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MICHAEL REAGAN

But What Is It That You Actually *Do*? (What It's Really Like Working in the Lab)

"But what do you actually do?" I have been asked that question about life in the lab as a working molecular biologist many times over the years. Often the question comes from students pondering the decision to go to graduate school, at other times the question is from interested nonscientists (civilians I call them), and sometimes the question is from a member of my family. That question used to be my cue to launch into a detailed explanation of the role of the nucleosome in transcription of the *GAL1* gene, or the role of the Rad27 protein as a flap endonuclease in DNA base excision repair. I found that my explanation often stimulated an intense desire in the listener to be somewhere, anywhere, other than trapped in this conversation.

After a number of episodes such as this, I gradually shortened my explanation until now my answer is simply "Trying to cure cancer" or, if I'm feeling particularly cheeky, "As little as possible." Neither of these answers is very satisfying and so, as I was engaging in a sabbatical in the fall of 2004, I decided to keep a journal of my days in the lab so that the next time someone asks me what I do, I can show them this series of vignettes of lab life as I experience it. So students, civilians, mom and dad, *this* is what I actually do in lab.

THE ROOM

The first thing that strikes me when I walk into a biology lab is how messy and haphazard it all looks. There is an incredible amount of *stuff* in what is usually a rather small space. My lab space here is typical. I have a lab bench, literally a flat countertop, about six feet long. The countertop is made of an impervious black substance that resists acids, bases, fire, and any other goop that might spill on it. Granite countertops have nothing on this stuff! On the bench is always a collection of instruments, the leftovers of yesterday's experiment, a variety of types and sizes of tubes, pipettes, bottles and flasks, and my lab book.

Labs are built with a whole lot of electrical outlets (there are 12 at my bench) and we need them! Plugged in at my bench are a microscope, several apparatuses for doing gel electrophoresis (a technique that separates nucleic acids or proteins by size using an electrical field, a mainstay of modern molecular biology), a hot plate, a vortexer (a really neat instrument that allows one to thoroughly mix liquids in a tube), a balance (that's a scale for you civilians), a pH meter, and a stir plate for stirring solutions. This space gets so crowded that I usually have to spend a few moments clearing off a work space on my bench when I arrive.

Above the countertop is a series of shelves carrying all manner of scientific-looking things: a variety of shapes and sizes of plastic tubes, glass beakers and Ehrlenmeyer flasks (triangular glass bottles), boxes of toothpicks (you'll see why later), a huge number of glass bottles with various clear and colored solutions in them (all having been labeled with a piece of fat colored tape on which is written the contents), a wide variety of plastic bottles of chemicals with scary warning labels, a few instruction manuals (page-turners like *Current Protocols in Molecular Biology*), a freebie T-shirt from a biological supply company, a collection of Sharpies for labeling bottles, a Bunsen burner, and the other detritus of a working lab.

Under the countertop are drawers and cabinets similarly full. A smell familiar to every biologist is faintly in the air, a combination of bleach, ethanol, and the rotten-egg smell of ß–mercaptoethanol. I walk in, take a whiff, and sit at my bench. I feel right at home.

PREPARING FOR RESEARCH

Time to get to work. First, negotiations must commence with labmates on what music to listen to. This is vital in the lab. As you will see, one spends many tedious hours sitting at the bench doing repetitive tasks, and music is a way to relieve the boredom. We share a common research room, so this summer I must find a compromise acceptable to two students, one colleague, and myself. Classical music works for me, although I often need something a little more peppy. My colleague is mired in the '60s and if it were up to him we'd listen to Bob Dylan and other folk music all day. Fortunately the students have as limited a tolerance for that as I do. Unfortunately the students like '70s hits, which I can no longer tolerate, having heard more than enough REO Speedwagon, the Who, and Led Zeppelin to last a lifetime. We settle on a pop station, and I listen to Jessica Simpson and Hilary Duff throughout the summer, with the occasional 50 Cent (pronounced "fittycent" according to my students) or Snoop Dog offering thrown in.

The first thing I have to do is decide what chemical solutions I need to make for the immediate future, and then make these solutions. This is one of the many tedious, repetitive, and downright boring tasks I'll encounter this semester. Solutions are like spices in the kitchen — you often don't need too much of any one but you have to have a whole variety in your cupboard to cook a meal. The difference is that rather than just buying a bottle of the stuff, I have to make each one.

First I look to see what my undergraduates have made. Okay, that bottle of 5*M* NaCl looks good and fairly full, I'll just use that. Hmmm, this bottle of 1*M* Tris looks dodgy — is that something growing in it? — better dump it and make another batch. I decide what and how much of each solution I need to make, then I calculate the amount of dry chemical I need to add to water in order to make each solution.

Now comes the tedious, frustrating part. To make each solution I need to precisely weigh the dry chemical, add it to a beaker containing a certain amount of water, stir until the chemical dissolves, and finally pour the mix into a graduated cylinder which allows me to add water to the exactly correct volume. So, I need to find the chemical, the beaker and graduated cylinder, and a small magnet covered in plastic called a stir bar. Should be simple, but because of safety concerns we have chemicals in about 20 different places, so I need to figure out which safety category a chemical belongs to — low hazard, irritant, acid, base, poison, and so on. I go to where the chemical should be and . . . it isn't there. #*$%! Why doesn't anybody return things when they take them! I have to go on a search mission to find the missing chemical bottle.

After a half hour I find it on a bench top and nab it. (Later I'll tackle an ethical dilemma: Now that I have the chemical do I put it back where it belongs or hide it so that it will always be available to me?) Now I need to find a clean beaker and graduated cylinder. If I'm lucky it's on a shelf of clean glassware, but usually I find it caked with some mystery goop which needs to be cleaned off. I put on my nylon gloves, pray the goop isn't hazardous waste, and scrub away. Finally, after half an hour, I'm ready to make the solution.

I precisely weigh, add, use the stir plate to spin the stir bar to dissolve the chemical, top off to the proper volume, find a bottle (I've finally bought my own bottles because of the frustration of never being able to find a clean one of the proper size), tear off a thick piece of colored tape and write the composition of the solution on the tape and stick it to the bottle. Forty-five minutes, one solution made, only ten more to go. I think, "For *this* I got a Ph.D.?" By the end of the day the solutions are made and now need to be sterilized in an expensive and finicky instrument called an autoclave. I set them on the "to be autoclaved" table and hope that this isn't one of the days the autoclave is out of commission.

THE PROJECT

After making solutions it is time to dive right into my project. I work on DNA repair in a particular species of yeast. My project is to discover whether several recently described proteins are involved in DNA repair. My strategy to investigate this is to delete the genes for these proteins from the yeast genome and check to see if the resulting mutant yeast strains have any identifiable DNA repair defects. Why yeast? It's not that I'm particularly interested in the arcane details of DNA repair in yeast cells, but I'm very interested in DNA repair in human cells, and it turns out that yeast cells do DNA repair in a way surprisingly similar to human cells. Why DNA repair? Well, if you can't do DNA repair you may accumulate mutations, and some mutations can cause cells to be transformed into cancer cells. So, *if* I can learn about DNA repair in yeast and *if* yeast repair their DNA like human cells do, then by studying DNA repair in yeast I'm probably learning a lot that can be directly applied to human cells, and some of that may impact our understanding of how healthy cells mutate into cancer cells.

Deleting genes from yeast turns out to be surprisingly easy, at least in theory. I need to synthesize a particular piece of DNA containing a gene called a selectable marker. If I add the piece of DNA to the yeast cells under the correct conditions, the cells will take it up and replace their own gene with the selectable marker gene. I then put the cells on a Petri plate with selective growth agar, and in theory only cells that have deleted the gene and replaced it with the selectable marker survive. Most cells won't take up the new DNA and delete their own genes, but a few will, and only these cells will continue to grow on the agar and form occasional colonies of cells on the Petri plate. Each colony starts with a single cell that deleted the intended gene and replaced it with the selectable marker gene.

In order to synthesize the piece of DNA I'll add to the cells, I have to order short pieces of DNA (oligonucleotides) to get started. Amazingly, you can order these from a company for about ten bucks. (Recently a paper came out in which the authors ordered oligonucleotides from commercial sources and stiched together the genome of the smallpox virus from these oligonucleotides. Sleep well tonight, everyone!) I go to the web site and fill in the order form with the particular sequence of DNA I need, and a day or two later I get an envelope with the DNA in a tube.

Since I'm on the web I go to the main search engine for biomedical literature (Medline) and type in a few DNA repair keywords to make sure I haven't been scooped on this set of experiments. Whew! Not yet anyway. While I'm at it I go to an interesting cicada web site and learn about the fascinating life of these creatures. Then I go to a website to check how the stock markets are doing, then to one of a golf course near Las Vegas I've heard about. Boy, those views are beautiful. . . . Oops — I have just wasted

half an hour internet surfing! Oh well, sabbaticals are for broadening one's horizons, right?

A few days later I receive the oligonucleotides and synthesize the pieces of DNA I need to make my deletions. I'm actually deleting five different genes, so I make the five pieces of DNA I need. Now I need to grow my yeast up to just the right point to make them most efficient at taking up DNA. I need to start the culture the night before, so I need to work backwards from the number of cells I want at 9 a.m. tomorrow (Friday) morning. If they double every two hours, then how many cells do I want to add now? I calculate then add the cells, but this is always a bit of a gamble. The cells aren't very predictable, and overgrown or undergrown cells are no good. Some people start three cultures with different amounts of yeast hoping that one will be right the next morning, but that seems wasteful to me so I just grow one flask. Unfortunately my penury has backfired; my cells are overgrown the next morning. I press ahead anyway: prepare the cells, add the DNA to the cells, and plate them onto the Petri plates. I'll leave them in the incubator over the weekend and Monday morning I'll see if cells grew. If they took up the DNA and made the deletion, they will have replaced their own gene with the selectable marker gene and will grow into a colony on top of the agar, appearing as a little white raised dot. I leave lab hoping for success.

Monday morning I come in to the building, unlock my office, drop my briefcase on the floor, and immediately walk into the lab to check the plates — no time for checking email until I open the gray doors of the incubator, pull the plates out, and see if there are white dots. This moment, when I pull the plates out of the incubator, is a moment of true lab excitement for me. These moments come rarely amidst the mind-numbing tasks like making solutions and cleaning glassware, so I savor them. If I see colonies it means that I may have created a new yeast strain, possibly one that has never before existed on earth. It also means that step one of my project may be complete.

Since doing an entire set of experiment may take months or years, I find I must break them down into single steps to feel a daily, weekly, or monthly sense of progress. I pull my plates out and eagerly scan them on the benchtop. Eureka! Well, partly eureka anyway. Three of the five plates have colonies on them. Two thoughts immediately occur in parallel: I have colonies(!), and, why the heck don't I have colonies on the other two plates? Now I can check my email and plan the next steps.

For the plates with colonies, the next step is easy. I must pick the colony off the plate and transfer the cells to a new plate, so that I'll have many colonies, all identical, that I can keep in the refrigerator and use for my experiments. The colonies grow on top of the agar in the Petri plate, so I use a toothpick to carefully scrape off the colony

and transfer the cells to a new plate and smear them on top of the new plate to yield many colonies. The toothpicks are sterile and are kept in a glass beaker. I actually pay attention to which end of the toothpick is at the bottom of the beaker, the fat end or the narrow end. Since the end of the toothpick I touch is no longer sterile, the end on the bottom is the business end. The narrow end is good for picking colonies off the top of the agar, but the fat end is better for smearing. Some people actually painstakingly arrange the toothpicks so that all of the narrow ends are down or up, depending on their preference. I myself prefer a mixed beaker, fortunate because that is the way the toothpicks are arranged in the box from the store (just check for yourself). One narrow tip for plucking the colony off the first plate and depositing it on the second, and one wide tip to smear. These restreaks go back in the incubator for a few days.

For the plates without colonies, I must sit down quietly with a pad of paper and think of all of the possible things that could have gone wrong, and then try to think of ways to test which of these might actually be the problem. In the ensuing days I confirm that the Petri plates were made correctly and have not gone bad, and the DNA I used for transformation is intact and correct as far as I can tell. I cannot easily identify an obvious gaffe in my previous procedure other than that the cells were a bit overgrown. Well, when all else fails, try again and see if it works the second time. Often, simply paying more attention to each individual step of the procedure will solve the problem even when you can't identify one crucial foul-up from the first failure. If it doesn't work twice in a row, you need to do some *serious* troubleshooting and rethink your experiment. Fortunately, repeating the experiment with cells grown to the proper density yields Petri plates with colonies on them a week later.

Now I need to check the colonies to find out if I've really deleted the proper genes and replaced them with the selectable marker gene. I've decided on a technique called PCR to do this.

TROUBLESHOOTING ANGST

I spend the next five weeks in an incredibly frustrating series of experiments to try to get the PCR to work. In theory this (as in so many things in science) should be easy. It has been done many times before and the procedures are well established — only I can't get it to work. Nearly every day I come to the lab, do the PCR (which takes about half a day), and run an agarose gel electrophoresis of the results. Each day after running the agarose gel electrophoresis, I eagerly look at the DNA in the gel . . . and see that the PCR hasn't worked. I either get no DNA, or lots of DNA that is clearly *not* the region of the DNA I want.

The first few times this is okay; there are lots of things I can fiddle with, and it is a challenge. However, after several weeks of this my mental state is something like this: I hate biology, I hate working in the lab, I hate these yeasts, I hate the company that sold me this yeast DNA purification kit, why wasn't I an econ major in college so I could be doing something useful with my life? By the end of this period I'm so discouraged that it is really difficult making myself show up in lab at all and I am seriously thinking about dropping this project and trying something else. Finally though, I get the right combination of factors and one day I look at the agarose gel and see the DNA I'm looking for. Because this worked I now understand the reason for the previous failures and I'm confident I can now get PCR to work routinely. This is another "Eureka!" moment (that's two so far in three months of work — pretty good).

In the next couple of weeks I confirm that I've made at least some of the mutant strains I need. If you recall, the point of the project was to determine if the genes I deleted made proteins involved in DNA repair, and so now I need to see if the mutant yeast lacking these proteins have interesting DNA repair defects. We are in mid-fall now, so there should be time for me to characterize these strains and still be ready for class in January. So, I rush to begin studying these strains. Well, actually I rush to decide exactly how I will do this, then I tediously collect glassware, calculate how much and what solutions I need, weigh, mix, autoclave. . . . Finally after a week or so of this, I'm ready to do some crucial experiments.

RESULTS — OH NO!

One of the mutant strains I created deletes the same gene that has been deleted before by another group in their yeast strains. The paper on which this work is based indicates that this deletion will be exceptionally sensitive to a particular chemical, MMS, which damages DNA. This is a good preliminary indication that the protein encoded by this gene is involved in DNA repair. So, one of the first things I'd like to do with these strains is to see if they're sensitive to this chemical.

I track down the chemical and find that it's in a small brown glass bottle in our poisons cabinet, and it has a skull and crossbones on it. I don't know as much as I should about chemical safety, but I know a bottle with a skull and crossbones on it is not good! This chemical is actually similar to the chemical warfare agent called mustard gas (although different enough that many of the perils of mustard gas are irrelevant for this chemical). In fact, the class of chemicals called the nitrogen mustards are a famous example of good cop/bad cop chemicals. The same chemicals that are used as chemical warfare agents are also (at lower doses) chemotherapy agents. We think that the DNA damage these chemicals produce causes rapidly growing cells (like cancer cells) to die. So the fact that people have chosen MMS for experiments like these is not a random choice, as it is related to a number of chemotherapeutic agents.

To determine if my cells are sensitive to the MMS, I simply grow two flasks full of cells, subject one of them but not the other to the chemical for 30 minutes, wash out the chemical (saving the chemical waste in a hazardous waste container which will have to be sent to the University of Minnesota for safe disposal) and put the cells from the untreated and treated flasks on Petri plates. The Petri plates then go in the infamous gray incubator for a few days. The idea is that the more cells that survive the chemical, the more colonies on the plates. If the mutant strain is sensitive to MMS, it should give me far fewer colonies after chemical treatment than a normal (wild-type) strain.

I perform this simple experiment and leave the plates in the incubator. I try not to check the Petri plates each day — a watched Petri plate never grows and nothing good can come of looking at these plates too soon. Finally, after four days I take the plates out. A very unpleasant shock awaits me. Not one of my strains is sensitive to MMS! I repeat the experiment, tweaking until I am absolutely sure — these mutant strains are not sensitive. What is particularly devastating is that even the strain with the gene deletion that has been reported in the literature to be sensitive is not sensitive. This is maddening. My investigations into these particular genes and proteins were guided by that paper, which indicated that lack of one of these proteins made the yeast cells sensitive.

Gradually, over the course of two weeks, I see my entire sabbatical project disintegrate before my eyes. None of my mutants have any identifiable DNA repair defect, even the one that should. There is really no publishable, or even interesting, result here. One can hardly publish a paper that says a protein is *not* involved in DNA repair; it would be akin to Chevy Chase's old *Saturday Night Live* bit, when he would announce during the news segment of Weekend Update that "Generalissimo Francisco Franco is *still* dead!"

STARTING OVER

I'm totally discouraged and disappointed. In a few subsequent experiments I never do find out why my results differ from the previously published results. It may be a difference in our yeast strains, it may be that the previous result is wrong (probably not interesting enough to publish), it may be that I'm wrong — although I can't imagine how. I finally make the difficult decision to give up on this entire line of research. Though a wrenching decision, when I do make it I feel a weight has lifted. I devote the rest of the sabbatical to writing a grant proposal for an instrument and devising a new line of research to pursue.

As I look back on this sabbatical some time later, I find it was worthwhile, though in a completely surprising way. My original project came to nothing. I did, however, learn a few new techniques and I've in fact devised a much more productive line of research. Happily, the grant proposal was funded. Finally, this experience confirmed by decision (if it needed confirming) that not having a full-time research job was definitely the right choice for me.

This entire sabbatical reminded me way too much of my time in graduate school. So, Mom, Dad, civilians, there you have it. The ups, downs, and sideways of life in the research lab, and the unexpected directions of sabbaticals.

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