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The Language of Life

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Abstract

Science is our best current approximation of the way things work. You cannot do science unless you believe there is a discernable truth inherent to the arrangement of our tangible world. The problem is, we in our given time, never know where exactly the asymptote lies or how far we are from it. My curiosity about the natural world is innate, but fate has variously gifted me with outstanding personal opportunities to indulge that curiosity through the study of viruses. As a woman of the boomer generation, professional paths were not always open-door, and to a certain extent, still aren't. Whether such points should now be viewed as obstacles or stepping stones is a matter of perspective. RNA viruses and the multiple, seminal mentors who taught me their secrets, have defined my career. Some of their stories are told here as they dovetail with mine. If there is any unity to this, it would be a pursuit of the language of life, or sequence analysis, as taught to us by natural selection. The intent here is not a legacy but an example. Science is a beautiful fate.

Nanuet, New York

Do you believe in kismet? I'm a scientist so it isn't cool to admit such stuff, but it seems at every stage of my life with key decisions pending, a clearly labeled path appeared with the message, "Do this!" I was born in Maryland, 1948, while my dad served as an army engineer at the Aberdeen Proving Grounds, designing optics for tracking missiles. Mom went into labor shortly after an officers' wives tour of the site's chemical warfare storage facilities, a protocol designed to reassure them of the safety of their nearby billets. Perhaps it was more of an oracle than fate, but as many of those materials were subsequently transferred to nearby Fort Detrick to enhance the nascent bio-warfare program, I guess you can say I arrived in the shadow of the nation's first BL4 facility.

My parents were born and raised in the same small town just north-west of New York City, and returned there after the war to a house they built and lived in the rest of their lives. The budding NY suburbs were close enough to the city for museum and arts forays, but far enough out that we were pretty sure we wouldn't be directly targeted in the first wave of cold war missiles destined to annihilate Manhattan. In the 1950s and '60s, right up through JFK and the Cuban Missile Crisis, if one lived on the US east coast anywhere near a Nike missile silo (we had 3 within a 5 mile radius), that specter was very real. It colored my whole generation's perspective, especially women, with a kind of self-reliant fatalism. Do it now, you might be dead tomorrow. I remember quite clearly my initial curiosity in science was in fact piqued by the specific technical differences between hydrogen and uranium bombs. It was not an uncommon question at the time. My dad carefully explained why those

differences had such important implications. His interest was in the engineering of the optical tracking mechanics. Whether you chose to drop a nuclear bomb from a plane or incorporate it into launched warhead were subtleties that complicated the projection of accuracy. The potential obliteration of our particular house basically came down to the standard deviation. He taught me to calculate trajectories before I was out of grade school. From him, I learned that physics was great at explaining how stuff worked, but it was clear that math made the physics possible, and chemistry was at the heart of actually blowing things up.

Great Uncle Oscar, my grandfather's brother, was a chemist. After the war he set up a business testing boiler water samples for heavy metals. If you didn't test regularly, the steel corroded and the boiler could fail. His township in nearby New Jersey did not permit the requisite materials in residential settings, so he set up a lab in our basement, visiting every few weeks to conduct his tests. My older brother and I were allowed to watch as he calibrated his burets, mixed his potions and annotated everything in a little black book. Oscar also did magic tricks with phenolphthalein to amuse us, after which we were required to leave him alone. The fundamentals of acid-base reactions were never a problem after watching him perform. Better still, when Oscar wasn't there, and after he retired, we had access to a fully equipped chemistry lab right in our own house. My brothers made gunpowder from scratch for their miniature brass cannons. I distilled fermented pineapple juice into the obviously useful product. Years later when I helped my parents finally clean out that lab, we found all kinds of stuff that shouldn't have been there. There was a half kilo of metallic sodium stored dubiously under oil, various unstable cyanide derivatives, azides, and a huge vat of liquid mercury with which Oscar had taught us to coat copper pennies. We should have all been dead! Because of this though, I never had the slightest fear of laboratories and chemicals, or any doubt about the neat things one could do there.

My first experience with biology, also while very young, involved prophetically, poliovirus. American Cyanamid's Lederle Laboratories was located in my home town about a mile from our school. Polio was ravaging the whole country, but was especially prevalent in the Hudson River Valley. FDR's infection several decades before was symbolic of the endemic specter of local virus. Several kids in my class developed the disease with consequent permanent leg and arm paralysis. Lederle was among the first companies to receive government vaccine contracts for the Salk trials and later the Sabin trials. Production required large-volume, high-titer virus growth as well as animal testing. The whole community was abuzz with the implications of these contracts. It necessitated a plant expansion and lots of high-paying jobs. My dad explained that the increased tax base meant our schools were going to improve. Moreover, our small town could shortly brag we had the vaccine plant that was going to cure polio. It was a really big deal! When I was in kindergarten, we took a class trip to see the resident test monkeys, some of which were housed in great pens like a zoo. Pop applied for a position designing their water treatment plant. He didn't get it as they didn't really need engineers. Instead, he came home with a pocket full of laboratory gerbils which we transformed into pets. Needless to say, biosecurity wasn't the highest priority then. It was only decades later, once the plant finally closed and they tried to build houses on the campus that the full extent of the waste problems were discovered. Seemingly, production materials were sometimes discarded after minimum

inactivation. On the positive side, we locals, the apparent canaries in the coal mine, were among the first in the nation to take part in the polio trials using the local products. We got our shots before anybody else, Salk injections, followed shortly by three oral Sabin doses. Everyone took part. Twenty-five years later when I joined Roland Rueckert's lab, antibody tests were required to ensure you were protected. I still had polio titers that were off the charts. My serum, even now, is good enough for microneut assays. You have to love big pharma! No one was going to question production practices unless there was a plant-related outbreak. At the time we knew only that we were now protected, polio was on the way to eradication, the company was providing great local paychecks, and I had developed a healthy (literally) respect for the society-changing power of biology innovation. At seven years old, I wanted to make vaccines and work for Lederle.

The promise of improved schools did actually materialize. In the 1950's, good teachers were moving from the city to the suburbs. The kids were behaved, and their parents supported quality education. There were no disciplinary problems. Mom taught 3rd grade so if we even looked cross-eyed at a teacher, we would hear about it from home. Although sadly the environment was to deteriorate in later years, during my high school tenure, we enjoyed excellent instruction in English, math, science and music. I especially liked creative writing and literature. Among the first of my kismet signposts, those directions were doomed by abysmal penmanship and an inability to spell (I really didn't care). Career options for women in those days, and more or less for the next 2 decades, were pretty much limited to teaching, secretarial work, or clerking in a bank. To my parents' everlasting disappointment, my defective skill set *de facto* eliminated any potential for the first two. My dad gave me a slide rule in grade school as soon as I learned to multiply, so math, geometry and algebra came as easily as breathing. Who gives a girl a slide rule? As I look back, I suspect they still held out hope for the banking option.

High school sciences were taken in sequence. Earth science was about map reading, i.e. volcanos, alluvial plains, seashores, glacial moraines, deserts, swamps and anything else you could think of. These were explored with an unending series of National Geologic Service full-color topographical maps from which we had to treasure-hunt a weekly list of features. The virus-surface "roadmaps" Michael Rossmann and I used many years later were exact duplicates of these exercises. Information inherent to depth-cue imaging or "ownership" plots is as applicable to the rhinovirus canyon as to the Grand Canyon. Biology was taught by Dr. Duffy. That's right, a PhD level research scientist teaching high school biology. She was a fantastic seminal mentor, instigating and launching any number of scientific careers from among the few years of students she was associated with. After I graduated, she left teaching to become President of a small liberal arts college. In her class, we dissected the usual assortment of frogs and sea urchins. The best lab though was "crustaceans", preceded by Dr. Duffy's morning trip to the Fulton Fish Market for a dozen live lobsters. We observed and drew them during class and then returned after school to cook them in 6 L beakers over Bunsen burners. The dissection and consumption lab included a lecture on the milk fat content of butter and the principles of why proteins precipitate when heated. Dr. Duffy also lectured on DNA, the structure of which had been published just 10 years before. The central dogma of DNA-to-RNA-to-protein struck me like a thunderbolt. I understood implicitly this was the language of life. The concept instantly parsed for me, every single

biological question into its absolute fundamentals. Why is grass green? Why is a dog not a cat? Why were my brothers weird? The answers were obviously in the DNA as transmitted through genetics. Resolving immediately to learn and master this magical language, I look back now at the better part of my life spent doing just that. After Dr. Duffy's epiphany, high school chemistry, physics and calculus were mostly intellectual formalities. These were excellent prep courses for college but I already knew that stuff from Uncle Oscar, Pop's engineering lessons, and the monthly Scientific Americans that arrived at the house.

St. Lawrence University

The secrets of DNA and genetics required university-level studies. The fall of our high school senior year was shaded by my classmates' angst-driven dramas over choosing colleges. The question was never a problem for me. I tend to make decisions easily. (Some would say, precipitously!) I had the academic creds to go where I wanted and it was just a matter of selecting a place with the right balance of science programs. Early in October, Mom dropped a St. Lawrence University catalog on the table. This private, upstate New York liberal arts school drew its students primarily from the smart-set of east coast prep schools. Just a few miles from Clarkson, where Pop had gone to engineering school, the recently appointed SLU President was none other than our previous high school principal, a close friend of Dr. Duffy and my parents. His new regime's agenda, no doubt to the substantial consternation of his Board, included recruiting more "rurals" to help balance the SLU student experience. It was an economic diversity initiative, long before such actions were considered mainstream. Again, the situation was kismet. SLU had outstanding chemistry, biology, math and biochemistry programs. Their graduates were teaching at major universities or working for big pharma. Mom, who had obviously conspired with her President friend, fervently hoped an exposure to the cultured preppie set would, "make a lady of you," or at the very least, snag me a rich husband. SLU offered a scholarship. Application, interview and early admission took less than 5 days. I never looked back.

Canton is very beautiful. The region epitomizes upstate New York dairy lands, studded with spectacular hardwood forests which extend all the way south through the Adirondacks. It is also very isolated. Once the snow flew in October, college life was pretty much reduced to drinking, studying, and ice hockey. I didn't have a car until my junior year. The bars were about a mile downtown, so that pretty much left the last two options. Women didn't play sports then. Although SLU is now NCAA in multiple women's sports, at that time, unless you were on the Olympic ski team (2 of my classmates), there were no competitive opportunities. The whole student body though was insanely dedicated to men's ice hockey. Whenever the team had a home stand, admission was free to students. The beer flowed (pre-21 age restrictions), and those Friday/Saturday series comprised a good deal of the obligatory campus social calendar once the Adirondack passes closed for the winter. In the spring, there was a short window for tennis or golf, an obligatory part of preppie professional development, but other than that, we pretty much spent our time studying. The curriculum was comprehensive and certainly lived up to my expectations. I could take as much science as I wanted. After my freshman year, I taught sections of the intro chem lab to earn tuition money.

SLU was heavy on the liberal arts and that meant even science geeks had to take substantial credits in humanities, arts and languages. At that time 4 semesters of “Scientific Translation” was a prerequisite for entry into any US graduate program. Since I couldn’t spell, French and Russian were non-starters. That left German, another finger aimed straight at my Austrian heraldic, “*Fraulein von Mütlich von Palmenberg*”. The problem was, our professor, a presumed post-war retiree from the OSS, in classic Teutonic fashion, loved 7 AM classes, pop quizzes and diabolical exams, all of which required a discipline I declined to extend outside of my beloved lab courses. I pulled the minimum “C” for 3 semesters. Near the end of the excruciating 4th semester, unaccountably, a new national consensus was announced, unilaterally abolishing the hated language requirement for US graduate schools. Outraged at the wasted effort, I carted two years’ worth of German books out to the Quad and burned them into ash. Had the temperature not been subzero, I would have danced naked around the fire. This was also the time of the Kent State shootings triggered by the raging Vietnam war protests, which by now had spread actively to SLU. In solidarity, all senior finals were cancelled that year, including Scientific German.

Vietnam was inescapable, a veil that overwhelmed the academic culture of that era. Institution of the military draft meant that young men who were not in school, were in the war. A number of my SLU classmates came home in body bags. Fear was pervasive. When the draft finally ended it was one number short of my younger brother being called, and one month before my older brother finished graduate school. I was seriously evaluating graduate school possibilities. Sorely disappointing my mother, I hadn’t met and married a preppie. In truth, had I been in school 5 years earlier or 5 years later, I’d probably have hooked up with a stock-broker and be living in Westchester with 4 kids. But at that time, in the midst of the free-love hippie-fueled national chaos of anti-war protests, no one I knew was getting married. It wasn’t a priority. We first had to save the world and even women had to do our part, or more correctly, were now allowed to participate with quasi-subservient footing. I realized I didn’t have the emotional stamina to practice medicine, although I would have dearly loved to study it, so I reverted to Dr. Duffy’s teachings, pursuing the language of life. Wisconsin and UC Berkeley both had internationally renowned programs in biochemistry and genetics. Molecular biology, as a named field was still a few years away. Instead, one had to major/minor in both subjects to span the gap. Wisconsin admitted me. Berkeley didn’t (citing those sub-par German grades) and I was off to Madison. The bombing of UW Sterling Hall aimed at the Army Math Research Center, had killed a physics researcher and injured three others, 2 days before I left New York. The Biochemistry building was only a block away. My mother lay down in front of the car to prevent me from going, but I drove around her and headed west. I knew this was my fate.

Madison, the first time

It was only after I arrived that I learned UW admittance was conditional. The Biochemistry Graduate Program had generic NIH funding for about 20 fellowships per year, but they admitted 60 candidates, expecting that after second-year oral and written comprehensives, more than half the class would be let go. For the men, dismissal meant instant Vietnam. With conditional admittance, more students at least had a chance to prove themselves. For the women (all 3 of us), it meant the competition would be fierce. I struggled mightily, not really

sure I shouldn't have married that stock-broker. While I aced the intro genetics and techniques units, with the core biochemistry course "601: Metabolism" I managed only a "D", meaning it would need to be repeated the next year. I later learned the men had formed Harvard Law-like study groups just for that course, from which the women were deliberately excluded. Live and learn. SLU had provided no opportunities for research experience. As a complete neophyte, I did trial rotations with Mattaiya "Sunda" Sundaralingam (protein crystallography), Bill Reznikoff (bacterial genetics) and Paul Kaesberg (plant and bacterial viruses); then choosing those labs, in that order as my preferred assignment. The first two Profs turned me down in favor of male students. Dutifully reporting to Paul or "PK" as he was affectionately known, I was greeted with, "I didn't want a student. They made me take you. What's with the 'D' in 601?" OK, this was a great start. Then he pointed to an empty bench and the postdocs nearby. "They know what they're doing. When you've done something useful, come see me."

Well, "they" were L. Andrew Ball and Jean Rohrschneider, working on mutagenesis of the Q-beta phage capsid protein. Their most recent virus screen had a series of amber mutants with odd properties. Ribosomes translating Q-beta RNA traverse the capsid cistron before scanning to the downstream replicase cistron. Native capsid protein binds repressively to an RNA stem between these regions, regulating the replicase-to-capsid ratio. Modern MS2 cloning kits are based on this exact phenomenon. The Q-beta amber panel was a Goldilocks paradox. If the unsuppressed capsid fragments were too big, replicase synthesis was repressed. If the fragments were too small, ribosomes couldn't jump between the cistrons. But if the size was just right, we hoped the replicase protein might translate unrepressed, accumulating to high levels. In the years before recombinant engineering, this was the mutational equivalent of an IPTG induction. If made to work, purified RNA replicase, the Holy Grail in protein biochemistry (at that time), could be isolated and finally studied. I tweaked the assays, ran the mutants, found the genetic sweet-spot ("amber 86") and started making buckets of active enzyme. PK was pleased. By the end of that first year, a first-author paper was in press in *J. Virology* (1).

I still had to retake Biochem 601. This time it went rather well. The 3rd semester written comprehensive exams were 1 week after the 601 final. At least 80% of the prelim questions were derived from that year's 601 exam. My classmates, having locked me out the year before, were nowhere near as fresh with the materials, so I scored near the top of the roster. After that the research prelim was a no-brainer. I alone had a major paper out. As promised, 60% of the class, but (surprisingly) none of the women, were dropped that second spring. Luckily the draft had essentially ended. Biochemistry never again took so many students. Somehow, I'd slipped in with the bolus and was now firmly ensconced in graduate school. Three weeks after my research prelim, where I proposed to characterize bacterial subunits of the replicase, Tom Blumenthal published their identity, making my project moot. This actually freed me to do pretty much whatever I wanted with my beautiful enzyme for the next 3 years. Field-stripping the system to see how it worked, I learned a lot of RNA virology and protein biochemistry. PK and I became the best of colleagues. My thesis was published in *PNAS* (2).

Zürich

Polymerase people were in demand. When it came to a postdoc, Paul pointed me towards Charlie Weissmann in Zürich, who said he would take me if I brought my enzyme. I was in a semi-committed relationship then, so the choice came down to a house with a white picket fence, or Zürich. The house would have been in Pittsburgh and the guy wasn't that special. With great chagrin, I marked the decision by slinking off to the UW bookstore to rebuy a German grammar book before setting off for Europe. Had it not been for those 4 semesters of 7 AM classes, the choice might have been different. My minimum defective language set provided enough for me to function. Zürich was just a few hours from many wonderful European cities. French men, German cars, Belgium beer, Dutch flowers, Swiss ski slopes, Italian food, museums, history, culture, what's not to love? It was a great environment to experience and learn. The lab was harder. Charles ran a very different shop than PK. Group meetings ran 8 AM to 12 noon on Saturdays, inconveniently overlapping the only hours the local stores were open. It was simply expected that your spouse took care of such things. Every experiment was discussed beforehand. Deviations were discouraged. Commercial enzymes, very precious at the time, were doled out from a locked freezer only for agreed upon protocols. Botched experiments required repentant *mea culpas* before repetition.

A few years earlier, Sol Spiegelman had published his amazing "Spiegelman Monsters" papers asserting that Q-beta replicase could synthesize random 6S RNA from free nucleotides in the absence of added templates (3–5). He said essentially, the enzyme could "create life." I already knew that my replicase, which was cleaner and more active than anything published, nonetheless maintained about a 1:1000 RNA signal that couldn't be scrubbed free. Nothing about this activity was *de novo*. The enzyme was self-templating on residual, tightly-bound viral fragments. "Prove it!" said Charles when I told him those three PNAS papers by a National Academy member were probably mistaken. Over the next year and a half I did just that, wasting many millicuries of self-made alpha-NTPs to show no three of them could ever get further than the endogenous contaminants. Furthermore, I showed that replicase made with Dr Spiegelman's protocols also had detectable RNase activity. Charles didn't like those results, so we never published. To this day, there are probably people out there still unaware those famous "Monsters" were just artifacts of relatively low purity replicase preps. Now deeply on the wrong side of the boss, I switched to an avian leukemia virus project and used the replicase to help sequence the first terminal repeats of a retrovirus by adding those alpha-NTPs one at a time (6). If you add Mn^{+2} to a Q-beta reaction, it will make dsRNA product on any ssRNA template. The first product base is always GTP, matching the ultimate 3' proximal C in the template. It was small progress, but it got a speaking invitation to a retrovirus CHS meeting during which I explored options for returning to the States.

Madison, the second time

PK missed me in Wisconsin (I threw a lot of parties!), so he coerced Roland Rueckert, another virologist in the IMV/Biochemistry Dept, into offering me a second postdoc. While at Berkeley in 1963, Roland had explored nascent PAGE techniques using urea to denature proteins. Independently, Jake Maizel showed SDS could provide uniform charge/length to

protein samples. Proper “Maizel gels” with both urea and SDS required complex circulating phosphate buffers on a water-cooled slab or tube apparatus (7, 8). Boy, could they fractionate proteins! Roland knew his techniques. He and others were using them to figure out how picornavirus RNAs of only 7200 bases could encode 300,000 Kd of protein bands (9–11). Obviously, there was a precursor and the plethora of bands represented intermediate processing products. The protease must be in there. Would I please find it? The method required timing the ribosomes to pause partway through translation so the progressive bands could be mapped (12). Neither polio nor rhinovirus genomes translated very well in rabbit reticulocyte lysates because (as we now know) they have a Type-1 IRES requiring additional human-specific factors. But encephalomyocarditis virus RNA (EMCV, a Type-2 IRES) made loads of full-length, fully processed proteins. Ding Shih from PK’s lab, showed me how to use retics (13) with Mark Pallansch’s EMCV RNA (14). Mark, who went on to a stellar polio-eradication career with the CDC, was then a grad student with Roland. But for me, fate struck again. I messed up one of my first gels, adding 5.0 M instead of 0.5 M urea to the acrylamide. The pattern didn’t look right because smack in the middle of the slab was a newly resolved band evident only at this urea concentration. It was the sought-after 3C protease (aka “p22”). We quickly established its activity, mapping it next to the 3’ polymerase gene, and with dilution experiments showed it would cleave the EMCV polyprotein in a magnificent cascade of monomolecular and bimolecular reactions (15–17). “OK,” said Roland, “We ought to get some funding for this.”

So I wrote up an NIH postdoc application. A week later a phone call from the NIH informed me that I’d used the wrong forms. That postdoc program was no longer active. Would I mind if they reviewed the project as an RO1? That’s how I got my first grant. Subsequently, “Cardioviral Proteases” was continually funded for 34 years. Back then, however, it created administrative problems because postdocs couldn’t hold RO1s. Wisconsin wasn’t giving the money back, so I was moved sideways as an independent scientist. Tenure track was not an option. In 1980, there were almost no female faculty in basic research departments anywhere on campus (or the US). Male protégés routinely had faculty positions created for them, or they were recruited from outside, directly into custom allocated slots to advance perceived departmental agendas. Women worked in parallel. It didn’t occur to me until several years later, with the first glimmers of equity promises, to ask why this was so. It just was. Still, I now had my own small lab space, funding, and an EMCV *in vitro* translation system, that to this day cannot be rivaled in beauty or efficiency. On a good day, the system could make at least a microgram of custom-designed recombinant protein per microgram of RNA template.

Poly(C) tracts

As non-faculty, I couldn’t take my own students, so I mentored some of Roland’s. Notable were Greg Duke, who later went to Aviron, Griff Parks, currently on the faculty at Wake Forest, and Neal Drake from PK’s group. We sequenced EMCV and later Mengo, the cardiovirus isolate Michael Rossmann used for structure determination. In the process, Greg discovered that the cardio/aphtho-specific poly(C) tracts in the 5’ UTRs were real problems for both sequencing and cloning. Maxim & Gilbert techniques would label the tracts on gels (C₁₂₅UCUC₃UC₁₀ and C₄₄UCUC₁₀ respectively) but no amount of effort established anything larger than C₂₄ as a cDNA, a requisite step to creating full-length native

recombinant virus (18). Poly(G:C) turns out to be toxic at the DNA level because tight stacking interactions prevent supercoiling, an important technical limit which eventually ended the early common ligation practice of G-C tailing. We knew at the time, only that the reverse transcriptase would preferentially delete, rather than plow through these peculiar non-coding virus homopolymers. Greg, a master cloner, configured everything except this tract into a plasmid. I convinced him to test the sequence for infectivity even though neither of us expected it to work. The plates were wiped out with plaques! Greg was beyond irate because I'd made him work 6 months cloning those poly(C) "artifacts" and clearly the virus was infectious without them. Then we had the bright idea to put the recombinant sequence into mice. Normally, the LD₅₀ for EMCV is 1 PFU. For recombinant vEC₉ with deleted poly(C), the value was >10⁹ PFU and you could really only kill a mouse if you went into the brains with about a microgram of virus (10¹⁰ particles) (19). Removing the poly(C) somehow severely attenuated Mengo/EMCV and instead, made a superb, highly-protective live vaccine.

Extensive subsequent studies were completed by Jorge Osorio, a DVM who eventually joined me as a graduate student to handle the animal models, and by Lee Martin, another graduate student. In the early going, we mostly probed the phenotypes of the poly(C)-deleted viruses in mice. EMCV actually infects many other animals too, essentially, anything with fur that deliberately eats mice, or has contaminating contact (dead mouse in the food bin). Consequently, one memorable afternoon, a filthy overall-clad farmer sucking on a hay stalk (he really was) pulled up in the back of our building. My name was on his invoice so the office staff raced to fetch me. "Where do you want these, lady?" he asked, waving vaguely at the 6 pink screaming piglets careening loose around the bed of his decrepit pickup. Uh, well, we'd just received protocol permission to test the putative vaccine for seroconversion in pigs. Native EMCV can be a big problem in commercial swine operations and the Vet School had asked us to prove our materials were effective in non-murine systems. Jorge had ordered these piglets, but with somewhat obscure delivery instructions. They were destined for UW large animal facilities, not my office. It took considerable persuasion to keep the farmer from dumping the pigs in our parking lot, but after proper rerouting, the trials were a complete success. All animals seroconverted to high titers and were protected against challenge with usually lethal wild-type EMCV (20). For 6 weeks, though, until we certified them as virus free, the pig poop, 2–5 kg/day, from each animal, including the controls, had to be bagged and autoclaved before disposal. We never volunteered to repeat that experiment. Instead, as zoo keepers and veterinarians found out about this work, they asked to do parallel protocols in their animals, including elephants, guanacos (sick mice apparently die in their hay and are inadvertently eaten), and many types of primates (21). We had only to supply the virus (or cDNA) and then help with the microneuts and serum titers.

One of the biggest regrets in my career is that we never solved the exact poly(C) attenuation mechanism for EMCV. It somehow involves inactivation of PKR in a poly(C) and 3C protease-dependent, anti-interferon activity, but I never figured out, mechanistically how that worked. Nonetheless, live recombinant vMC0 virus (Mengo without the tract) remains an excellent cross-protective vaccine against all known strains of EMCV in every type of

animal ever tested. Sadly though, as a small market “exotic” animal reagent, no one in big pharma ever chose to commercialize the idea.

Interlude in Buenos Aires

From early on it was recognized that our protease and poly(C) work with EMCV had direct biological analogs with the closely related foot-and-mouth disease virus (FMDV), a highly-contagious, much feared animal pathogen. In the 1920's FMDV was eradicated in the US by wholesale slaughter of hundreds of thousands of farm animals. (Remember Paul Newman shooting his cattle in the movie “Hud”?) Because of this, FMDV work has since required BL4 containment at the USDA Plum Island facilities. From the 1950's through the 80's, the virus was endemic to much of South America, thanks to Argentine imports of Spanish bulls during WWII. Luckily for the US, the Darién Gap in Panama provides a natural effective barrier to contact transmission because native carriers like deer and wild pigs, can't easily cross the swampy terrain. The Pan-American Highway, originally designed to link the continents, was deliberately never completed in this region. The break provides an ideal isolation zone, containing fauna on both sides. But FMDV was and is, always a threat to the US. Inactivated vaccines are now reasonably effective, albeit in their early stages, they had significant technical problems arising from a lack of basic virology.

In 1982, César Vásquez of CEVAN, an Argentine vaccine company, invited me to Buenos Aires for 6 weeks so we could run FMDV translations in parallel with EMCV in my retic system (22). He sent a personal check for \$20,000 to cover 15 cases of related supplies, including microfuges, radioisotopes, chemicals, gel rigs and yellow tips. When I asked about customs regulations (it took 2 vans to get it all to the airport), he simply stated, “No hay problema.” Apprehensive and completely worn out after the 18 hr flights, as I set foot on the tarmac I was intercepted by 2 gold-braided Generals guarded by a full squad of stone-faced aids with submachine guns. They had a small picture to identify me. Cut out like a calf at branding time from the other passengers, my passport and documents were confiscated as we marched to a small windowless room in a building some distance from the main terminal. One aid served me a nice cup of mate. Two more guarded the door. No one put their guns down. Presently, the Generals reappeared. Behind the opened door was a presidential limo into which the last of my materiel was being loaded. César obviously had some serious pull with the reigning junta. No customs, no import documents and apparently no biocontainment either. The lab refrigerator contained an open beaker with a full liter of 10^9 PFU/ml live FMDV, presumably skimmed from the vaccine plant. “Is that enough?” he asked.

The RNA extractions, translations and EM grids paralleling our EMCV *in vitro* assembly work, were done on the benchtop (BL1), with filtrates, including isotopes, going straight down the sink. The only things recycled were those precious yellow tips, each washed individually and reused. It was a glorious time in a beautiful city, teaching and working on a virus that was impossible in the US. The Southern Cross gleamed spectacularly in the night sky! But with just a week to go, the Brits invaded the Malvinas (Falkland Islands) and all hell broke loose. During the ensuing pro-war riots, it was certainly safer to speak German rather than English in the streets. I could get by in Spanish, but my American accent

continually betrayed me. The flight home was one of the last before the airport closed. It's a sobering experience to watch the start of a war. Arriving in Chicago supremely relieved to be home, I checked "No" on the customs form, "Have you recently been on a farm?" The USDA requires this question to ferret out people with potential FMDV on their shoes. I'd made daily trips to the lab, but never to a farm. Good thing they didn't ask about that beaker or I'd probably still be in quarantine. I knew enough not to pet any cows for the next few months.

Madison, finally

Eventually, with a second grant pending for poly(C), I took a hard look at my professional situation. For 8–9 years I'd been teaching graduate courses for Roland and PK, mentoring their students and my lab was well funded. But faculty status, although long promised, was continuously assigned to other more pressing Institute directions and male protégés. The lack of women faculty nationwide and especially at the UW was being noticed by the NIH. There were veiled threats to pull elements of campus funding unless the science departments assumed a minimum patina of equity. In 1980 JoAnne Stubbe had been (briefly) hired by the Biochemistry Dept before leaving (1987) for a permanent home at MIT. Naively, in 1986 when Biochemistry posted a junior faculty opening on "Mechanisms of Disease," I took my packet to the search chair. I'd taught 2 years of fall semester Saturday labs for that man during grad school, freeing him to attend Badger football games. Smiling, he handed it directly back. "I'll save you the time. We've met our quota." I knew exactly what he meant. Until the mid-90's, that attitude was pretty much the norm in most high-end research departments across the US.

Female or not, I was really getting tired of being treated as second class. I sent CVs elsewhere and quickly got 3 recruitment invitations at big east/west schools. The very week I showed these to PK, I received a visit from Virginia Hinshaw, our local avian flu virologist, and at that time the only woman in the Veterinary School. She was soon to become the Dean of our Graduate School and she didn't want me to leave Madison. Somehow, unexpectedly, she'd learned of an "emergency" vacancy in the UW Veterinary Science Dept., and if I would just give them a seminar, she was optimistic about the outcome. They too were under pressure from the Deans and funding agencies, having never hired a woman (or minority) in 75 years. To sweeten the pot, the faculty there was willing to vote for immediate tenure. I liked Madison. I was productive there and didn't (really) want to leave. It took about a month of paperwork, support letters and committee votes, but in late 1987 I found myself with a tenured UW faculty appointment in a modest lab a few blocks down the street from the Institute for Molecular Virology (IMV). My faculty assignment was the first and only one with IMV affiliate status, not to be housed in the IMV building itself. The full extent of my startup package was ~\$35K applied towards an ultracentrifuge and a Revoc, to be shared by 5 other Vet Sci faculty, none of whom contributed additional funding. All other lab and animal equipment including my office furniture and the remaining \$15K for that ultra, came from my grants or from scrounging among other lab's discards. It was back-door, overdue and way under budget, but at least I was now within the established University core. Perhaps it was fate, or I just finally found the backbone to force something loose. When IMV/

Biochemistry made their next hire 2 years later, his IMV lab was well equipped and the package included ~\$100 K of startup funds.

Within the context of VetSci (aka: Dept of Animal Health and Biomedical Sciences) my group spent the next ~8 years playing with poly(C) tracts (lots of mice), and walking up and down the EMCV genome with recombinant techniques, probing for secrets. In 1994, during a campus restructuring, AH&BS moved into the Veterinary School. I asked that my tenure home be transferred more correctly back to Biochemistry, where attitudes towards women had adjusted somewhat in the interim. My program was accelerating on many fronts; including the IRES technologies (see below). The Dean of our College (CALS) owed me multiple favors. Having served on endless College and campus committees (yes, women do carry a heavy service burden), I knew where his skeletons were buried and it was important to keep them so. He encouraged me on behalf of the progressive members of the Biochemistry faculty, to “reapply” and backed it up with political incentives to the department. As a result of that transfer, our group was ousted from AB&BS and assigned for the next 2 years, to an abandoned, decrepit Biochemistry lab, scheduled to be torn down. We finally relocated back into the IMV itself, only when Virginia Hinshaw decreed accommodations must be made for equitable space after a building renovation project. This was one of her last acts as Graduate School Dean before leaving to become Provost at UC-Davis. Throughout this and into the present, I continued to maintain an appointment affiliation in the IMV, assuming the directorship of that unit from 1997 until 2012. The IMV and Biochemistry have been my home ever since. Can’t say it was easy, but all those “Do this” kismet paths eventually led to where I was supposed to be. Upon assuming the IMV directorship, Steve Tracy, at the University of Nebraska-Omaha, a close personal friend and keen wit, gifted me, as a presumed joke, a copy of Machiavelli’s “The Prince.” It was a fascinating read, the definitive description of male political mechanisms and motivations. I sincerely recommend it to any woman assuming a leadership role. This IS how it works!

Sequences

Until recently, the majority of the US picornavirus field, with the exception of the Plum Island FMDV people, has been centered on poliovirus, with a lesser emphasis on rhinovirus. The cachet of those systems, especially in the nascent years of the polio eradication campaign, ensured funded grants and status publications. Certainly, from Roland’s tutelage, I knew those viruses well. But because I’m female, or just stubborn, I never bought into the logic of studying a specific virus, over an alternative system more suited to answering a particular scientific question. “I don’t do polio”, is an abiding personal principle. I prefer to leave those well-trodden paths to excellent colleagues who are more vested in the politics of the field.

Polio aside, I found a lot of room on the open scientific range. From the beginning, I collected and analyzed sequences, my reason for becoming a scientist. Our earliest EMCV/Mengo autoradiographs (indeed, until ~1990, one can and did sequence whole genomes by hand), were lent to John Devereux and Fred Blattner of the Genetics Department, soon to be the respective founders of the Genetics Computer Group of Wisconsin (GCG), and DNASTar, respectively. A number of their commercial software suites were developed and

trained on those datasets. In return, they funded multiple world-renowned speakers for my graduate bioinformatics courses. Nobel Laureate Howard Temin was in charge of our Advanced Virology class. He certainly didn't need my meddling, so I taught Sequence Analysis. James Crow, Fred Cohen, Michael Gribskov, David Swofford, Michael Zuker, Walter Gilbert, Walter Fitch, Temple Smith and Craig Venter are just a few of the folks who came to Madison to lecture. Some of it was pretty deep for the students, but I reveled in this stuff. If you teach, you learn. Bathed in the glorious lore of comparative analysis, cutting edge at that time, I found the core of my scientific soul. That and the thermodynamics I was force-fed in that fateful Biochem 601 class, were instrumental in providing creative tools that subsequently helped attack research problems from multiple angles.

Perhaps it's kismet again, but I've always preferred to work in single-stranded RNA systems. To me, DNA is really just a storage mechanism. The 2' or 3' sugar conformers create life-altering differences between A-form and B-form helices. Proteins can read sequences directly from the broad B-form major groove, but not from the narrower A-form crevice, where the bases are twisted tighter (11 per turn) and more highly compacted (28 Å per turn). Therefore, while DNA makes an excellent filing cabinet, RNAs are the curios in the cabinet and the reason the cabinet was built. Aside from transcription regulation, I've never been creatively curious about a linear storage system which accumulates sequences, useful or not, and perpetuates them regardless of current significance. With ssRNA, there's immediate relevance, both in shape and at the business end of a ribosome. The thermodynamic constraints of folding and packaging make any viral ssRNA information-dense and sensitive to every whim of evolution. It's like the brilliance of the Gettysburg Address verses Edward Everett's plodding 2 hr introduction. I can still spend long hours lost in multicolored alignments, examining anomalies, trying to speak this language. Why exactly is that particular base different? When did that change happen, and for what purpose? My students, wisely, have learned to leave me alone when I get into a full-blown mind-meld with the computer. It's not unlike entering the Matrix.

Our earliest sequencing systems required hand-made primers from DNA synthesis kits, assembled base-by-base in a fume hood. It took about 16 hrs of continuous 15 min steps to create a 20-mer. The entire lab worked in rotating shifts. For data entry, one typed on a VT-100 keyboard while reading films on a light box. I could do 1000 nucleotides per hour using my state-of-the art B&W terminal (bought with my first grant) connected to a remote mainframe running the nascent GCG and Staden suites. The entry algorithm beeped if on the second pass you make a typo needing resolution. Fitting contigs together took a considerable finesse to maintain the presumed reading frame. The EMCV-R genome took us about a year and a half. Mengo-M, a few years after, needed only 9 mos because custom primers became commercially available, albeit at \$20 per base. This all marked huge technical advances relative to the T1 RNase-based sequencing pioneered by PK and his group in the late 1970's. As I recall, his ~900 base BMV-4 component, ate about 5 full years of a postdoc's life.

After finishing the cardioviruses, we mostly just sequenced for cloning protocols or to confirm engineered mutations. But sometime in early 2008, I happened to mentioned to Jim Gern, our UW Medical School collaborator on rhinoviruses (RV) projects (see below), that our work on 2A proteases, specific to those species, was somewhat hampered, because most

labs only reported capsid region sequences. I needed full genomes to understand the cleavage proclivities. Jim said he had a way to fix that with his freezers full of typed, clinical RV isolates, augmenting the known panel of classic ATCC reference strains. Moreover, he knew that Steve Liggett (University of Maryland) and his colleagues at the Craig Venter Institute were looking for a good project to occupy their expensive ABI systems. In short order our collaboration turned out 80 full-length RV genomes, completing the collection of known RV-A and RV-B types (99x), as well as making substantial headway on the 55 types of the newly discovered RV-C. The study and its virology implications made the cover of Science (23). My mother called when she saw the TV news blurb announcing this feat, running as a live update, along the bottom of her Yankees pre-season game. “I thought you were studying cancer. Didn’t that work out?” Three years later, our team added 180 more full genomes to the master RV alignment. This time the impact was limited to 3 oh-by-the-way manuscripts in Genome Announcements. *Sic transit Gloria!*

Moscow collaborations

In 1983 I gave an update on the EMCV project at the Urbino European Study Group Meeting on the Molecular Biology of Picornaviruses (EUROPIC). Shortly after returning home, the lab phone rang. It was Prof Vadim Agol, the legendary picornavirus guru, calling from a “secure station” in Moscow, Russia. Vadim’s group at the Institute for Poliomyelitis had paralleled our work on the 3C protease a few years earlier. I had never met him. His government didn’t allow him to travel (the Berlin wall didn’t come down for another 7 years), but he’d heard of our sequencing through a colleague at Urbino. His group was doing the EMCV capsid proteins by Edman techniques (24), and our RNA determinations would be most helpful. I offered to send copies, but as it might take months for uncertain delivery, he asked me to read the sequence over the phone. Grabbing a printout, I started slowly, “M-A-T-T-M, etc” for about 200 amino acids. Almost instantly, the scratchy connection picked up echoes of breathing, clicks and background shuffling. I swore I heard someone mumbling in Chinese. After a second repetition for error checking, Vadim thanked me, and as the call was costing him a lot of money, we hung up. The next morning, while preparing a follow up mail package, 2 guys in gray suits and buzz haircuts showed up at my office. They inquired politely about why I’d been on the phone to Moscow, and “What secrets did that message encode?” Phone taps were ubiquitous on both sides of the cold war. I told them if I had the answer to their second question, they should give me a Nobel Prize. As to the first, I explained the value of scientific collaboration. They poked at a few things on my desk and in the lab until I reminded them we worked with infectious virus. They really shouldn’t be picking stuff up without gloves. That led to a quick exit, hands in pockets. I never learned if they were FBI or CIA, but to this day, when my passport is scanned, there is still an occasional raised eyebrow.

By 1988, the political situation had loosened sufficiently that Vadim was allowed to convene a small meeting at the USSR Academy of Medical Sciences in Moscow. Anne Mosser, Roland’s chief scientist, Eckard Wimmer, Stan Lemon, Ellie Ehrenfeld, Bert Semler, Marc Girard and I were among the handful privileged invitees. The flights were exhausting. By way of greeting, Vadim had arranged a superb home-cooked banquet at his apartment, starting as soon as our American contingent arrived. The apartment wasn’t large enough for

everyone, so European guests were feted on the following night. Course after course of delicious Russian delicacies were served, each accompanied by obligatory tumblers of kvass, champagne and of course, vodka. I have no idea how Vadim managed to acquire all that stuff, but it was overwhelmingly generous and gracious. The happy party went on and on, toasting new collaborations. Someone eventually mentioned that the meeting itself was starting in less than 3 hours so we bundled off to the hotel. This was late May and the beginning of the amazing White Nights, where it never really gets dark. It's a bizarre, disorientating phenomenon. What with the party, jet lag, sensory overdrive, and no visible biorhythm cues, I don't recall sleeping at any point in 4 days. That might have been planned. Vadim's team was prepared! For some, this was their first interaction with Western counterparts, and they had questions, reams of questions, about techniques, instrumentation and projects which paralleled theirs. They mostly worked in pairs. Every break in the meeting, or even walking to the rest room, we were shadowed by 2 or more eager scientists firing relentless inquiries from written lists. They knew every paper I'd ever published and wanted experimental specifics. It was a first-rate interrogation. I found the process was not unlike being phenol-extracted. It was highly intense to meet people so informed and so eager to learn. My respect for Vadim, is very, very deep.

Stan Lemon was there because the Moscow Institute was also working on hepatitis A. I'd been fooling with the HepA sequences and knew a bit about unique characteristics of those proteins. Stan and I were invited to an especially early morning "debriefing" with that Institute's unit, somehow managing to dodge the stern government minders who were required to accompany us Westerners everywhere. We were glad to help with their questions. In gratitude, one of the locals extended 2 tickets to that evening's Bolshoi performance. His wife was a dancer. The rest of the group was slated to go to the Moscow Circus, so our minders were not yet not at the hotel when our host escorted the pair of us through the labyrinth Metro system, directly to the door of the Bolshoi Theater. There he left us so he could catch the last train home. The performance was unbelievable! When we emerged at about 11 PM, it was still bright daylight, so Stan and I strolled a bit around the city, taking in the sights, before following our host's careful Metro instructions back to the hotel (Fig 2). The lobby was in turmoil! The minders and hotel's babushkas (they keep an eye on everything), were working the phones trying frantically to locate the missing foreigners. Losing a pair of Americans was pretty serious! We'd just been having a delightful time wandering about, off the leash, but realized later our host could have lost his job if something happened. Safely back in the fold, we were never again out of sight of the minders, right up to the gate of the airplane. The flight was to Vienna. The local University people, including Dieter Blass, Tim Skern and Ernst Kuechler, corralled the Moscow group to piggyback a 2 day mini-symposium on picornavirus proteases. The culture contrast after the intensity of Moscow was mind-blowing. Instant mental decompression into brilliant opulence and streets full of Mercedes was an almost physical shock. (Part of that though, may have been residual hangovers.) I probably slept for a week after arriving back in Madison. Vadim and I have since exchanged many reagents and projects. My 3 Russian postdoc/scientists, Vladimir Frolov, Aleksey Aminev and Yury Bochkov, were among the hardest workers I ever hired.

Roadmaps

In 1986, Jean-Yves Sgro arrived in Madison for some BMV postdoctoral work with PK, and subsequently (1989+) as a scientist with Roland who needed help with his virus visualizations in the wake of the rhinovirus B14 structure (25). French to the core of his being, and one of the kindest souls in the universe, Jean-Yves combines that rare essence of artiste and analytic. Before him, no one in the IMV had ever seen a PDB file. After him, everything was digital. He taught himself UNIX by reading the 10 volume manual that came with our first Silicon Graphics machine. Computers bend to his will. He can find and exploit the unique strengths of any existing algorithm to extract biology in beautifully rendered images and creative applications. Visit his current VirusWorld web site (26) and you'll get the idea. No matter what biological question I dreamed up, he found the computational tools to solve it. This included RNA folding at the full genome level, alignments based on analogy, and particularly, structure analyses. He also taught in the Sequence Analysis courses. When I became IMV chair, Dean Virginia Hinshaw, gifted me with Jean-Yves' permanent University position. He remains a cherished colleague and irreplaceable resource.

Roland had developed wonderful relationships with structure masterminds, Michael Rossmann and Jack Johnson at Purdue, contributing in 1985 to the resolution of the first rhinovirus (25) with the simultaneous mapping of the immunogenic epitopes by Roland's student, Barbara Sherry. It was an exquisite lesson, dovetailing function and structure. For some time thereafter, the Wisconsin and Purdue groups continued to concoct numerous collaborations involving picornaviruses, nodaviruses and bromoviruses, at yearly informal "WisPur" meetings which commonly ended with beer parties at my house. I participated experimentally, when the Purdue sights expanded to cardioviruses in 1987. Doug Scraba, from the University of Alberta, contributed many milligrams of material for the first cardiovirus crystals before it was realized that Mengo (unsequenced) and EMCV (our recent sequence) were not the same thing, and in fact differed by ~10% in protein identity. Greg Duke cranked up our sequencing gels and we managed to stay just ahead of that structure resolution (27), in completing our second genome.

To marry my comparative sequence interests with Michael's virus structures, I needed a method to assign and visualize residue ownership to specific territories on a capsid surface. Michael's VSURF program already had the core of this. It would parse PDB coordinates to whole number grid points, selecting those with the biggest atoms and highest z-values (surface) for plotted outputs, while recording the ownership and height of each node. Michael sent me large-scale paper plots, which initially I redrew by hand onto gridded McDraw templates (pre Adobe Illustrator), outlining and labeling nodes from the same residues. Color printing was in its infancy. To achieve dimension, Marchel Hill, my invaluable lab assistant (for 28+ years she has run my lab), helped hand paint each of 1000 little squares with graded tempura colors denoting topography or biological conservation (Fig 3). Those original "roadmaps", beautiful but painstakingly wrought should hang in a museum! The first published gray-scale versions, also hand drawn, helped me with a Nature editorial on the perils of rhinovirus peptide vaccines (28). Michael and I used others for some early comparative structure illustrations (29, 30). Hand work is prone to errors, though. In short order, Jean-Yves and Tom Smith automated the VSURF data conversion and color

assignments. After that, Michael Chapman (31) and more recently Chung “River” Xiao’s RIVEM (32) took the concepts much further, and now implement easy, stunning surface illustrations with just a few keystrokes. My office drawer still holds those tiny paint brushes, in the eventuality modern CPU ever fails.

IRESeS

For my 8th birthday, Uncle Bob Pugh, a horticulturist, gave me a bushel of bearded iris bulbs. These were dutifully planted around a stump near the house. Every June until Pop rerouted the driveway through that garden, elegant fronds of purple, white and yellow flowers graced the yard. Next to roses, I think I like irises the best!

Somewhere around 1985, Griff Parks, working with Greg’s EMCV cDNAs, was trying to study the 3C protease in our retic extracts. Before recombinant proteins were individually available, the effective technique was to make labeled capsid substrates (easily obtained by truncated RNAs), and mix them with unlabeled retic-translated enzyme. But to make 3C alone, ribosomes had to jump about 4000 bases, skipping the whole capsid and P2 sequences. Griff and Greg had identified a unique *BalI/MscI* restriction site within the polyprotein AUG context, and a reasonable cognate near the 3C gene. They blithely deleted about 60% of the genome. To my astonishment, transcript translation was even better than from genome RNAs. The magical translation property, characteristic of EMCV, was still intact despite the absence of any 5’ cap, or most of the genome (33). The 5’ proximal UTR segment upstream of the poly(C), and the poly(C) itself, were not required either, as Greg was still struggling with those regions of the cDNA. Griff quickly established that almost any cistron would work if linked similarly, as long as one respected the native AUG and its upstream context of ~450 bases. The “Cap-Independent Translation Enhancer” plasmid (pCITE) was a great boon to our polyprotein processing work. It made superb radiolabeled proteins to be used individually or as defined markers for gels.

Commercial interest in extract kits was growing. Among our local companies were Promega Biotech and Novagen. Each was interested in the utility of pCITE units as potential transcription-translation controls. Licensing required *a priori* intellectual property (IP) protections. In yet another turn of kismet serendipity, the Wisconsin Alumni Research Foundation (WARF), our IP unit, had just hired Dr. Jean Baker as a patent attorney. Jean had done a year with me as a postdoc, then attended law school, preferring to litigate science, instead of practicing it. For IP, though, there is nothing like having a lawyer fluent in one’s geek-speak, especially one with experience in your own lab. While I had imagined our constructs as potential gel markers, she wrote coverage (as her first patent ever) that was very much broader, completely airtight, and anticipated multiple protein expression uses that ultimately proved prophetic. Wisely, WARF chose to retain her for many future biotech IP applications (mine and others), to the significant financial benefit of the UW and our research programs. With the IP secure, Novagen licensed, Promega declined and the pCITE plasmids went on the market.

While this paperwork was shuffling, Eckard Wimmer, a dear friend and respected colleague contacted me about Griff and Greg’s enhancer paper. Most probably it was sent to him for

review. As the senior and most influential player in the polio field, he wanted to know if perchance our EMCV segment might at last allow similar cell-free translation of analogous polio proteins, were they properly configured into our constructs. I assured him that would (should) work, and took the opportunity at the imminent 1986 Positive-Strand RNA Virus Symposium, to present him with a small gift-wrapped box containing 2 nestled tubes of the pLVP0 parental plasmid. The package was tied with a ribbon and accompanied by a folder of maps, cloning instructions (Don't mess with that AUG! Keep the upstream ~400 bases intact!), and cell-free protocols. In subsequent experiments by his Stony Brook team (Sung Jang, Hans-Georg Kräusslick, Martin Nicklin) the translation system continued to work with amazing efficiency. This was reassuring but not surprising. But then, Eckard had the brilliant idea of also testing our segment in a bicistronic context. When this too proved functional, he coined the moniker, "Internal Ribosome Entry Site" or "IRES", to describe the phenomenon (34). The term has stuck to EMCV and similar elements ever since.

Once the idea of an IRES was launched, there was no putting the genie back in the bottle. Cap-independent translation became an immediate rage for many eukaryotic protein expression applications. Novagen sales of pCITE skyrocketed, not for its utility directing gel markers (duh...!), but for our included high activity EMCV IRES segment that could make any (almost) protein in (almost) any cell type. Derivative after derivative passed lab to lab, in the first throws of wack-a-gene cloning technologies. Common failures, directed pointedly back to me by e-mail ("Your *&^%\$ plasmid doesn't work!"), usually involved inane deletions or inadvertent commercial permutations (e.g. CloneTech pIRES) that ablated segment continuity and therefore, activity. Many such pitfalls were later explained by Greg and Michael Hoffmann, whose thesis with me, precisely mapped the EMCV sequence and structural elements, without which our IRES would not function (35). More recently, a best-use BioTechniques article by postdoc, Yury Bochkov, demonstrated how common, albeit erroneous, cloning practices led to several published failures (36). To this day, that EMCV IRES remains the best cap-independent eukaryotic translation enhancer available. That 1 PFU of the parental virus can kill a mouse quickly and brutally, should be a testament to that efficiency. Eckard and his team deserve enormous accolades for coining the IRES acronym and popularizing its use. Even so, Griff, Greg and I originated that concept. Our founding WARF patents are still being administered on behalf of the University.

ASV

The 1980 ASM meeting was held in Miami Beach. Driving down in van load of 7 Madisonians we took a short detour through Georgia to pick up Olen Kew, an alumnus from Roland's lab, recently hired by the CDC. Along the interstate, we were pulled over by a cop who asked me, the singular female, "Y'all OK in there ma'am? Jez check'n fo' yo' safety!" Virology was somewhat underappreciated in ASM, so ASV formed as a separate society in 1981. The inaugural meeting was at Cornell in 1982 (37). PK and Roland were among the Society founders, huge proponents and active participants. They served as respective Presidents in 1987 and 1989. (I was privileged with that honor in 2007.) Everyone in the IMV was encouraged to join. Roland volunteered Madison for the 3rd Annual Meeting (1984), then enlisted Ann Gordon-Walker, our highly capable IMV administrator, and June Osborn, a colleague and Assoc. Dean of the Graduate School, to set up the show. Except for

the symposia at a downtown theater, all other activities were held on campus. Unfortunately, Roland took sick with HepA during a trip to Colombia a few weeks before the meeting, and missed most of the fun. His obvious success brought ASV back to Madison in 1994 and this time I got the organizational nod (Roland having had his fill). Again, I enlisted Ann Gordon-Walker as my wingman. With almost 1500 registrants that year, the most ever, it was clear ASV was growing fast. We struggled mightily with rooms and AV facilities for 490 workshop talks (~90 simultaneous slide carrousels), and 250 posters. Registration was \$50 for students and \$90 for members. The real problem was lining up sufficient adjacent classrooms for convenient workshop access, since multiple UW units controlled them. One week before the meeting, we were almost displaced from our largest room by a music student who decided to hold a spontaneous recital. The staff and 80 student volunteers gritted through like champs, very proud of what the UW was doing. I admit occasionally retreating to an alcove for a stiff G&T.

Professional service is drummed into everyone at the IMV. You have to give back. It's part of one's responsibilities. ASV loved Madison and wanted to return every 5–6 years. So after a reasonable decompression period, I started thinking about the subsequent meeting. Jean-Yves was enamored with 2001, an auspicious date with fantasy connections to the Space Odyssey movie. Additionally, that year would be the 20th anniversary of the Society. We hoped we could do something special so we booked that year in the ASV queue. In the fall of 1997, Biochemistry convened a retreat at the newly opened Monona Terrace Convention Center, a Frank Lloyd Wright conception that had taken Madison 60 years to build. I gave a brief talk, but then spent most of the morning admiring the magnificent facilities. Lordy! What if we could hold ASV here? I cut the afternoon short to find their business offices and inquire whether the MT might be interested in a 5 day conference for 1500 people. Really? Most certainly! Would I care to name the terms? Initially, their price structure seemed hopelessly beyond reach. Volunteers verses professionals have a cost. The MT brochures and price lists filled a small binder. I put a verbal hold on our preferred 2001 dates, just in case, and went home to ponder.

That year, over Christmas-New Year, our family splurged on a cruise to honor Mom and Pop's 80th birthdays and their 52 wedding anniversary. My mother never did anything half way. She chose the HMS Queen Elizabeth II, one of the most elegant ships ever built. The IRES royalties were put to good use. It was like being on the newly launched Titanic but without the iceberg. Caribbean days were filled with sunny ports of call, but in the evenings, there were classes in the ship's small computer lab, designed to teach pearl-crusted doyens how to balance their checkbooks with Excel, a spiffy new spreadsheet software. I settled into a corner with my MT binder and started to put in numbers. Tuxedoed stewards glided past occasionally with martinis on silver trays. The instructor couldn't answer my questions about reiterative nested-IF statements, but he did lend me his manual. By New Year's Eve, I'd run dozens of scenarios. The MT room prices were on a sliding scale dependent upon consumables. As the bottom line, if ASV ate enough and drank enough, we could afford a meeting at the Monona Terrace. "Enough" could be solved, fundamentally, with an inclusive banquet and a sufficiently liberal beer budget. Neither was an obstacle in Wisconsin. The MT administration was willing to deal. In July, a plan was put to the ASV Council (without mentioning the beer part). They approved it, taking somewhat of a risk in assuming my

spreadsheets were correct. Registration fees increased to \$90 and \$160, to provide a margin of error, but that included a banquet ticket, augmenting my consumables. During the 2001 meeting itself, Madison temperatures soared into the high 90's, thankfully ensuring we would easily meet the beer quota. Combined with an unexpected surge of walk-ons (267), the final finances were well in the black. An alcove in the MT Meeting Headquarters housed a well-stocked cooler until I became convinced of that. The 20th meeting marks the first time every workshop, symposium and state-of-the art presentation (636) was electronic. No more slide carousels. The year before (19th) in Ft Collins was my first invited plenary. The computer and InFocus projector used for that talk were hauled all the way from Madison.

The 2006 25th anniversary meeting was a reprise of 2001, although this time, I gave the reins (and the spreadsheets) to Paul Friesen. I was his wingman. For 2012, the roles were supposed to revert, except that in late 2011, just when final preparations heated up, I collapsed with cancer. It was stage IV aggressive T-cell lymphoma, probably from a virus. UW Carbone Cancer Center saved my life with 6 months of intensive chemo. Despite 4 more months of physical rehab, I was only barely walking again when the meeting rolled around. Paul and everyone at the IMV stepped up like champs. The 31st ASV went off without a hitch. I'm very proud and very grateful for their unselfish responses. It's a heck of a lot of work to put on one of these meetings. My cancer has been in remission now for 4 years. ASV is scheduled to return to Madison in 2017, but like Roland, I think I've had my fill. Kristen Bernard and Paul are taking over (with updated spreadsheets), and the tradition will continue. The ASV legacy is in fine hands.

As a final note about meeting organization, in 2004, 2006 and 2010, I was invited by Margo Brinton of Georgia State University to help with the prestigious Positive-Strand RNA Virus Symposia. Margo and Roland had started this excellent series in 1986. Since the inception, every detail of site selections and arrangements was orchestrated personally by Margo, with very high, hand's-on standards. Web and speaker technologies became ever more advanced, however, and eventually she needed some assistance. I knew how to program html for registration and abstract collection, and my people, Marchel Hill and of course, Jean-Yves, were experts at on-site AV applications. The lessons I learned from watching Margo, were the need for absolute attention to every detail, in order to ensure superb, seminal scientific and amenity experiences for all attendees. More important though, she insisted on, and implemented speaker rosters that were balanced by nationality, gender and topics. *Status quo*, poor speakers, or repeat cliques were not tolerated. The programs were fresh, innovative, balanced, and state-of-the art. I don't think Margo has ever received enough credit for teaching us all how to behave like engaged, responsible professionals when entrusted with activities like this.

Nuclear pores

Returning to science adventures, from 1978 until last year (2015) my group focused mostly on EMCV/Mengo, touching on proteases, poly(C), IRESes, and RNA structures. At that point, recognizing my cancer, although in remission, had permanently sapped strength and mobility, I chose to downsize the lab. The cardiovirus grants were retired and we continue

only with rhinoviruses (below) where a great deal of low-hanging fruit still remains unpicked. The last EMCV hurrah though, was technically the most challenging.

Projects on virus protein fates come sooner or later to their impact on virus-host interactions. I used to hate “Virus-Host” symposia talks, preferring catalytic mechanisms with PDB files, to the “why’s” of making cells sick. It’s usually the little auxiliary proteins that carry this load. For 25+ years we tiptoed around the Leader (L) and 2A proteins unique to the cardiovirus genus. The C-terminus of 2A presents an odd ribosomal pause cassette (NPG/P) that was fun to map, but the N-terminal L protein of only 67 amino acids, defied logical utility. Acid-rich (3.3 pI) with a unique CHCC zinc finger motif, deletion of L reduced plaque size, but didn’t kill the virus. Our IRES ablation studies had identified a single nucleotide compensatory reversion in the L gene, but that could have been an RNA structure trick impacting the nearby polyprotein AUG. In 2006, Vadim Agol and Frank van Kuppeveld reported that picornaviruses induced profound disorders in nucleo-cytoplasmic trafficking (38), and that the cardioviruses were somehow doing this differently from the enterovirus 2A proteolytic mechanisms. For EMCV, the L protein, its zinc finger, and phosphorylation status, were clearly complicit.

As a minor contributor to that study (we supplied some reagents), our group was nonetheless well-placed for obvious follow-up experiments. Fred Porter, a very creative graduate student, tried standard pulldowns, only to discover that L, because of its tiny size and massive charge, is not readily detectable with normal Western assays. It slips right through 4 layers of intended capture membranes, explaining how it had been missed by us and other groups trolling for L activities. But Fred then found, if coupled to GST, the L biochemistry remained intact and the protein could be easily monitored. The recombinant chimera readily extracted RanGTPase from lysates. Ran is the cellular regulator of all nuclear pore complex (NPC) transport mechanisms (39). The tight coupling (3 nM Kd) with L inhibited RanGTP/GDP cycling, but this partnership alone, couldn’t explain how a few molecules of L, synthesized by an infecting virus, could titrate out Ran, one of the most abundant proteins in a cell. The binding had to be leveraged. The logical candidate was a phosphorylation cascade. I cringed at this idea because the impenetrable morass of phosphorylation pathways is not a direction any mechanistic biochemist ever volunteers to navigate! We knew L itself was not a kinase, nor was Ran. Still, I was amazed when Fred produced intensely-labeled autoradiographs showing that multiple nuclear pore proteins (Nups) became hyper-phosphorylated when GST-L was added to *in vitro* assays (40). Paul Bertics, a local kinase expert more fearless than I, helped Fred, Ryan Petty, Jessica Ciomperlik and Holly Basta with inhibitor and reconstitution studies. Eventually the active players (p38 and ERK-1/2) were identified for those Nup phosphorylation events, and for required modifications of the L protein itself (CK2 and Syk). Fitting the pieces together though, required NMR of the L:Ran complex (41) a superb technical feat by Valjean Bacot-Davis, a microbiology student who self-taught himself structural biology, just for this project. The structure(s) showed that L hijacks Ran, locking it into an irreversible GTP conformer. Stuck like a leech on the Ran surface, exposed residues of L become phosphorylated. The new charges snare Crm1/CAS karyopherins carrying activated kinase cargos into pseudo-exportin complexes. Unable to dissociate, the total unit hangs out in the NPC, catalyzing debilitating Nup hyper-phosphorylation. The consequence is absolute active transport failure. It probably should

scare us all, that one tiny viral protein, produced from a single infecting RNA can utterly ablate a 124 MDa NPC edifice, bringing transport-dependent antiviral activities to a full halt within 2 hours of infection. But that's what this virus has evolved to do. The EMCV 2A, by the way, the other little auxiliary protein, piggybacks L to the NPC, while on its own way into the nucleolus to shut off host translation.

Rhinoviruses

Our current experimental directions, where presumably I'll eventually wind things up, return full-circle to my origins in picornaviriology. As a postdoc with Roland, I worked on EMCV, but the group meetings (for everyone else) were heavily focused on the nuances of the RV-A and RV-B capsids. My curiosity then was directed at the language of the RNA genomes. Roland was more interested in the particles themselves, the virion "wrappers" as I called them. Still, with exposure, one does to soak up at least some key stuff, and today, deeply immersed in RV-C biology, those capsid lessons come back to haunt me.

Fred Porter's EMCV L work needed controls for NPC inhibition. In 2006–2007, Kelly Watters purified several RV 2A enzymes after Vadim and others reported that enterovirus proteases would cleave Nups, instead of phosphorylating them. The cellular outcomes were similar in that the L or 2A-treated NPC then became open to large-molecule diffusion, but not active transport. Oddly though, Kelly observed her 2A from different RV species did not respond identically. Existing RV alignments showed puzzling variability in this protein, almost equivalent to the capsid epitope regions. Viral enzymes with important anti-host functions aren't supposed to diverge like that. I touched base with Jim Gern, an awesome pediatric research-clinician at the UW-Medical School (this is the guy you want treating your kids!), who had spent years investigating virus links to asthma. He and his team, including Anne Mosser, Wai-Ming Lee (both trained by Roland), and more recently Yury Bochkov (my ex-postdoc), had freezers full of Wisconsin-circulating RV samples and were studiously cataloging them. Jim's question, "Why don't we just sequence them all?" led to our collaboration with Steve Liggett, and eventually that 2009 paper (23). But Jim didn't stop there. Responding to an RFA on asthma, he coerced me into writing a subproject so he could demonstrate connections with a hard-core virologist. For our group, this was an easy way to fund the 2A protease work. Via that omnipotent finger of fate, it also positioned us with exactly the right resources when the RV field took sudden leap forward.

The world-wide SARS surveillance efforts, combined with clinical sequencing from many labs (like us), had stumbled across a new species of rhinoviruses. The RV-C were first reported in 2006, and quickly linked to the severest forms of childhood asthma exacerbations and many other adult/childhood respiratory ailments. The whole species had been missed during 50 years of RV research because they don't grow in known types of differentiated tissue culture. Now though, we had the experimental power of cDNAs, a forte of my lab, and particularly for Yury. He quickly showed that recombinant C15 RNA was infectious to HeLa cells. The progeny couldn't re-infect new monolayers, but they did allow reagent development suitable for organ culture validation and tests with air-liquid interface (ALI) cells. The methods permitted crude antiviral drug testing, highlighting distinctions from the RV-A&B, or for that matter, all other enteroviruses. The growing sequence pile

confirmed the anticipated variability in 2A proteases, but also flagged unexpected indels in the capsid proteins, particularly VP1, which might profoundly affect RV-C receptor binding and epitope display. The deletions were conserved in every RV-C isolate (Fig 4). Curious about the structural consequences, Jean-Yves, Holly Basta and I used bioinformatics to calculate a capsid model for the C15 sequence (42). The pseudo-coordinates, while not exactly “real” did predict observed drug-resistance profiles, and projected how the RV-C, might interact with their unknown cellular receptor(s).

Models though, are conjecture. To get further, we needed the actual receptor, which elusively disappeared whenever susceptible cells were transitioned from *in vivo* to *in vitro*. Cadherin-related protein 3 (CDHR3) popped out of Yury’s gene screens (43) simultaneously with Kurt Bonnelykke’s report linking a specific allele of this gene, Tyr₅₂₉ versus Cys₅₂₉, with severe asthma exacerbations in Danish children (44). Phenotype sound familiar? Apparently, these alternate alleles change by a factor of 10, the surface display of CDHR3 in pulmonary tissues, or in transfected/transduced cells. The high-display allele (Tyr₅₂₉), is the ancestral genotype in nearly all organisms with lungs. It marks kids as hyper-susceptible to RV-C infections, the initiating triggers of their asthma attacks. The alternative Cys₅₂₉ allele, predominant in modern humans (but not Neanderthals or non-human primates), is instead protective of asthma, even in those with other propensity indicators. Cys₅₂₉ reduces the CDHR3 surface display, shielding cells from RV-C attack. Cultured monolayers are mostly refractive to RV-C because CDHR3 expression is pulmonary-specific, and/or most human cells don’t encode the Tyr₅₂₉ allele. With these new tricks in hand though, it will be exciting to spend the next few years dissecting this system, from biochemistry, to cell biology, to clinical and anthropological perspectives. The first few micrograms of C15 from transduced Tyr₅₂₉ CHDR3 cultures were delivered recently to Michael Rossmann and his student Yue Liu. Within 3 days they had an initial cryo-EM map. The current version (at ~2.8 Å), suggests our crude bioinformatics model actually got a bit of it right! After all, sequences don’t lie.

Personal

Yes, I do enjoy sports, particularly the NY Yankees and anything involving UW Badgers. That started with the hockey craze back at SLU. In grad school, I was recruited by friends into fast-pitch softball, initially for recreation. But like my mother, I rarely do things half-way. Once I discovered the wherewithal to pitch with effect is an activity which fundamentally requires only focus, heft and practice, I was locked in for the next 15 years. Until the mid-1990s the Wisconsin tavern leagues paid modest bonuses to their teams for weekend tournament wins. That required pitchers. I was usually brought in as a ringer. If I threw and won a few games, I could make \$50–60. That was good money as a postdoc/scientist. Distilled to its essence, from the mound, one learns each pitch is independent of the one before and the one after. Whether you just threw a third strike, or a home-run mistake, the subsequent pitch is the one you now need to face. Perform, or do not, the deed is yours alone. Success or failure is measured only at the end of the game, when those individual events are added in sequence. Science is similar. Define the next thing you need to do for any given situation and then execute it. If you screw up (don’t do that too often), just throw the next pitch and make that one count. Another thing intrinsic to playing ball, is you

find there are a great many people out there who are not scientists. The best players on my teams were clerks, truck drivers, police women, waitresses or stay-at-home moms. Not one of them cared if my experiments that day had worked in the lab, or maybe a major paper review was unflattering. They just gave me the ball and expected me to throw.

Communication obviously was at a level very different than we erudite academics use with each other. In the real world, you need to bring your stuff to your audience. My teammates taught me all of this, and also gave me a genuine deep respect for the lives of all women outside of our privileged, sequestered realm. Tough as it is, basically, we lucky few, generally do have it pretty good.

There are many people and their many stories left untold here. Continuity dictates that plot weaving must be mindful of the thread count. Some tales therefore await a different knit. Mom, Pop, Roger, Bruce and Karen deserve unsung credits for personal support while I pursued my fate. I don't think any of them ever really understood what science is about, but they always had my back, regardless. Wisconsin, my home for 45 years, has brought a long series of influential characters, only a few of whom are mentioned above. The IMV and Biochemistry Dept. have changed significantly relative to the culture of my early days. I would not have stayed or been as productive, without those mentors and colleagues. Roland, PK, Jean-Yves, Marchel, Virginia Hinshaw, Paul Friesen, Rob Kalejta, Nate Sherer, Paul Ahlquist, Jim Gern, etc, as well as members of their groups and mine, created the environment and resources where science could be our passion. I am fortunate to have been led on this path.

On being a woman

In my experience, the current scientific infrastructure has made only modest overall progress in altering the innate steady-state difficulties encountered by typical women with career aspirations, such as those which colored my early professional options. To frame that in biochem-speak, the present system, similar to that of 45 years ago, serves to reduce the effective K_m required for career V_{max} whenever a new Y chromosome intercalates into the matrix of similar genetic components. The mechanism is clear, catalytic and reciprocal. Thermodynamics teaches that for any such state, a higher K_D can be made equivalent only with a significant increase in the substrate concentration or by altering the effective K_{cat} . This means that women, with the exception of a few extraordinary superstars, are rarely included in the full matrix, socially or collaboratively, and have to work much harder and more efficiently, generally with fewer resources, to achieve the same benchmarks of productivity and recognition. Despite professed intentions to the contrary (by the politically correct), the intrinsic K_D at all levels of science, is quite simply, much lower for men than women, from entry to retirement.

Madeline Albright, former US Secretary of State, said much the same thing at a 2013 Pennsylvania Conference for Women. "Women do have to work much harder than men. There is plenty of room in the world for mediocre men; there is no room for mediocre women."

To be sure, there have been some improvements. These days, a search chair would be summarily fired for returning an unread resume. Moreover, even the most anointed of protégés usually now has to apply and interview before being handed the keys. Neither in the past nor in the present, have I ever experienced the slightest hint of gender (or minority) bias in initial funding opportunities at the national level, especially at the NIH. Getting hired is no longer the problem it used to be. Assuming even a modicum of equity in startup packages, although by no means is that a given (!), the continuing hard part is advancing from there. Ongoing assessments of a program's perceived qualities, as evidenced by visible recognition at significant meetings, or at grant continuation time, or upon submission of manuscripts to high impact journals to support that continuation, or even in the application for prestigious (and lucrative) awards, are all processes relying on the prevalent standards of peer review. Therein lays both the strength and the rub of the system.

Peer review is the cornerstone ideal of honest science. But most of our current implementations still choose to administer it with anonymity from like-minded cliques in the Y-based matrix, and that opens everything to subjective instead of objective criteria. For most subfields of virology, and it's probably true for most of STEM science, there are only so many binding sites, after which the field becomes saturated. The historic turf occupied by those who had insider access, perhaps facilitated by a low K_D or protégé status, develop heightened protectiveness of any new competition (male or especially female) which might threaten to displace them. If one's invested 20 years or more of education and training to break into the establishment you're going to fight like heck to maintain your status. The resulting dominant units in such fields frequently become insular, self-protective, self-reinforcing and predominantly male. Ironically though, senior women also can be among the most vociferous turf defenders. The inhibitors turn on pretty quickly, if one inadvertently strays into those perceived territories. Displacement is resisted by all possible means, unless the incoming ligand proves of undisputable quality, or naturally, of exceptionally low K_D . "Average" or "good" doesn't stand a chance. Too often, "very good" or even "excellent" is also scourged from the highest journals or overlooked for podiums and grant renewals unless reviewers, organizers and editors are threatened by exposure, to look outside their tight little circles. Few people I know have not experienced unfair savage reviews or meeting rejections that fit that bill. When hit like that, most women instinctively back off and try easier paths. I know I did. Most men, instead, get even. In retrospect, I probably should have read a bit more Machiavelli earlier in my career, to better understand these mindsets.

Admittedly, the benchmarks in science, especially STEM science are very tough. You aren't measured by the energy you put in, but by the product you produce. Even for talented (low K_D), efficient (K_{cat}) women, and let's face it, we have deep populations of superbly trained female graduate students, career substrate turnover (productivity) is still absolutely proportional to the time available for these reactions. Without co-catalytic synergy from peers, you just have to do it on your own. And it can be done, if you can grind away with all of your time and energy. Time though, for many women, is a significant rate-limiting parameter. I laugh when I read articles thanking the "little woman" for her "unselfish support". Who championed that woman when she wanted the same career and not just a significant-other niche? Taking care of the kids, walking the dog, soccer practice, cleaning the house, buying the groceries? Maybe it was kismet again, but in my life I never really met

a man who was looking for a supportive life partner, instead of an enabling mother/wife (and wasn't already married). I recognized I wasn't strong enough or good enough to carry their career and mine too, so I chose alone to follow the science that burned in me, instead of raising a family. That's a really hard call. I think it's why many young women (probably the sane ones!) drop out at the grad school or postdoc levels.

The professional bar doesn't drop because you are female. If anything, it's set higher. To play for the Yankees instead of their farm team, you must consistently generate hits. I don't know if that's right or wrong, but it's the current state of our profession. Unless you're superwoman, or manage by some superb stroke of luck to draw the rare gem of a fully engaged partner, it's very, very hard to sustain the energy it takes to do high-power science for 30 years and also have a fulfilling home life. Maybe the infrastructure will change? Maybe it is changing? Maybe we just need more transparency in identifying the obstacles, and compassion or leniency in overcoming them. Certainly at the peer review level, I'd endorse that. I'm not in favor of anonymous reviews because ingrained bias tends to have a short half-life when exposed to light. Own up to your critiques the same way you take credit for your science. Put your name on it. The same goes for meeting organizers. Every time a speaker roster is drafted, someone must ask, "Where are the women? Where are the non-US scientists? Why, if our field is 50% female, do we have 1 in 20 on the podium? Why is the matrix core continually entitled to recycle itself?" You know and I know that these self-nominating reselections only rarely represent the current best science, year after year after year. Shake it up a little and pride yourselves on showcasing new people.

Pride yourselves also on giving entry level scientists an equivalent shot. My startup in real purchasing power was more than 5x lower than the next hired male. I was savvy enough to make do and claw through with that. Why should I have had to? Had I thrown a hissy fit, the offer would have been withdrawn, that's why. It really does take longer to reach V_{max} when substrate is that limiting. A man would never tolerate such a package, or ever be offered one. While I'm a realist, and admittedly a little jaded after traversing my peculiar paths, I only hope when the next lady writes one of these articles 20 years from now, she'll record somewhat different reaction parameters.

Lessons learned

Science is our best current approximation of the way things work. You cannot do science unless you believe there is a discernable truth inherent to the arrangement of our tangible world. The problem is, we in our given time, never know where exactly the asymptote lies or how far we are from it. Aristotle is perhaps the last man who thought he knew every "fact." He pushed extant boundaries as far as logic and observation could take him. But given where science is now, relative to his timeframe, or that of Galileo, Newton, Darwin, Einstein, Curie or any of the other giants, it is clear their major findings were only on consistent trajectories towards "fact" while not exactly intersecting it. One hundred years from now, one thousand years from now, where will your work stand? If you think you alone have solved some fundamental question of Nature, then carve it in stone, polish it with your ego, and bury them both somewhere for the bemused discovery by future generations. They'll have a good laugh! There is always more to learn or ideas to refine. Nevertheless, truth is the only

defensible objective in our work, to say nothing of the only defensible position. I learned from PK and Roland, the most guile-free man I ever met, that ethics, honesty and experimental transparency are uncompromising values for a scientist.

To be sure, when I report my work, especially in front of an audience, I'm a shameless Queen of hyperbole. Many reviews, even of our best work, frequently use the word, "over-extrapolate". It's an admitted character flaw inherent to my joy of discovery. The data we published, the data you publish, must stand on their own, advancing (or not) the putative continuum of the curve. When writing a paper, the creative bits are recorded in Methods. The ideas and interpretations go into the Discussion. The guts go into Results. Any self-serving Conclusions will wax and wane over time because ultimately, you may be right, or maybe not. The Results alone are your legacy. In the end, these singularly chronicle your success or progress. The future will need to explain your findings, while trying to advance them further. If you're lucky, and indeed I have been, perhaps some spark of inventive serendipity may eventually register as a miniscule tic-mark on the evolving approximation curve, but only if you keep the priorities of truth-seeking straight. Remember also, whenever you find yourself at the head of a line, you are either leading, or you are the SOB holding up everyone else. Learn the difference.

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Figure 1.
ACP, photograph (2015) by R. Palmenberg. Background portrait (~1760) is Albertus von Ramdohr my great⁴-grandfather.

Figure 2.
Red Square, Moscow, Russia, May 1988.

Figure 3.
Hand-painted picornavirus roadmaps, ~1986.

Figure 4.
Alignment fragment of RV sequences shows relative deletions in key RV-C capsid surface loops. ~2014.