

Linking experiments

Key points:

- Results sections tell stories
 - Connect introduction to discussion
 - Connect each experiment to the one before and the one after
- Research strategy sections explain how experiments address the aims
- Both require motivating each experiment
 - How it relates to overall question or aim
 - What you want to know
 - How experiment addresses that question

Both results sections of basic papers and research strategy sections of proposals are about a series of experiments. These sections can thus easily become lists of what you did and what you found (or what you will do and what you expect to find), but that doesn't serve the purpose of each section. A results section is the bridge between the introduction and the discussion, and has to help the reader get from the overall question to the conclusions. Research strategy sections are somewhat different—they convince a reviewer that the experiments you propose will accomplish your aims.

Results sections as stories

The most natural way to help a reader see how you got from the overall question to the conclusions is to tell it as a story. This story might be the actual story of why you chose the first experiment, how its results led you to do the next experiment, and so on (omitting the inconclusive experiments and technical problems), but most likely the actual story involved some dead ends that you would leave out of the published version. Additionally, sometimes your experiments might make more sense in a different order than that in which they actually happened; for example, when a result leads you to ask a more general question (the general experiment works as an introduction to the earlier one). This natural order determines the order of the figures.

The logic you use to determine the order of figures is the story that forms the results section. To introduce the first experiment, explain why you chose to place it at the beginning—how does knowing the result of that question help you get to the later experiments? How does it relate to the overall question? Similarly, to introduce the second (and third, and fourth...) experiment, explain how it follows from the previous experiment. What question did the results of the first experiment bring up? How does this experiment address that question? This approach to composing a results section meets the needs of its audience: those new to the field. Experts tend to skip results sections since interpreting a figure leads them to ask questions that later experiments address, but novices may not see the connections.

→ *Explain your reasoning to lead the reader from one result to the next*

Motivating each experiment: results

Let's apply this approach to an example, the first two results from Nomura DK et al., *Cell* Jan 2010. The overall question of this paper is how aggressive cancer cells generate free fatty acids (FFAs) to support cancer-promoting lipogenesis.

Example 1 part 1: unclear rationale

To identify enzyme activities that contribute to cancer pathogenesis, we conducted a functional proteomic analysis of a panel of aggressive and nonaggressive human cancer cell lines from multiple tumors of origin, including melanoma (aggressive [C8161, MUM2B], nonaggressive [MUM2C]), ovarian (aggressive [SKOV3], nonaggressive [OVCAR3]), and breast (aggressive [231MFP], nonaggressive [MCF7]) cancer. Proteomes from these cancer lines were screened by activity-based protein profiling (ABPP) using serine hydrolase-directed fluorophosphonate (FP) activity-based probes (23, 24). Serine hydrolases are one of the largest and most diverse enzyme classes in the human proteome and play important roles in lipid metabolism (25, 26). Among the more than 50 serine hydrolases detected in this analysis (Tables S1, S2, and S3), two enzymes, KIAA1363 and MAGL, were found to be consistently elevated in aggressive cancer cells relative to their nonaggressive counterparts, as judged by spectral counting (23, 24). We have previously shown that KIAA1363 plays a role in regulating ether lipid signaling pathways in aggressive cancer cells (27). On the other hand, very little was known about the function of MAGL in cancer. The heightened activity of MAGL in aggressive cancer cells was confirmed using the substrate C20:4 MAG (Figure 1B). Since several enzymes have been shown to display MAG hydrolytic activity (28), we confirmed the contribution that MAGL makes to this process in cancer cells using the potent and selective MAGL inhibitor JZL184 (29). JZL184 (1 μ M, 4 hr) dramatically reduced the MAG hydrolytic activity, but not other lipid hydrolytic activities, of cancer cells (Figure 1B). These data demonstrate that aggressive cancer cells display highly elevated MAG hydrolytic activity and most, if not all, of this activity originates from the MAGL enzyme.

We'll pause here to consider how well this works as a narrative. The beginning is somewhat challenging—there's not a clear connection between generating free fatty acids and a screen to identify enzyme activities. Since the authors don't specify what sort of enzyme activity they looked for, it seems at first that they're looking for any activity, which is an awfully general approach to identify a mechanism to support lipogenesis. They later explain that they screened only for serine hydrolases, which are a large class of enzymes known to be involved in lipid metabolism. Explaining this first would avoid our initial confusion; the authors could have said that generating free fatty acids would require a lipid hydrolyzing activity, and that the broadest class of enzymes with such activity are serine hydrolases, so they screened for those. This example illustrates the importance of not just indicating your reasoning, but explaining it as specifically and explicitly as possible.

→ *Make explanation of rationale correspond to method*

Example 1 part 2: missing rationales

MAGL Regulates Free Fatty Acid Levels in Aggressive Cancer Cells

MAGL is perhaps best recognized for its role in degrading MAGs in brain and peripheral tissues (28, 29, 30). Consistent with this established function, blockade of MAGL by JZL184 (1 μ M, 4 hr) produced significant elevations in the levels of several MAGs, including 2-AG, in each of the aggressive cancer cell lines (Figure 1C and Figure S2). Interestingly, however, MAGL inhibition also caused significant reductions in the levels of FFAs in aggressive cancer cells (Figure 1D and Figure S2). This surprising finding contrasts with the function of MAGL in normal tissues, where the enzyme does not, in general, control the levels of FFAs (29, 30).

Curiously, we noted that the magnitude of reduction of FFAs greatly exceeded the corresponding elevation in MAGs. We hypothesized that this apparent discrepancy in mass balance might be accounted for by the conversion of elevated MAGs to alternative metabolites in JZL184-treated cancer cells. Consistent with this premise, lipidomic analyses revealed significant increases in lysophosphatidyl choline (LPC) and lysophosphatidyl ethanolamine (LPE) in JZL184-treated cancer cells (Figure S1). The cumulative magnitude of elevation of these lysophospholipids matched closely the reduction in FFAs observed in JZL184-treated cells.

We next stably knocked down MAGL expression by RNA interference using two independent shRNA probes (shMAGL1, shMAGL2), both of which reduced MAGL activity by 70%–80% in aggressive cancer lines (Figures 2A and 2D). Other serine hydrolase activities were unaffected (Figures 2A and 2D), confirming the specificity of these reagents. Both shMAGL probes caused significant elevations in MAGs and corresponding reductions in FFAs in aggressive cancer cells (Figure 2).

Together, these data demonstrate that both acute (pharmacological) and stable (shRNA) blockade of MAGL cause elevations in MAGs and reductions in FFAs in aggressive cancer cells. It is furthermore noteworthy that aggressive cancer cells were found to express higher basal levels of FFAs (and conversely lower levels of MAGs) than nonaggressive cancer cells (Figures 1C and 1D), and this altered metabolic profile was largely eradicated by MAGL inhibition. These intriguing findings indicate that MAGL is the principal regulator of FFA levels in aggressive cancer cells.

This subsection continues where the previous one left off, with more information on MAGL. This information justifies the measurement of JZL's effect on MAG levels, but not the measurement of FFA levels. The reader must recall the goal of the paper, to identify the lipolytic enzyme that liberates FFAs, for the latter result to make sense. The reader may not bother to return to the introduction since the placement of the FFA result suggests that it is less important than that on MAG. The FFA result is in fact the major finding of this subsection, so the authors' omission of rationale for this is an even more significant problem, since it obscures the importance of this result to the paper's conclusions. Following the major storyline would be easier if the authors placed the result on FFAs first, introducing it with the reason they tested whether MAGL regulated FFA levels.

→ *Introduce each experiment as it relates to the overall story*

Similarly, the authors switch to the results of the second experiment without explaining its purpose. They've already established that blocking MAGL increases MAG levels and reduces FFAs, so the reader may wonder why they used an additional method to test the same thing. The reason for using RNA interference remains unclear until the conclusion (the authors were interested in the effects of *stable* reduction in MAGL activity). The authors may not have noticed this omission since the subsection began with rationale, or they may have assumed that their reasoning was obvious, but explaining even a secondary experiment will help the reader. Further, it would only require a phrase: "to determine whether stable reduction in MAGL activity causes similar effects."

→ *Include a rationale for EACH result, even secondary ones*

Though the authors omit their rationale for measuring FFA levels early in the subsection, the reader will likely still get the point of it and the corresponding figure because the heading and the final sentence state it directly. Including both rationale and conclusion help to ensure that readers don't miss the logic and can compensate for lack of clarity in one or the other.

→ *State the conclusion of each subsection*

Research strategy sections connect experiments to aims

Though research strategy sections aren't stories (since independent aims do not build on one another), explaining your logic may still be worthwhile. Whether explanation will help reviewers depends on how direct and obvious the connections between the aims and experiments are. For example, if the experiment is to determine the extent to which ADP-ribosylation affects an RNA-binding protein's ability to bind RNA and the aim is to determine the molecular consequences of ADP-ribosylation of the protein (from the Alfano proposal at NIAID), the relationship is fairly obvious. Nonetheless, the authors include some rationale: their preliminary evidence that the protein is ADP-ribosylated within the RNA-binding domain. In other cases, some explanation is necessary for the reviewer to understand how the experiment serves to accomplish the aim. Explanation requires more than simply stating the evidence that supports your reasoning, as this example shows:

Example 2: incomplete rationale

Aim 1: Test the hypothesis that *E. histolytica* has a phagocytosis receptor specific for the collagenous collectin tail. **Rationale:** The ability of *E. histolytica* to ingest both apoptotic cells opsonized with C1q and colonic bacteria opsonized with collectins suggests they may have features in common that facilitate *E. histolytica* phagocytosis. One possible feature is the ability of collectins to bind to both. [both C1q and collectins have related collagenous tails.] Further, *E. histolytica* ingested apoptotic cells opsonized with human C1q better than control cells, and latex beads coated with C1q, MBL [a collectin], and C1q tails better than control beads. To understand the significance of these data, it is critical to determine the mechanism of the C1q-ameba interaction. We hypothesize that C1q and the collectins facilitate *E. histolytica* phagocytosis by interaction with an amebic receptor specific for their conserved collagenous tail, providing a mechanism for engulfment of both host cells and bacteria.

1.3: Determine if *E. histolytica* recognizes sugars or proteins on C1q and the collectins: The collectins are modified by N-linked glycosylation [65-67, 120]. Calreticulin [the candidate phagocytosis receptor] is a chaperone and has lectin activity [81].
From the RO1 application titled “Molecular Mechanism of Entamoeba histolytica phagocytosis” by Huston CD, available at <http://funding.niaid.nih.gov/ncn/grants/app/default.htm>

The reader has to guess at why the authors propose experiment 1.3, since it's not clear how determining which part of C1q and the collectins *E. histolytica* binds to helps show that binding is specific, causes phagocytosis, and occurs through a common receptor. Perhaps the authors imply with the information about glycosylation and lectin activity that this similarity between the two proteins means that it's likely that the *E. histolytica* receptor binds sugars? It seems just as likely that the receptor binds protein since the proteins' collagenous tails are related. Reviewers would be more likely to find the experiment necessary if the authors explained better how this experiment serves the aim.

→ *Explain how the question of each experiment serves the aim*

In other cases, the relationship of the experiment's question to the aim is simple, but the choice of method to answer that question is not. Another example within the above aim is the experiment to determine whether collectin tails stimulate phagocytosis. In this experiment, the authors propose to use purified c1q tails bound to latex beads but do not explain why testing the ability of other collectins to cause this effect is unnecessary. Are the domains of the proteins that closely related? Have collectins already been shown to induce phagocytosis by *E. histolytica*?

→ *Explain why your method is appropriate for the question*

These examples show that understanding how each experiment contributes to an aim requires two steps of logic: why you need to answer the question of the experiment to accomplish the aim, and why this experiment is best to answer the question. If you're unsure whether explaining this logic is necessary for each experiment, consider discussing the proposal with those in your field. Alternatively, you could compose the rationale for every experiment and edit out those that are redundant with the rationale for the aim or the sub-aim heading.