Maguire's Aphorisms, Rules of Grant Writing and Grantsmanship

M.E. Maguire

July, 2008

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. Many, many grants get triaged because the applicant (you!) failed to state, up front, a clear overall biological question and context for their proposal.

Following is one possible way to think about organizing your proposal. I must admit that the majority of my own grant applications do not follow this outline. They don't follow it simply because the biological question and appropriate experiments to test it cannot be shoehorned so neatly into such an outline. Nonetheless, I present them because it's a way to think about organizing your proposal and a way to think about presenting experiments. For example, whether or not you have a set of experiments that would fit into the suggested Rule I/Aim 1 format, you do not want to start off your grant proposal with the most technically challenging method. Likewise, you do not want be in the position that if Aim 1 fails, Aims 2 and 3 are toast. I've appended my most recent Specific Aims page where I've basically gone 2/3/1 rather than 1/2/3.

RULE 1

AIM I should be, methodologically, like falling off a log. It asks relevant but straightforward questions. Your training and your preliminary data should 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. Some (though not all) of the methods for Aim II might have to be developed; however if you present them carefully with controls (including alternative approaches!!), the reviewers will usually give you the benefit of any doubt 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 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. Second, there's a good chance that 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.) Third, never assume that you are so smart that no one else will think of 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 just do the darn 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!" By the way, I've broken this rule is the Specific Aims page that's appended. See sentence 2 of Aim 2. In addition, I've basically gone Aims 2/3/1 rather than 1/2/3 as suggested above.

2. The Rules of Grantsmanship

a. 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 usually not very good, the bedrooms are noisy, the meeting rooms are hot, the coffee is terrible, your breakfast is that same lousy coffee and even lousier 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. One presumes that this is not the impression you wish to convey.

b. 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"). Never get defensive at this point. You do yourself a disservice if you won't tolerate criticism, even if you think it's trivial or due to lack of knowledge.

   ii) Give the grant to at least one colleague to read, preferably one who is not an expert in your area. Even better, give it to 2 or 3 colleagues. 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) After writing a complete first draft, *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 paragraphs 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[1]

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 need.

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. Conversely, 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 then 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 far 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, they are not

[1] Now that we've gone to electronic submission, undoubtedly a few of these pointers and ideas will have to be modified. But these are meant to be general ones anyway. Besides, you're a biologist – **Evolve**!

for single words. Don't abbreviate drug names. If you're not the expert in the field, no one will know what all of them mean. Most abbreviations are just irritating. All of them make the grant harder to understand. *Avoid abbreviations!*

b. Content

1. For a basic research grant to NIH/NSF, my personal preference and, in my experience, the preference of the large majority of reviewers is that they neither need nor want to see gory details of your methods. (This will be particularly true as NIH cuts page limits to 12 pages.) Buffers, protocols and the like do not need to be put into the grant unless they are highly unusual. You have a Ph.D. or M.D. and have most likely 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?

   Other agencies may or may not want more details (usually they don't). Ask someone who's submitted to, or better yet, reviewed grants for that agency.

2. ALL independent divisions of your Experimental Section should almost always start with a paragraph entitled *Rationale*. This is akin to a hypothesis section for the particular set of experiments but with a justification (**not** an explanation but a rationale or justification). WHY are you approaching the problem in this manner? As examples, I have appended a rationale 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.* The order of these two sections will depend on the science and the specific experiments proposed.

   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 or if the method works but doesn't give a sufficient or relevant answer, the study section will want to know what you'll do about it. These sections 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 experimental results are anticipated; in other words, what answer do you expect? Then tell them what you'll do if you get that answer. Perhaps more importantly, then 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 this answer I will interpret to mean…" Then *briefly* 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 all the "we", "our", "my" phrases. 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 at http://www.americanscientist.org/template/AssetDetail/assetid/23947 for details on passive *versus* active voice and other similar issues.

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). (If you don't have the Cherokee font installed on your computer, the previous parenthetical text will likely display rather strangely.)

)

   Any grant written in Courier font has 3 strikes before the reviewer even starts. It's ugly on the page, it's hard to read, and Courier font for some reason never prints/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!).

3. 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.

4. 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 display in the above lines.)

5. 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 although sparing use in the text is okay. Avoid overemphasis, don't italicize everything.

6. 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.

7. If you use a 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.

8. *Separate paragraphs by a blank line.* This makes the grant much, much easier to read and follow. It also makes it look more organized and neater. 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; alternatively you can set your paragraph style to insert 6 pts after each paragraph.). This document uses that type of spacing between paragraphs. I have a number of Macros written in MSWord to do this as well as other shortcuts like super/subscript font sizing.

9. 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. Most figures I see on grants (and papers), have data points and lines that 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 entire page of sequence.

10. Now that we have gone to electronic submission, you can afford to make your gel photos, micrographs, *etc*. very high resolution. The size of the Word file will go through the roof, but the pdf document will still be relatively small. Moreover, the higher the resolution to start with, the better the pdf conversion will look.

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 in Word at the very end just before pdf conversion. You don't have to accept it, but use it, especially for things like "that" *versus* "which", "presently" *versus* "currently", "since" *versus* "because", "while" *versus* "whereas" and the like.

5. Your reviewer will *love* you if you do not use the full 12 page limit. If you've said what you needed to say, don't worry that it's only 11 pages. In my experience there is a strong negative correlation between the quality of a grant and excessive length. Conversely, make sure you say everything that needs to be said.

6. Some reviewers (Who? Me???) are rather anal-retentive about proper grammar, spelling and usage. Following is 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 trailing commas; "*et al."* is italicized and has a trailing period but no comma after it since *"al."* is itself an abbreviation.
    c. Get your compound sentences and dependent clauses correct. "This sentence is about grammar, and should *not* have that comma after the word "grammar". OR "This sentence is about grammar, and it should have the 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 so. I'm sure 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

Obviously the more the better. Nonetheless, reviewers prefer fewer papers in higher quality journals as opposed to many papers in poor or mediocre journals.

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 proposal 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 60) over the last 30 years has been about 25th percentile. But the two I excluded from that average received 91st and 88th percentiles. On the 88th percentile grant, I thought and still think that the one reviewer was just plain wrong scientifically. On that 91st 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? If not, it's your fault.

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 more than 70 study section, program project, and fellowship meetings is that the study section gets it right >90-95% of the time. The remainder is 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, fairly and 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 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 on the study section. (By the way, current reviewers don't usually know who reviewed the grant previously.)

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. 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 attack the reviewers, scientifically and certainly personally. There are few human beings on this planet that 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 the 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 (this may change) in addition to the 12 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, and did you reorganize? That's what the reviewer wants to know.

5. *Reviewer's Rule 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 enjoys 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 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 several 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 wish. 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.

A. INTRODUCTION and SPECIFIC AIMS

Three classes of Mg^{2+} influx systems are known in prokaryotes (1): MgtA/B, CorA and MgtE. The MgtA/B class is a P-type ATPase system. CorA is a Mg^{2+}-selective cation channel. Its ubiquitous eukaryotic homolog is Mrs2p, the mitochondrial Mg^{2+} channel. This laboratory in collaboration with the Structural Genomics Consortium at the University of Toronto recently published the crystal structure of CorA, the first divalent cation channel structure solved (3,4). Two other groups subsequently published identical CorA structures (5,6). MgtE is the third prokaryotic Mg^{2+} transport system (7-9). Its widespread eukaryotic homologs are the SLC41 family of solute carriers (10).

An elegant crystal structure of MgtE from *Thermus thermophilus* was recently solved by Hattori *et al.* (11), presenting a second and structurally quite different example of a Mg^{2+} channel. However, in both systems, Mg^{2+} ions are bound at specific sites in the cytosolic domain poised to potentially control Mg^{2+} flux, an indication of similar homeostatic regulation. Conversely, the basis for Mg^{2+} selectivity appears different in the two systems. Uniquely to date among ion channels, CorA selects for Mg^{2+} in part by initially binding a *fully hydrated* Mg^{2+} ion to a periplasmic charged loop before delivery to the pore (4,12). During membrane passage, Mg^{2+} is partially dehydrated and interacts solely with backbone carbonyls of pore residues. In MgtE, the molecular basis for Mg^{2+} selectivity appears reversed and has a different molecular basis. Again, however, we propose selectivity involves the fully hydrated Mg^{2+} cation. An electrostatic sink apparently attracts cation but does not provide Mg^{2+} selectivity. Just within the opening of the membrane pore, a "ladder" of 5 sequential backbone carbonyls from each monomer appears spaced to select a *hydrated* Mg^{2+} but not other cations. Subsequent movement through the pore after partial dehydration involves interaction with both backbone carbonyls and charged residues within the pore. Thus, in both MgtE and CorA Mg^{2+} selectivity seems to involve interaction with a hydrated cation, but evolution appears to have found two quite distinct means to accomplish this goal.

This proposal seeks to extend our study of Mg^{2+} systems to MgtE, an important prokaryotic Mg^{2+} carrier with mammalian homologs, through investigation of its properties, energetics and structure-function relationship, with focus on examining the differential basis of Mg^{2+} selectivity in MgtE *versus* CorA. Continuing structural work will be performed in collaboration with Hattori and Nureki, who originally solved the MgtE structure (letter attached).

SPECIFIC AIM 1: Characterization of the MgtE Pore and the Basis of Mg^{2+} Selectivity

Site-directed as well as alanine and cysteine scanning mutagenesis studies will investigate *a)* the hypothesis that 5 precisely positioned backbone carbonyls at the entrance to the pore confer Mg^{2+} selectivity and *b)* the role of specific residues within the pore hypothesized to interact with Mg^{2+} during passage through the membrane. The initial site of interaction of the Mg^{2+} cation with the external face of the pore will also be identified by hydroxyl radical mapping. Selected mutants will be purified and crystallized by our collaborators.

SPECIFIC AIM 2: Mg^{2+} Regulation and Soluble Domain Movement in MgtE

Using site-directed and alanine and cysteine scanning mutagenesis, the role of the Mg^{2+} ions bridging membrane and cytosolic domains of MgtE will be studied. A hybrid channel will be constructed where Mg^{2+} regulation of Mg^{2+} transport is replaced by anion regulation. Similar approaches will be used to determine the (functional) role(s) of the N and CBS domains. Again, selected mutants will be purified and crystallized by our collaborators.

SPECIFIC AIM 3: *Properties of MgtE transport in* Bacillus *and* Thermotoga

T. thermophilus and *B. subtilis* MgtE will be expressed in *E. coli* and/or *Salmonella typhimurium* strains lacking all endogenous Mg^{2+} transporters. Basic transport properties and energetics of ion movement of wild type and various mutant transporters from ***Aims 1*** and ***2*** will be dissected using whole cell transport assays and, with purified MgtE reconstituted in liposomes, counter-flow/concentrative uptake assays.

SPECIFIC AIM 2: Mg^{2+} Regulation and Soluble Domain Movement in MgtE

Rationale. Our collaborators, in their initial work (11), solved the crystal structure of the soluble domain of TtMgtE in the presence and absence of Mg^{2+}. The overall N domain structure does not differ in the two forms, nor does the overall CBS domain structure although the individual tandem CBS domains within each monomer change slightly. Strikingly, in the Mg^{2+}-free structure, the N and CBS domains are markedly rotated away from each other by 120° relative to the Mg^{2+}-bound form (***Figure 11***). This rotation provides an obvious means of promoting movement of the connecting helices in turn moving TM2 and TM5 to allow Mg^{2+} entry. Alternatively, it is possible that this marked movement is an artifact of crystallization in the absence of Mg^{2+}. The bound Mg^{2+}'s in the full length structure appear to provide a mechanism to keep the various soluble and membrane domains together. The paired Mg2/3 ions and likewise the paired Mg4/5 ions would appear to play distinct roles in maintaining structure.

Many questions are possible about the role and structure of the soluble domain of MgtE. Rather than extensive mutagenesis of multiple individual residues within the N domain or the CBS domain, we will concentrate on experiments designed to *a)* confirm the role of the Mg^{2+} binding sites in the cytosolic domain through mutagenesis and conversion of the Mg^{2+} regulatory sites to anion regulatory sites, *b)* investigate how the "connecting helices" move in the absence and presence of Mg^{2+} through site-directed and cysteine-scanning mutagenesis and *c)* determine whether or not large movements of the soluble domains are relevant using crosslinking and mutagenesis. In the course of these experiments, we hope to provide Professor Nureki's laboratory with stabilized forms of full length MgtE that are potentially locked in an open conformation for crystallization. The structure of an open form of MgtE would be of obvious value in elucidating its mechanism and in evaluating various hypotheses discussed in this proposal about Mg^{2+} recognition and ion flux.