

# Maguire's Aphorisms, Rules of Grant Writing and Grantsmanship

M.E. Maguire  
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## RULE 0 – Rules are made to be broken!

### 1. *The Rules of Grant Organization*

The biological question being asked is absolutely paramount. You must convey both the question and its importance to the reviewers clearly and succinctly. There should be no question whatsoever of its importance. The large majority of reviewers will forgive some methodological issues if they can clearly see and **like** the question being asked. A very large percentage of the grants that are triaged result from failure of the applicant to state, up front, a clear overall biological question and context for their proposal.

#### RULE 1

AIM I should be, methodologically, like falling off a log. It asks relevant but straightforward questions. Your training and your preliminary data leave no question in the reviewer's mind that you can do these experiments. Aim I may not be exciting, but the data is needed, and **you** can readily get that data.

#### RULE 2

AIM II is more challenging both conceptually and methodologically than Aim I. Accomplishment of Aim II would add some quite interesting and valuable information to the literature. It should indicate your ability to ask interesting questions and to approach them in interesting though not necessarily novel ways. One or 2 of the methods for Aim II might have to be developed, but if you present them carefully with controls (and alternative approaches!!), the reviewers will usually give you the benefit of any doubts they might have. "Well, the applicant hasn't thought of this problem with his/her approach, but it is clear from the discussion that it would be recognized if it occurs, and they have the training to solve it."

At this point, the reviewer should be quite favorably impressed, though not necessarily overwhelmed. If you got the grant and accomplished only Aims I and II, your competitive renewal would be reasonably solid.

#### RULE 3

AIM III is the place where your intellect and conception of the biological problem should shine. This is the place for the really "cute" experiment or the ambitious approach (assuming you have one). If you have structured Aims I and II properly, even if the reviewers don't buy the cute experiment or ambitious approach in Aim III, they will be inclined to let you try because if you succeed, you've hit the proverbial home run. If you don't succeed, the data in Aims I and II should give you a good chance to renew the grant, and the reviewers should feel that the data from Aims I and II are worth obtaining. The key is in being cute or novel but not too cute or novel.

#### RULE 4

I recently learned of an addendum to my first three rules. As a friend put it after serving on the study section my grants go to, "I unfortunately did not even have time to read your proposals. It did provide a new Maguire's rule--never allow two proposals to be reviewed by the same group at the same time."

#### RULE X

If you have a really, really, really “cute” experiment, NEVER, NEVER, NEVER tell the study section, just do the experiment!!!!!!

First, the odds are that it won't work and moreover someone on study section will likely know something that you don't know that would tell you that it won't work if only you knew it. (Or they've done it themselves and know it doesn't work.) Second, never assume that you are so smart that no one else will think or ever has thought of the experiment. Either someone has already tried it, and it didn't work, or, someone is doing the experiment right now. So your best option is to do the experiment.

Study sections are fairly conservative and tend to react negatively to what at first glance are wild ideas or approaches. *Conversely, if you present them with positive results from that really, really, really “cute” experiment, almost anything will be forgiven you.* No one but you has to know that 16 different “cute” ideas failed. Never give up on those flashes of inspiration. One of them will probably pay off. Even jaded reviewers have been known to say “Wow, that's a cool result!”

## 2. **The Rules of Grantsmanship**

Grantsmanship is not a pejorative, it is common sense. Ignore it at your peril. Remember, study section members get less than minimum wage for their work. The hotel in Washington is not always very good, the bedrooms are noisy, the meeting rooms are hot, the coffee is terrible, your breakfast is coffee and lousy pastry, and your other 2 meals are at the equivalent of a Holiday Inn Restaurant (or worse). Most study sections meet from 8 a.m. to 8 p.m. We then grab a late meal and either hit the sack or hit the bar. Anything you can do to make your reviewer's life and their review of your proposal easier will, more often than not, get you a better priority score. This is not cheating, gaming the system or any other negative you might wish to apply. If you make your reviewer's life easy, it basically means you've written a clear grant.

A sloppily presented, poorly organized, badly written grant strongly suggests that your experiments are performed the same way. This is obviously not an impression you wish to convey.

The single biggest present you can give yourself is to get a *rough* draft finished at least a month in advance of the deadline so that you will have time to put the following prescription into practice.

- i)* have *everyone* in your lab read it (including the research assistants) and give you comments. This is often best accomplished verbally in a group meeting rather than one-on-one (strength in numbers may make it easier to criticize the “boss”).
- ii)* Give the grant to at least one colleague to read, preferably one who is not an expert in your area. Give them at least a week. Then sit down with them and LISTEN. You do not have to agree with them, but do not get defensive and waste your and their time defending and explaining. If they did not understand it, **YOU** did not write it well. When they don't understand what you're trying to say try out alternative explanations on them. Having a non-expert try to explain it to you may tell you where the misconception or misunderstanding is.
- iii)* **Do NOT read your grant for at least 3-4 days, even a week.** Time may or may not make the heart grow fonder, but it sure has a tendency to make sections you previously thought were okay or even wonderful suddenly seem muddy or even stupid. If you read the same things day after day after day, you are much less likely to step back and look at the grant as a whole and see a lot of little (or not so little) flaws.

### 3. **General Pointers**

Listed below are a variety of comments, pointers and instructions. They are mine. They may or may not be yours. Some have a real basis in experience on several study sections. Other comments are simply my “bias.” If something makes no sense to you, don’t use it. If you format it “my” way, and you think it looks terrible, then change it. If you just don’t work that way, don’t work that way. On the other hand, trying a new approach might be the spark you needed.

**a. Do not make the common mistake of assuming that your grant will be reviewed by expert(s) in your area.**

The large majority of the time, this is **not** true. While I get lots of transport grants to review, that does not mean I know a lot about the particular transporters that influx or efflux this ion or sugar or whatever, nor do I know the *biology* associated with them. I also review grants that have nothing whatsoever to do with transport.

As examples of what I mean, at a recent study section I reviewed the following (areas are generalized for reasons of confidentiality):

- amino acid transporters
- Drug resistance in eukaryotes (x2) and prokaryotes (x1)
- Proton transport protein structure
- Transcriptional regulator for a transporter
- Crystallographic structure based mutagenesis of a transcription factor
- Bacterial efflux pumps

Even when the reviewer knows something of your area, they may only know a part of it. For example, I once reviewed a grant on eukaryotic lipid transport containing genetic selections for a variety of proposed mutations. While I know the transport field in general, I only know the basics of lipid transport, and I sure don’t know much more than the basics about genetic selection in the eukaryotic system in question. Likewise, the other two reviewers were geneticists who knew nothing about transport.

**Write a grant that the non-expert will understand.** That does not mean write for a non-scientist; write for a reasonably well-educated biologist. Tell them your assumptions, tell them what you think the problems are, tell them what the alternative approaches are. If a good scientist, but one who is not an expert in your area, reads your grant and understands it, you’ve done a good job. If they can’t understand it, as evidenced by their questions, keep trying. The best present you can give yourself is to have the grant written well enough in advance that a colleague can look at it for you (Yes, I said this above. It bears repeating.). For example, one colleague in my Department often reads my grants and papers, but he is an enzymologist and not in my area of bacterial genetics/pathogenesis. He is normally my most valuable reviewer, because if I can’t convince him, I’d better rewrite.

A corollary to the above concerns abbreviations. Reviewers hate abbreviations (at least this one does). Keep them to a minimum. ALWAYS define them even if they’re obvious. ATP and EDTA you don’t have to abbreviate, but most everything else shouldn’t be. Abbreviations and acronyms are for LONG, LONG phrases. If you’re not the expert in the field, no one will know what all of them mean. Some are just irritating. All of them make the grant harder to understand. Avoid abbreviations!

**b. CONTENT**

1. My personal preference and, in my experience, the preference of the large majority of reviewers is that they do not need nor want to see gory details of your methods. Buffers, protocols and the like do not need to be put into the grant unless they are highly unusual. You have a Ph.D. and have been through a

postdoc. The study section will assume you can pipette and make solutions. What is critical in terms of methods is that you demonstrate an *understanding* of this or that method. What does it tell me? *And perhaps more importantly, what doesn't it tell me?* Is it appropriately sensitive? What is the crucial control? What *alternative* methods are available and appropriate if the first method doesn't work?

2. ALL independent parts of your Experimental Section should almost always start with a section entitled *Rationale*. This is akin to a hypothesis section for the particular set of experiments but with justification. WHY are you approaching the problem in this manner? As examples, I have appended two Rationales from a recent application at the end of this document.
3. Likewise, at the end of each section of experiments and again at the end of each Aim, write two sections entitled something like *Alternative Approaches* and *Expected Results*.
  - a) In *Alternative Approaches*, tell the reviewers what alternative methods you will use or what entire approaches you will take if you simply don't get an answer at all (*i.e.*, your method doesn't work). If your method doesn't work, if the method works but turns out not to give a sufficient or relevant answer, the study section will want to know what you'll do about it. This is a section that can make or break a grant. If your experiment fails and if that data is crucial to the rest of your proposal, you just got triaged. Especially if you are a new investigator, the study section wants to know that you've thought through the approach and have multiple routes to answer your questions.
  - b) In the *Expected Results* section, briefly tell the reviewers what results you expect; in other words, what's the answer? Then tell them what you'll do if you get that answer. Then, perhaps more importantly, tell them what you'll do if you don't get the expected answer. You run into trouble if you've set up a set of experiments that must give a positive answer to be useful. Think about every set of experiments and ask yourself if you get useful information from *both* a positive and a negative result. "This answer I will interpret to mean...., but that answer I will interpret to mean..." Then tell the study section what comes next, based on each set of answers.

### c. Presentation Pointers and Fonts

1. After you have written your grant, go back and remove as many "we", "our", "my" phrases that you can. This does change active voice to passive voice, but it's shorter, clearer and avoids giving the impression that you have a very high opinion of yourself. You might conceivably really be that wonderful, but don't ram it down the reviewer's throat. See the paper by Gopen and Swan that was distributed for their take on passive *versus* active voice.
2. Never-never, ever-ever use Courier font for anything except gene/sequence data and keep even that to an absolute minimum. You might as well be writing the grant in Cherokee (Χηεροκεε).

Any grant written in Courier font has 3 strikes before the reviewer even starts. It's ugly on the page, it's very hard to read, and Courier font for some reason never copies well so that the printed copies are often unreadable. I've never met a reviewer who does not detest Courier font. It also effectively cuts 3-4 pages off your maximum length since it is not proportionally spaced (strike 4!).

In my experience, reviewers seem evenly split between preferring Times Roman or Arial/Helvetica (although Arial font at 11 points takes more space than Times Roman at 11 points, so as in this clause, use 10.5 point). The reviewers don't get upset at either. Likewise, some reviewers prefer paragraphs with ragged R justification and some prefer paragraphs with even justification like this document. I personally strongly prefer an even R margin because I think it looks far more professional. If you use hyphenation, it also gives you a few more lines, perhaps 1/3 page.

3. Use an 11 or 12 point font (10.5 Arial is okay), never 10 point font for the basic grant text. However, 10 point font is useful for figure legends. It sets them off and will not offend the reviewer. (Notice how different 10.5 point and 10 point Arial displays in the above lines.)
4. Use **bold**, *italics* or **both** to set off your headings, but use them sparingly. Never use underlining if you can possibly avoid it. *Italics* are better for emphasis whereas underlining "crowds" the page. Avoid **bold** when you want to emphasize something within a paragraph, *italics* are better. **Bold** and **Bold Italics** are for titles and headings.
5. Don't be afraid to indent sections, generally only from the L margin, but occasionally from both R and L margins simultaneously. This applies especially to numbered or bulleted lists. It makes your grant look much more organized and is easier to follow.
6. If you use an 10.5/11 point font but force single space for all paragraphs, it will look more open and less crowded since the spacing for default single space is for 12 point font size. Subscripts and superscripts should be 2 points less (*i.e.*, 11 to 9 point). If they are the same size, (in my opinion) they look ugly and the line spacing is uneven. This lends a sloppy air to the grant.
7. *Separate paragraphs by a blank line.* This makes the grant much, much easier to read and follow. It also makes it look more organized. You can get the same effect but also get back half the lines if you record a macro in MSWord that automatically searches for 2 consecutive paragraph marks (^p^p in 'Find and Replace') and replaces the second one with a half line (a line set to 6 points rather than the default 12). This document uses that type of spacing between paragraphs. I have a number of Macros written in MSWord to do this and other shortcuts like super/subscript font sizing.
8. Make graphs and figures **LARGE**. Far too many grants try to cram far too many figures into far too small a space. The result is usually unreadable. If your data can't stand enlarging into the light of day, don't show it. Likewise, **NEVER** use small data points, especially when more than one line is shown on a single graph. By the time the proposal gets reproduced, the data points and lines cannot be told apart. If you aren't sure how large is large enough, find some old person (like me!) and ask if they can read the figure comfortably and see all the data. See last page of this document for an example.

A corollary is never to show more data than necessary. If I show you a dose response curve for  $Mg^{2+}$  and  $Ca^{2+}$ , you will accept my claim that  $Mn^{2+}$  and  $Ni^{2+}$  did the same, but  $Co^{2+}$ ,  $Fe^{2+}$ , and  $Cu^{2+}$  did not. But I do not want to slug through 7 dose response curves, or the endless gel, or the page of sequence.

9. If you have gel photos, micrographs, etc. that do not normally xerox well, make multiple photo quality copies of everything and **paste** them into every single one of the required copies. Likewise for the Appendix. Alternatively, print every page on the Tektronix color printer or a high resolution inkjet printer.

When the required copies of your grant get to Bethesda, one copy goes to the Study Section Executive Secretary for his/her file during the review process. Two and sometimes 3 original copies are sent to the 2 or 3 reviewers (although every round, for unknown reasons, I always get 1 or 2 grants to review where I get xeroxed copies rather than the original). One or 2 copies go who the heck knows where into the bowels of NIH. The final copy goes to the printer. Remember that the printer who does this work is the low bidder. They do the job quickly on low quality paper. Results are, shall we say, "variable" in clarity. These are the copies that the rest of the study section gets. If all your required copies have high quality pictures this ensures that at least good copies are available to the actual reviewers and makes it easier to pass copies around to the other study section members.

The Appendix goes ONLY to the reviewers. In general, virtually nothing should be in the Appendix except publications and manuscripts. You are *not* allowed to put most of your micrographs and figures here, only material that cannot conveniently be put in the main body. Technically, the NIH-CSR can reject your application if you put lots of data into the Appendix that they think should be in the main grant. You **might** get away with one large figure, but no more.

**d. Organize your grant.**

Organize your grant carefully and *obviously*, preferably using an outline format. This makes it much easier for the reviewer to follow, cross-reference and find things in the front of the grant that they previously read, but now that they're in Aim III, need to check their memory on.

1. Use simple outlining, do not use references like "Section 3.2.1.3.4" (anathema!!). The classic alternating form of number-letter is the best (I/A/1/a/etc.). Use different font sizes for the various headings but don't go overboard. Perhaps 14 point for titles (**EXPERIMENTAL, BACKGROUND AND SIGNIFICANCE**), 13 point for other titles (***AIM I***), 12 point for main sections of each Aim (e.g., under Aim I, *A. Development of transfected cell lines*).
2. Once you get 3-4 levels down in the outline use italicized or bold words to start a paragraph (like Figure Legends in some journals) rather than impose another level of outline. These headings are to guide the reviewer, and they tell the reviewer what's coming. They are not a substitute for a topic sentence of your paragraph, but they help.
3. Don't trust your spell checker. READ the grant. Few scientists are good typists. You will be surprised how many places you have 'in' when you meant 'is'.
4. Use the grammar checker. You don't have to accept it, but use it, especially for things like "that" versus "which", "presently" versus "currently" and the like.
5. Your reviewer will *love* you if you do not use the full 25 page limit. If you've said what you needed to say, don't worry that it's only 21 pages. In my experience there is a strong negative correlation between the quality of a grant and excessive length.
6. Some reviewers (Who? Me???) are rather anal-retentive about proper grammar, spelling and usage. Following are a (partial) list of some of the things that can irritate.
  - a. Too many abbreviations, especially for short words.
  - b. Improper use of many common abbreviations, especially Latin abbreviations. Anything that is Latin should always be italicized.

For example, “*versus*” is always in italics (and is preferred to “vs.”); “*e.g.*,” and “*i.e.*,” are always italicized and have following commas; “*et al.*” is italicized and has a period but no comma after it since “*al.*” is itself an abbreviation.

- c. “This sentence is about grammar, and should not have the comma after the word “grammar”. OR “This sentence is about grammar, and it should have a comma after the word “grammar”.

**e. Reading and rereading your grant**

1. It's your grant. You're free to take or reject advice. But look at the advice with an open mind, be willing to change, even change radically. On one grant, one week before it was due, having thought long and hard about comments from a couple of internal reviewers, I threw the whole grant in the trashcan and reorganized and rewrote it virtually from scratch. It turned out far better (and got funded), even if I didn't sleep that week.
2. ***Imitate Hemingway.*** This is (or should be) your mantra. Chant it to yourself every night and every morning. Look at one of your previous papers or grants. Pick out all the long compound sentences. Do *you* really understand the point of each sentence? Break it up. We all tend to write long, convoluted sequences. That's fine for a first draft to get your ideas down. Rewrite as if you were Hemingway. I've gone back through this document to do this. I'm sure however that you can find places where I didn't try hard enough.
3. If at all possible, put the bloody grant down for 3-4 days and do something else. See some terrible movies, go bowling, sleep (!). THEN come back to it. You'll be surprised at how some sections read. Read a paper you wrote 1, 2, or 5 years ago. Upon rereading at least a few sections I suspect you'll say, “My God, I really wrote that!” (Yes, I know I also said this above, but PLEASE believe me, it's true.)

**f. About those manuscripts and papers**

1. Obviously the more the better. Nonetheless, reviewers prefer fewer papers in higher quality journals as opposed to many papers in poor or mediocre journals.
2. Don't be afraid to send in a manuscript, even if it isn't quite ready for submission. Just don't send in more than 1 or 2. Make them look complete even if they're not. The reviewers rarely read them, but if you send in a professional looking manuscript that you actually don't submit until a month later, you'll be forgiven.
3. Try not to have more than 1 or 2 “manuscript in preparation” listings in your CV, preferably none at all. Few if any reviewers will believe that such draft manuscripts have any existence in reality. Similarly, if you list a “manuscript submitted”, *specify* a journal and *include* the manuscript, otherwise it falls into the same “wishful thinking” category.
4. Provide a cover sheet in the Appendix listing the manuscripts individually. I know you also list them in the *Progress Report*, but this is another aid to the reviewer. Place a label on the first page of each paper/manuscript with your name, the grant title (and number if known) and something like “Appendix: PAPER #3.”
5. In the *Preliminary Data/Progress Report* section, refer to the labels you've just pasted on in #4 above.

**g. Rebutting the reviewers when they've trashed your grant.**

You will be in an unbelievably small minority (possibly unique) if you never have a grant rejected. And the majority of us have had at least one grant "trashed", that is, at least one reviewer really did not like it at all. Excluding two grants, my average scores on all my NIH grants submitted (around 25) over the last 27 years has been about 20<sup>th</sup> percentile (in other word, about half (eventually) get funded). But the two I excluded from that average received 91<sup>st</sup> and 88<sup>th</sup> percentiles. On the latter, I thought and still think that the one reviewer was just wrong. On that 91<sup>st</sup> percentile, in retrospect, it really was that bad. So, rule of thumb when you get a poor score back is to take a deep breath and realize that it isn't the end of the world. Go have a drink or do something to relax for a day or two or three. Then, and only then, go back to the critiques and try as objectively as possible to read them. There is always a grain and sometimes a boulder-sized truth in the critique. Don't get bogged down in the details of the critique; look to see that they got the concept first. Did they get the big picture?

Upon resubmission, you will almost certainly go back to the same study section unless you can give a *very good* reason not to. Avoid shopping for study sections (and you should have done that upon the first submission anyway). Study section members, perhaps unfairly, tend to look at a revised grant from another study section with some suspicion, as if you're avoiding criticism. In addition, in my experience, if your grant got reasonable reviews with some favorable comments, regardless of priority score, going back to the same study section retains those favorable comments. Believe it or not, the vast majority of the time, reviewers of a revised grant are looking for some excuse to say that the grant has improved. Going to another study section negates that tendency.

All that said, there are a couple of study sections that have bad reputations. Your colleagues will let you know. If one of my grants got put into one particular study section in all of NIH (which shall remain unnamed), I would immediately call up and strongly request a transfer, beg on my knees to get it and if that did not work, I would actually request that the grant be withdrawn so I could try again next cycle. THIS IS RARE. The system really works! My rule of thumb (from about 75 study section, program project, and fellowship meetings) is that the study section gets it right 95% of the time. The remainder are evenly split between a grant we treated too harshly and a grant we gave a present to. If the rest of the world worked that well, that much of the time.....

The vast majority of study sections do their job well, do it fairly and do it very conscientiously. It may be hard to believe, but on the study sections, we really agonize over many of the grants, trying to find some way around a criticism or find something positive that outweighs the criticism. We lament that we can't give a better score to this or that grant. When we really criticize a grant, most of us try hard to write a helpful critique, to suggest things, to give a reason why we don't like a section rather than just say it's poor. I have also rarely seen any favoritism to "established" investigators. Indeed, if anything, it's the opposite; we tend to expect more of the more established people. Study sections I've been on have a tendency to give young investigators the benefit of the doubt.

Once a revision is received back in the previous study section, the most common pattern is that the Executive Secretary will assign your revised grant to one of the reviewers who read it previously and will tap a new, second reviewer whether or not the previous other reviewer is still on the study section. This is done to ensure fairness both to the reviewers and give some checks and balances to the applicant. It is not uncommon for a new reviewer to state baldly that they disagree with the previous review, though it's prudent to say so tactfully in case the previous reviewer is still there.

1. Reviewer's Rule 1: The reviewer is more likely to be correct than you are. You may think they've trashed your grant, but far more often than not, there really are some flaws in that grant you slaved months over. Most often, it is less a flaw in the grant but rather that *you* did not adequately explain things. When the reviewer clearly did not understand what you meant, always consider the possibility that it is your fault because you did not explain it well.
2. Reviewer's Rule 2: Even if they're wrong, don't ever say so. The corollary is NEVER to attack the reviewers, scientifically and certainly personally. There are few human beings on this planet who will not react negatively to such comments, with the obvious consequences for your priority score.
3. Reviewer's Rule 3: Always *thank* the reviewers for their "constructive" comments that have helped you focus and improve the proposal (whether that's true or not). Don't gush or go overboard, but do remember that they are not out to get you and that they are spending their time to do these reviews. They know you're probably cursing them under your breath, but "observe the formalities."
4. Reviewer's Rule 4: Be *brief* in the "Introduction" to a revised grant. You are allowed 3 pages of introduction in addition to the 25 pages of the grant. Try to use no more than one page. Don't explain much, and don't defend yourself. State succinctly what you've changed or reorganized or dropped. I've yet to meet a reviewer that enjoys wading through 3 pages of detailed defensive statements and explanations. Did you listen, did you change things, did you reorganize? That's what the reviewer wants to know.
5. Ignore the NIH instructions that say to change font or put a line in the margin to indicate a changed section. I've never met a reviewer who enjoyed wading through different fonts, completely italicized paragraphs, etc. Besides, you're a fool if you don't completely rewrite the grant. Just state in the Introduction that you've completely rewritten the grant and therefore that marking individual sections won't accomplish anything. If there is a particular section that was criticized, and you want to draw the reviewer's attention to it, use the Introduction, e.g., "The reviewers' concern over this approach has led to a complete revision of the original experimental method with additional controls as suggested by the reviewers and additionally to consideration of alternative approaches. These are discussed in section ??? on page ???" This type of response lets them look specifically at a response if they want. Alternatively, many reviewers, myself included, prefer simply to read the revision *de novo* without even reading the previous critique or knowing the previous score. Afterwards, I read the critique and see if I agree or not and whether the applicant has improved the grant.

## EXAMPLES OF RATIONALE SECTIONS

(Just as an aside, virtually none of the hypotheses stated below turned out to be correct. Don't ever assume that you're a genius. Even Newton and Einstein got it wrong sometimes, in fact, a lot of the time.) Note that these are in 10 pt font only to fit on one page.

### b) The Oligomeric State of CorA

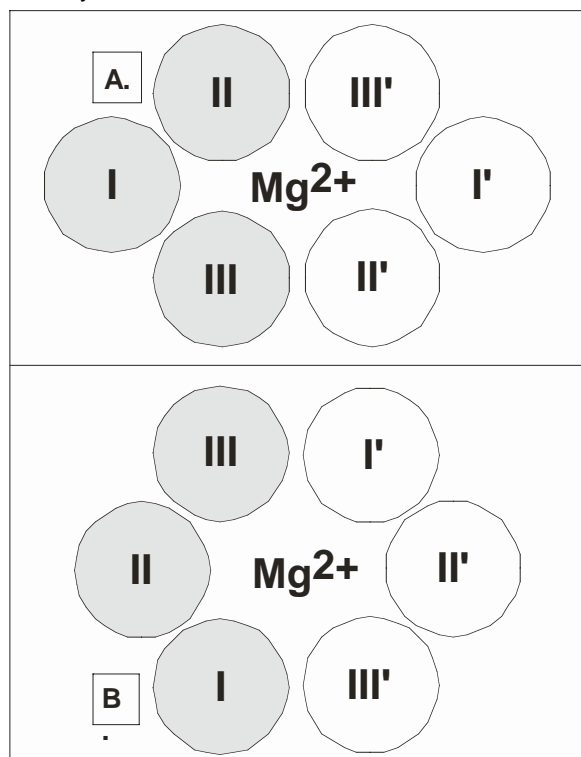
**Rationale.** An outstanding problem with CorA is its oligomeric state. Strong evidence that CorA forms at least a dimer has been presented above. While our working hypothesis is that CorA is homo-oligomer, the evidence that it forms only a dimer is based on negative data. That is, using various crosslinking agents, we have not detected a form of CorA larger than an apparent dimer. Therefore, the largest possible membrane pore that a homodimer could form would contain 6 transmembrane helices. But, given the sequence data discussed above, it is highly likely that many CorA family members contain only 2 transmembrane segments per monomer. Thus, if all members of this family function as homodimers, this implies that only 4 transmembrane helices form the membrane pore or channel. While certainly possible, this seems unlikely based on the presumed structures derived for other transporters which use a minimum of 5 helices to form the pore. In addition, although we currently have no evidence that any other proteins interact with CorA (*i.e.*, a heterodimer), the presence of doublet bands in some experiments suggests we should keep an open mind on this aspect of CorA's structure. Therefore, we plan to continue study of CorA oligomeric state.

### 2) Core Structure of TM Segments and TM2-TM3 loop of CorA.

The mutagenesis and topology experiments coupled with the analysis of the apparent oligomeric structure of CorA allow a simple model of the membrane domain to be constructed and used as a working hypothesis for further experiments. In this section, proposed mutagenesis experiments within the transmembrane domain are discussed followed in later sections by experiments on oligomeric state and reconstitution.

#### a) Mutagenesis of TM3 and Modeling of the Membrane Domain

**Rationale.** The data presented in the *Progress Report* and appended manuscripts suggest that CorA is a homo-oligomeric possibly a homodimeric protein and that residues Y292, M299 and Y307 of TM3 form part of a  $Mg^{2+}$  transport pathway or channel. The simplest models for the transmembrane domain of a homodimeric CorA protein are presented in *Figure 14*. One model proposes that the TM1 domain is outside the pore formed by TM2 and TM3 from the two monomers, while the other model puts all 6 TM segments together to form a hexameric pore. We prefer the latter model since it would allow any charged residues in the TM1 domain to have access to a presumed water filled pore and provide additional chances for charge distribution, thus minimizing the charge density.



**Figure 14. Model of the transmembrane domains of a CorA dimer.** Model A (top) suggests that the pore or channel through the membrane is composed of the TM2 and TM3 domains of a CorA dimeric protein. In contrast, Model B (bottom) forms a pore from all 6 transmembrane domains of the homodimer. Other models are possible, and these are meant only to illustrate that a limited number of possibilities exist.

We wish to test these models using experiments to map the interactions between transmembrane segments. Cysteine scanning mutagenesis coupled with crosslinking has been used extensively to map membrane structure and interactions within the aspartate chemoreceptor, the UhpT transporter and other membrane and soluble proteins (103-117). It provides not only information about whether transmembrane segments are physically adjacent, but also can provide information about  $\alpha$ -helicity (113), accessibility of intramembrane residues from each membrane face (105), and accessibility of intramembrane residues in the presence or absence of substrate (103;105;106). In addition, further mutations within TM3 (and eventually TM1 and TM2) will allow testing of the hypothesis that Y292/M299/Y307 in TM3 (*Progress Report*) and T270 (data not shown) in TM2 form a transport pathway through the membrane.