed. For sake of clarity, choose the most common one and use that throughout your paper. For example, the calcium-activated potassium ion channel BK_{Ca} is also called MaxiK and Slo-1, and the human gene encoding the pore-forming subunit is *KCNMA1*. In this case, you would choose one, introduce the others in case the reader knows the gene by another name, and then only use your chosen name throughout the rest of the paper. Also be careful with the use of jargon. If your subfield uses specific terminologies that are not known generally, define them in the introduction.

The specific question

Describe the specific question your paper addresses and how it answers some aspect of the broad question. This must be stated clearly. If not, the reader will be confused, guess what question the paper addresses, and judge your paper according to inappropriate standards.

Rationale

Why is your study system ideal for addressing your specific question? What advantages of your model organism are relevant to this question? What general approach are you taking to address the question? What assumptions have you made about the question? Often, introductions will conclude with a brief (one to three sentences) summary of the key findings.

Discussion

The discussion is often the hardest part of the paper to write because it is open-ended and requires substantial knowledge of the literature. Think of the discussion as being the opposite of the introduction; start narrow and expand. The first paragraph should restate the question posed at the end of the introduction and succinctly sum up the key conclusions of the experiments in the paper. “In this report, we tested the hypothesis that . . . ." or, “Here, we have presented four lines of evidence that . . . ” Without restating all of your results, list, in a few sentences, the key findings that support your conclusion.

The next paragraph(s) should be a little more general but still focused on your data. You have many options here, depending on the specifics of your findings and your field. You might explain how others’ results support your conclusions, or how your conclusions are consistent with others’ conclusion. If your results contradict those of others, discuss the differences in experimental set-up or data analysis that might explain the discrepancy. However, avoid implying that the other authors did something poorly. You might also discuss trends in the data, discrepancies that arose, unexpected findings, and ways in which the experimental design was inadequate to address a particular issue. You might suggest future experiments that could be done to resolve discrepancies or address remaining gaps in knowledge. If your data could be explained in more than one way, discuss why you favor one explanation, or suggest what would be required to distinguish between two or more possible explanations.

The next paragraph(s) should be broad and put your work into the context of your subfield. What do your results mean, and how are they relevant to the broader question posed at the beginning of the introduction? Next, describe the relevance of your work in the broader field. In the ion channel example cited above, you might discuss how data regarding the function of the BK channel in the uterus informs our understanding of BK function in the heart. Many journals, especially clinical journals, require a paragraph describing strengths and weaknesses of your study. Finally, discuss the new questions raised by your work. The last paragraph is a good place to suggest potential future experiments to address new questions raised by your work.

Generally, reviewers are pretty forgiving of the discussion. As long as you don’t over-interpret your data or mis-cite others’ data, you can speculate fairly freely, but don’t get too carried away. Three to five pages of double-spaced text is a good length.

Abstract and Title

The abstract is a mini version of your entire paper. As services such as PubMed display abstracts, this will serve as the teaser to allow the reader to decide whether to pull up your entire paper. First, provide one sentence that introduces the broad biological question in terms that any biologist will understand. Next, write a couple of sentences to introduce the system you are using and the specific question addressed in your study. Then, briefly describe your approach and list the major conclusions of your work. Conclude with one sentence that sums up your interpretation of what the results mean. The journal *Nature* has an annotated summary (***https://www.nature.com/nature/authors/gta/2c_Summary_para.pdf***) that provides an excellent example of how to structure an abstract for most journals. Some journals, especially clinical journals, require a "structured abstract" with labeled sections such as Introduction, Objectives, Methods, Results, and Conclusions. Finally, many journals have specific guidelines involving length, content, and use of references in the abstract, so make sure you read the journal's instructions to authors.

The title is enormously important, as many readers scan through journal tables of contents and Pubmed searches, spending half a second or less on each title before deciding whether or not to read the abstract. Thus, the title should convey the main conclusion of your work and contain key words that will attract the scientists you hope will read your paper. Keep in mind that as soon as a scientist reads your title, he will begin to decide what experiments and findings must be presented in the paper to justify the stated conclusion. Thus, the conclusion in your title should be absolutely defensible by the data and contain little interpretation. Keep the title succinct and free of jargon and abbreviations that non-specialists won't understand. Unless your paper is about developing a new method, avoid mentioning methods in the title. Resist any urge to be cute—humor is not a virtue in research article titles. Finally, pay careful attention to grammar and unintended interpretations of your title. Write three title options and ask all of your co-authors and as many others as possible to weigh in.

PUTTING IT ALL TOGETHER

Once you generate a complete first draft of your paper, the hard part can begin! First, you must decide whether the paper actually tells the story you want to tell. Maybe you started writing your paper with a clear idea of what it was about, only to find along the way that the data addressed a different question. How can this be? When you were in the discovery phase of your work, you designed each experiment to address the next logical question. You considered the results of each experiment carefully, but often in isolation. But when you write a paper, your collective analysis often results in new insights. Additionally, as you write the introduction and discussion, you have to read and reread a lot of papers from other groups, which puts you in a better position to clearly see the open questions in your field. Thus, you may come to realize that the data you generated to address one question could be used to address a different, and more interesting, conundrum in your field. You may thus want to rework your paper with this new focus in mind. This may require rewriting the introduction, reorganizing the results section, and revising the discussion.

Once you convince yourself that your paper tells the best possible story, make sure that your story makes sense to a reader who knows nothing about what you have been doing in the lab for the last year or so. Try to put yourself in that person's place. Consider carefully the order in which you present the figures. Could you tell the story more clearly with the figures in a different order? Consider how the plot of your story progresses from one figure to the next—are all the transitions logical? If your reader finishes reading about figure 2 and thinks to herself, "I wonder if that result means X? Hmm, they could test that by doing Y" and Y turns out to be exactly what you present in figure 3, then you have done an excellent job writing your manuscript. At this point, you will be so intimately familiar with your paper that you will have a hard time seeing it as your reader will. For that, you need fresh eyes.

SOLICITING AND RESPONDING TO CONSTRUCTIVE CRITICISM

First, give your manuscript to your mentor to read. For your sake, as a young scientist learning to write, your mentor is hopefully of the patient variety who, instead of simply rewriting your paper, will take the time to point out problems, suggest solutions, and be willing to go through many rounds of revision with you. At each round, save a new version of the paper and do not delete the old versions—you never know when you might decide to reinsert a deleted paragraph.

When you and your mentor are done with revisions, recruit others to read your paper. Start with people in your lab, as they are knowledgeable about your work. Once your lab is satisfied with your paper, give it to one or two colleagues who work on related but not identical topics. For this, your mentor may need to contact someone at a different university. Although you may be tempted to skip this step, don't. You, your mentor, and your labmates are so immersed in the ideas presented in your paper that you won't clearly see the flaws or gaps. Informed non-specialist readers, however, might point out holes in your logic that could lead you to do another experiment, or they may make suggestions about ways to present your data more effectively. These are the type of people who will review your paper, so their views are extremely valuable. Be sure to thank them in the acknowledgements.

Accepting constructive criticism can sometimes be emotionally painful and bruising to one's ego. One way to avoid these feelings is to make sure you have had enough time completely away from the manuscript so you can detach yourself emotionally from it and look at it more critically through the eyes of your reader. Additionally, remind yourself that your readers are criticizing your paper, they are not criticizing you! Often, readers write comments in the margins that make sense to them but may not be clear to you. Therefore, if possible, meet one-on-one with the person who has read your paper to discuss his suggestions and criticisms. Make sure that you understand not just what he wrote, but why he wrote it. Do not dismiss any critiquer as just uninformed, assuming that a more knowledgeable reader would certainly understand. Even if you don't fully agree with a criticism or suggestion, it points out a problem with a particular part of your paper that would improve with revision. If one person finds something to be unclear, others probably will as well. Sometimes a reader's confusion about one part of a manuscript is due to lack of clarity in another section, which then needs to be revised. As a reader may have a hard time articulating how you should change your paper, it can be helpful to instead ask the person to explain to you what he thought you meant. Then, when you describe what you were actually trying to get across, the reader might say, "Oh, so if you had explained such and such in this earlier section, I would have understood the point you are making here."

THREE STYLE ISSUES

Voice

Many well-meaning, brilliant, and authoritative scientists will insist that the only way to write a proper scientific manuscript is in third person and passive voice. The logic for this is that science is an impersonal pursuit of knowledge, so personal pronouns have no place in a scientific paper. However, writing in the 3rd person, passive voice is often stilted and dull. Furthermore, scientific questions do not ask themselves, the logic does not exist without human input, and the experiments do not perform themselves. The human mind and hands are integral parts of all aspects of what goes into a scientific manuscript. Active voice and use of the pronoun "we" allow you, the writer, to more easily portray your thought process to your reader. That said, if your mentor is a die-hard passive voice writer, do what she says.

Tense

For ease of reading, follow a simple rule regarding the use of tense in your manuscript. Accepted facts should be written in the present tense, whereas descriptions of experiments that led to the conclusions you state (be they yours or those shown by others) should be in past tense. Thus, much of your introduction will be written in the present tense, e.g., "Nucleoli are the sites of rRNA synthesis and processing." If you are describing experiments done in previous papers, they should be in the past tense, e.g., "Baker et al. (1993) demonstrated that gene X is required for gastrulation." Your results section should be written in the past tense, e.g., "To determine the transcriptional start site, we performed 5′RACE" or, "In this mutant, transcription initiated at an aberrant site five base pairs downstream of the site used in wild-type cells."

Plagiarism

I will assume that you know that copying another's work is wrong, so I will only focus here on a couple of issues that may present difficulties in avoiding plagiarism when writing a scientific manuscript. First, copying your own work is self-plagiarism and is unacceptable. Just as it would be wrong for Charles Dickens to have reused a paragraph from "Oliver Twist" in "David Copperfield," it would be wrong for you to reuse text from one of your lab's previous papers. If you or your mentor previously published a paper or a review article in which the topic of your current work is described, you cannot reuse sentences from that earlier paper. Think of another way to say it! Second, your introduction and discussion will cover a lot of material that has been presented in earlier papers written by your lab or other groups. You must determine how to describe that information in a new way. The closer you are physically to a paper and the closer you are to the time when you read a paper, the harder it will be to put descriptions into your own words. So, a good approach is to do your reading, then set all the papers aside. Do not have the paper you are referencing sitting open on your desk while you write.

COVER LETTER

You have your final draft ready to submit, but you're still not done writing! Every paper you submit to a journal should be accompanied by a cover letter addressed to the editor. This is your first chance to educate the editor about why the journal should be interested in your manuscript. The first sentence should ask the editor to please consider your paper for publication in the journal. Next, give a short description of the major findings of the paper. Point out the important question that your paper addresses and why your system and approach are amenable to tackling it. If your paper is the first to identify a particular gene, use a new technique, clearly prove a favored hypothesis wrong, etc., be sure to point that out. This is a sales job, so make the cover letter clear and concise. End this paragraph suggesting why you think your paper would be of particular interest to the readers of that journal. The more broadly appealing you can make your paper sound, the better. Next, write a brief paragraph stating that all authors have approved the manuscript and that the paper is not currently under review at any other journal. Regardless of who types the letter, it should be signed by the corresponding author (usually the PI).

SUBMISSION

Most, if not all, journals now use online submission systems. Most require you to establish a login name and password. Some journals allow any of the authors to be the submitting author, but others require the corresponding author (usually the PI) to be the submitting author. If that is the case, either your PI will have to submit the paper, or you will need to get your PI's permission to use his login information.

The online submission systems vary from one journal to the next. Some take about 15 min, whereas others will take two hours, so plan accordingly. Some journals will require you to submit your text as a Microsoft Word document and the figures as separate TIFFs or PDFs. Others may require you to convert the entire manuscript, including text and figures, into one merged PDF. At the end of the process, most systems will ask you to carefully look over a merged PDF created by the system. Make sure everything looks right, fix any problems, and then confirm your acceptance. Within a short time (minutes to a few days), you should receive a manuscript number and information about how you can follow the status of your paper.

THE REVIEW PROCESS

After you submit your paper, you should receive some confirmation that the journal received it and assigned it to an editor. The editor or systems administrator usually states when to expect reviews and a decision on your manuscript, which is typically between 2 and 10 weeks. If you don't receive a decision within this timeframe, the corresponding author may contact the journal to determine whether there is a problem. Keep in mind that most editors are working scientists and thus very busy, so don't pester the editor unnecessarily. The review process generally produces one of five outcomes:

1. The reviewers love your manuscript and the journal wants to publish it immediately as is. I've seen this happen only once. Don't get your hopes up.
2. The manuscript is pretty good, but the reviewers would like you to make a few changes before publication. In this case, the revised paper may not have to go back to the reviewers, but rather can be approved by the editor.
3. The reviewers are somewhat positive but require additional experiments and must see the revised manuscript again for re-review. In this case, you should definitely try to address the reviewers' comments and resubmit the paper to the same journal.
4. The reviewers are extremely negative, and the journal completely rejects your paper. They do not want to see it again. Although you could do more experiments, address all the reviewers' concerns, rewrite the paper with a new title, and resubmit it to the same journal, it's generally not worth it. The paper will likely go to the same editor who already has a negative view of your paper. Thus, your best bet is to submit your paper to another journal. In this case, you should still try to address as many of the reviewers' concerns as possible so as to improve the paper. Additionally, reviewers at the next journal are likely to have some of the same concerns. Moreover, occasionally the same person will be asked to review your paper at the second journal. If you have not dealt with the previous criticisms, the irritated reviewer will likely reject your paper at the second journal.
5. The journal chooses not to review your paper at all. Although you could argue with the editor in an attempt to convince her to change her mind, this is not advisable. She already dislikes your paper, so even if it does get sent out to review, she will read the reviewers' comments with a negative impression. Keep in mind that this rejection does not necessarily reflect the quality of your work. The highest-profile journals often make editorial decisions about which papers to review based on space considerations, the kinds of papers they have published recently, and what they view as the current hot topics. The best thing to do in this case is to submit your manuscript

to another journal. If you still believe it belongs in a broad-interest journal, send it to one of a similar tier. If the editor there says the same thing as the first, it's time to reconsider and submit your paper to a slightly more specialized journal.

Unless the reviews of your paper are fairly positive, your first reaction to reading them will likely be to get defensive and declare, "These reviewers don't know what they're talking about!" I assure you that, for the most part, this is not the case. Reviewers are working scientists, who, with rare exceptions, are intelligent, knowledgeable people. If your reviewer did not understand something in your paper, it likely means that you did a poor job of explaining it. In almost every case, the review process, however painful it may be, improves the quality of the paper.

So, after you have calmed down—perhaps the next day—reread the reviews. This time, go through each comment carefully, and try to understand what the reviewer meant. Often, reviewers provide specific comments (e.g., you need this control, you should do the analysis this way, or you need to perform this specific experiment). Other comments may be vague but often imply that (1) you did a poor job of explaining something, (2) the reviewer doesn't think your data sufficiently support your conclusion, or (3) your study, even though done well, does not represent a significant advancement from previous work. Ideally, the editor will provide some guidance about what he thinks are the important aspects of the reviews. This is not to say you can ignore the other comments, but focusing on the main aspects should help you decide which things you must address with another experiment and which you can address with a change in the text to make something clearer. Generally, you will be given two or three months to resubmit your paper. If this timeframe is not sufficient, contact the editor as soon as possible to request an extension.

REBUTTAL LETTER

When you resubmit your paper, you will need to include a detailed letter to the editor in which you outline the changes you made to the text. In the first part of the letter, be sure to thank the editor and the reviewers for their thoughtful consideration of your manuscript and their helpful comments (even if you still don't believe this to be true, write it anyway). Next, indicate that you understand the main criticisms of your paper and briefly describe the most substantial changes you made to the paper to address this concern. Then go point-by-point through each of the reviewers' comments, indicating how you changed the paper to address each issue. Some journals will ask you to number the lines of the revised manuscript; this is a good idea even if the journal doesn't require it to make the editor's and reviewers' jobs as easy as possible. For example:

"The most substantial changes to the reviewers' criticisms are as follows: As suggested by reviewers 1 and 2, we completed a new analysis of the data in Table 1. Additionally, in response to the concern of reviewer 2 about X, we added a new figure 3. Finally, to clarify our model, we added a schematic (new Figure 8). Responses to specific comments from reviewers are as follows (reviewer comments in *italics*, added text in **bold**):

Reviewer 1:

1. *For their argument that the levels of protein X are different in different tissues to be believable, the authors should include a loading control in their western blot.*

We agree and have now standardized our blot by using actin as a loading control. These data are included in a new Figure 1. On page 3, third paragraph, lines 123–124, we amended the text to read, 'Western blot analysis indicates that the levels of protein X, **standardized to actin as a loading control**, differ by an order of magnitude between brain and thymus.'"

Make sure you respond to every single comment. If you disagree with the value of an experiment, think that a requested experiment is technically impossible, or consider an experiment to be beyond the scope of the current paper, explain your reasoning in the rebuttal letter. You need to let the editor and reviewers know that you carefully considered all of the points and made a good-faith effort to respond to them.

AFTER YOUR PAPER IS ACCEPTED

The journal may require you to submit a copyright transfer agreement form. The corresponding author will need to sign it, asserting that the journal now owns the paper and all authors have approved the manuscript. Additionally, you may have to provide a method of payment for page charges. You may also be offered the opportunity to order reprints, which are essentially pages from the print journal.

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CONCLUSION

I wish I could tell you that when your paper is finally accepted, the page proofs have been submitted, and you see your work on the journal's website, you will be extremely happy. Unfortunately, such is rarely the case. So much sweat (and possibly tears) went into doing the experiments, writing the paper, and responding to reviewers (potentially at more than one journal) that the actual acceptance is pretty anticlimactic. That said, you MUST celebrate! Scientific research involves so much hard work and is fraught with so much frustration that when things do go well, the occasions must be marked. Go out for lunch, buy milkshakes for your lab mates, bring a bottle of champagne to lab meeting, or at least go out for a nice dinner with a friend or loved one. Then get right back into the lab, as your work surely raised more new questions than it answered!

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