This Week in Microbiology

With Vincent Racaniello, Michael Schmidt, Elio Schaechter, and Michele Swanson

Episode 182: A micro story with macro implications

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Vincent: This is TWIM, This Week in Microbiology, episode 182, recorded on July 26, 2018. I'm Vincent Racaniello and you're listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaechter.

Elio: Well hello there.

Vincent: How have you been?

Elio: I'm doing okay, I'm doing okay.

Vincent: We haven't been together for a while, good to hear from you again.

Elio: That's true, I missed you guys.

Vincent: Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello!

Vincent: I just saw a release of Meet the Microbiologist with Michele Swanson.

Michele: Oh, yeah!

Vincent: On asm.org.

Michele: I haven't listened to the polished show but I'm eagerly, anxiously (laughs) going to do that.

Vincent: I'm sure it's great, with Julie Wolf. Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello everyone!

Vincent: How have you been?

Michael: I've been good, I got our executive director for ASM, shared with me one of his viruses at the microbe meeting, so I got over the virus but I've got this paroxysmal cough that has not decided to go away yet.

Vincent: I'm sorry.

Michael: But I've been assured by the physicians in charge that it's just my immune system cleaning up the debris from Stefano's virus.

Vincent: That's right, it's always the immune system.

Michael: Yes.

Vincent: Today we have an in studio guest, Zarina Akbary, welcome to TWIM.

Zarina: Hello, great to be here.

Vincent: Zarina is an undergraduate at Drew University, which is in New Jersey, right?

Zarina: Yes.

Vincent: Drew is not too far from where I live, actually, and Brianne Barker, who is often on TWIV, is there.

Zarina: Yeah, she's my professor for four semesters.

Vincent: And you are a rising senior and your goal is to eventually get a PhD in microbiology, is that right?

Zarina: Yes.

Vincent: This is great, so refreshing.

Elio: Good for you, good for you.

Vincent: And of course you are a listener of TWIM.

Zarina: Yes.

Vincent: And can you tell us what Zarina means?

Zarina: Zarina means the one who has gold in her veins.

Vincent: The one who has gold in her veins. Your family is from Afghanistan, right?

Zarina: Yes.

Vincent: Welcome to TWIM, we hope you enjoy it. As Michael said, you can see how the product is made (laughs) The product.

Michael: Yes.

Vincent: We haven't been together for a while, it's been a few weeks.

Michael: Well you've been on your roadshow, I've been listening to your TWIVs, and they have been absolutely fascinating. You've been literally going hither and yon.

Vincent: We've been all over. I'm glad you like them. We have been all over the US for about six weeks, yeah, doing all road shows, in fact tomorrow is our first back home show. It's good to be back. But it is fun to go out and meet the people who are listening.

Michele: You know, I wanted to say that, I was able to go to a Gordon conference and saw many colleagues but also a lot of trainees I was meeting for the first time and I was really delighted that a number of them came up and wanted us to know how much they appreciate the work that we do, so I want to up my game now (laughs)

Vincent: Yeah, knowing that people are listening, Michele.

Michele: Now that I actually know these people who are listening, yeah.

Vincent: It makes a difference to have people.

Michele: Yeah but it was really great, so thank you, all, you know who you are. I appreciate it.

Vincent: So we are back to the regular TWIM which means we have a snippet and a paper. And Michael is gonna give us a little snippet.

Michael: Well, I'm gonna start off, I've of learned this paper from one of our faithful listeners from Sweden, Dr. Volkan Ozenci and he is an associate professor and consulting physician in clinical microbiology at the Karolinska University Hospital and he pleaded with me after our TWIM taping at Microbe in Atlanta that he really enjoys TWIM but he would like to learn more about antibiotic sensitivity and testing and just a little bit more clinical microbiology. So I said great, if he got a paper that you want us to look at and he, good to his word, sent me a paper look at and that's how we got today's paper which is entitled "Antibiotics susceptibility testing in less than 30 minutes using direct single cell imaging." It is also a paper from from Sweden and appeared in the Proceedings of the National Academy of Science and was authored by Ozden Baltekin, Alexis Boucharin, Eva Tano, Dan Andersson, and Johan Elf.

And it is a really cool paper but the science of the paper hinges on the key concepts we all learned in introductory microbiology namely the growth curve and the growth rate of a population. Now, there are some things that you need to keep in the back of your mind as you think about this paper, as you are reading it or even just listing along. First, there's no such thing as synchronous growth. A population can get close with a chemostat when you are maintaining the pH, the nutrients, getting rid of the waste, all with the intent of keeping the population growing at a defined rate. The true term is balance growth, as Elio well knows because he came out about the famous Copenhagen school that defined all the kinetics of balance growth, but as the authors point out in their discussion there's too much noise in monitoring population dynamic in this point of care test that they are attempting to develop. So what they're trying to do is to determine whether or not a microbe will be sensitive or resistant to an antibiotic.

And literally the way this test is intended to work is if you are suspected of having a urinary tract infection, which is a condition that will affect 100,000,000 people on this planet each year and unfortunately more and more of the UTIs that we're seeing have become resistant to the typically nine different antibiotics that folks used to treat UTIs. People are concerned such that you don't wanna give a patient the wrong antibiotic that won't have any effect. So what they wanna do is to produce your urine sample, and then while you're in the physician's office, they will be able to assess whether not the organism is resistant or sensitive to the antibiotic in question. So how is this system going to work?

Elio: The point here being that if you don't have such a mechanism you have to wait 24 hours, meanwhile the doctor feels like he has to do something, so he prescribes a broad spectrum antibiotic, which makes probably little sense in terms of efficacy. So the idea of having a 30 minute test is revolutionary.

Michele: And it wallops your microbiome and your GI tract, too, which we now appreciate is not good.

Michael: And all of these things are coming into this technology. So how does this work? The first thing that their test requires that you need to know is the base growth rate for an individual cell. Not a population but an individual either growing in a standard antibiotic assessment medium like we currently use for most of the instruments that are in the clinical lab, which uses Mueller-Hinton broth, which is a complex medium composed of a little bit of beef, lots of milk protein, a bit of starch, and then water. Or, this test will actually work even if you'd grow the microbe in urine, the stuff that came out of the patient. So it's even more clever when you think you don't even have to grow this, you just literally squirt the urine into this microfluidic device that they have developed. So then you determine the base growth rate for the microbe without any antibiotic present, and then you expose the single cell organism to the same medium with the antibiotic that you're assessing and ask, did the drug impact the growth rate?

So the hypothesis is that if you are sensitive to the antibiotic you will grow slower or not at all. If you are resistant to the antibiotic your growth rate should not be impacted by the presence of the antibiotic. You are effectively, as an individual cell you would be indifferent to that. And so the way their system works, and I'm gonna use this description since we don't, unfortunately, have videos that we can because we are an audio show and not a video show. Imagine a very large grocery store, or a Costco or a Sam's at Thanksgiving, with 2,000 fully staffed check out lines. You're one of the microbes and the management is asking how quickly you move through the line. So that's how effectively the technique works, they're just asking--

Elio: That's a nice analogy. Good for you.

Michael: They're just asking how fast the microbe can move through the checkout line. Now, the antibiotic is effectively price check on aisle four. If you're sensitive you're gonna sit there while they go back into the back and figure out how much a can of peas is really costing. And so their technique relies, just like when you go to an empty checkout and then you're gonna stand in line while they get ready for you. Once in line, the microbe, just like you in a checkout line, cannot leave. And they've engineered it, so once the microbe gets trapped in its line it can't get out. And eventually we want all the checkers to be filled up. So they did their experiment with their checkout lines, with their microfluidic device, which is 1.25 microns wide by 1.25 microns deep and 50 microns long.

Elio: Just enough room for a single bug.

Michael: Yeah, just like the lines at Costco. They're just about as wide for the cart and the people.

Michele: The engineering is amazing.

Michael: The engineering--

Michele: The engineering of these chips.

Michael: And in fact, the show notes actually will take you to a video not from these authors but a site that actually pioneered where they got this idea, and this is comes from the mother machine which is this cute microfluidic device that was originally developed for this. And on that particular website--

Elio: The author, the father of the mother machine is Suckjoon Jun, who happens to be at the University of California San Diego, and whom I know pretty well.

Michael: And his website that I put in the show notes actually will take you if you wanna make your own mother machines, it shows you how to do all of this, so it's very very cool. So the first thing they have to learn is how fast a checkup lines will fill up, because it's important in order to get the statistics to assess whether not the organism is truly indeed sensitive or resistant to the drug of choice. So they learned and they did the experiment that an 80% of the checkout lines were filled within 30 seconds when the concentration of bacteria in the urine were two million per mL. And at two million per mL the urine will effectively look turbid.

Elio: Will it really?

Michael: Yeah. Well, it's actually a hundred thousand.

Elio: Barely, barely.

Michael: A hundred thousand you get to see barely turbidity. But at two million it's turbid. And at a concentration of 1,100 per mL, approximately 160 of the 2,000 checkout lines and their little grocery store microfluidic device are all filled within 10 minutes. And this is an important statistical component that they go through in greater detail but since this is a snippet this recognize that the authors figured out that they need about 50% of the checkout lines full of a single cell microbe. And then what happens is using a straightforward phase contrast microscope using a 20x objective hooked up to a video camera, they just do a slice every 30 to 60 seconds and ask how far the microbe has moved in that 50 micron channel. That in turn allows them to calculate the growth rate. It's beautiful, you watch the little video that they have made available in the public domain and you literally can see the microbes moving down the checkout line which is published--

Michele: They're getting pushed, right, each time one goes to two and two to four they're pushing each other down the channel.

Michael: That's exactly right. And so again you're simply comparing the growth rate of the single microbe in the presence and in the absence of the drug. If the microbe is resistant to--

Elio: You do it over and over again, you do it for a thousand bugs.

Michael: You do it for a thousand bugs because you literally have the 2,000 channels full and you have a control lane that effectively never gets the antibiotic. They start off their story illustrating the proof of concept by presenting two beautiful experiments, one with a standard E. coli strain where they tested against the nine different antibiotics routinely used to treat a UTI. And and this is where they actually show you all the beautiful data on the growth rate and they normalize it to the control, they add the antibiotic, and what you quickly see is that the growth rate for individual microbes is a little bit different but statistically, and this is why you need the power of the number, it will effectively level off. And as you can imagine you can see a slope of a line going downward, indicating that the microbe is sensitive to the drug.

And then their second elegant experiment is they take an E. coli strain that was engineered to be resistant to Ciprofloxacin, one of the common antibiotics that is used to treat a UTI. So here they effectively evaluate this single microbe that has the resistance determinant to make it resistant to Ciprofloxacin. And they ask the question, what happens to the growth rate? Well, initially the growth rate dips a little bit but if you think about it it's effectively asking the question at the checkout line, you know, is the price of this actually that, and it's engineering all the things you need to be resistant to Cipro. And then quickly, the back guy in the stockroom goes yeah, peas are on sale. And then the growth rate resumes to the base rate and the grocery store metaphor

really works well for that because in their data they beautifully show how it dips a little and then comes back to normal. And they factor that in to their statistical proof that this will work as well as assessing antibiotic susceptibility as the gold standards currently in use.

Now the proof of the pudding is whether or not this will work with clinical strains. So they ask the hospitals to send them 50 strains, the hospital sends them 50 strains, 25 of which are sensitive to the antibiotics, 25 of which are resistant. All of the 50 strains but one grew normally and the one that didn't they just threw out. So there's only 49 strains that they tested. And they ask themselves the question: can this system discriminate between drug resistant bacteria straight out of urine? And drug sensitive isolates recovered from urine? And the answer was yes. They named their test the Fast Antibiotics Susceptibility Test, and it truly does indeed work for the isolates they have selected.

Now, there are some caveats that we all have to think about. Urinary tract infections are often not mixed infections, they are typically one organism that's in this unique environment, and that actually aides in the effectiveness of this. But in their discussion they go in to effectively if it is a mixed infection they would expect the individual organisms, and again this is why it's important to use individual organisms, and if there is a staph microbe in there with an E. coli you can actually exclude it if you load the microfluidic or you change the microfluidic device, but that's getting in to the inside baseball of how this system is going to work.

So in a nutshell, they present elegant data, their figures are crystal clear, green for go is clinically susceptible, magenta is for resistant. And if you think about it the normal patient will go to their health care provider sit in the waiting room typically for about 30 minutes, or even the process of getting intake where they test your blood pressure, they checked for fever, they review your history, they interrogate you about insurance. So if you produce a urine sample, when you walk in they hand you the cup, you fill it up, and they send it back to their device that can assess this, they were able to discern susceptible and reference isolates after the first 10 minutes at a concentration of bacteria in the urine as low as 10,000 microbes per mL. That is just remarkable.

Elio: Wow. It's mind boggling. Two questions, one of them is, won't everybody want this, wouldn't every doctor want to have this in his office? Besides a microscope, take this microfluidics device, which I am sure you would be able to buy in a pump, in a little something. Doesn't it sound like it is feasible to have this in every doctor's office?

Michael: I think you're gonna see this actually happen because you know everybody has a camera in their pocket today. It's called a phone, and that uses a CCD camera, which is a charge couple device, so you can imagine that they can put a 20x optic on this, they may be even able to reduce this to a cell phone and just put a cell phone on top of the microfluidic device. The trick is the software because the software is doing some fancy statistics where they are integrating the number of lanes. And the other remarkable thing is it's actually a urinary tract infection which is effectively pure culture microbiology more or less, because it's not a mixed infection. But, given that there's a hundred million of these infections and our antibiotic armamentarium is weak, we are understanding with each passing day the importance of the microbiome, and we don't wanna be prescribing antibiotics like Cipro and Levofloxacin which seem to be the default antibiotic that then gets given to patients because it will cover most everything. You can understand the wonder that this device actually offers the point of care system.

Elio: That's true. Right now we don't know how applicable this is to other infections, right?

Michael: No.

Elio: Infections from other sites, where you draw liquid from other places like blood, sputum, and so forth. We still don't know that, right? But at least theoretically it is possible.

Michele: In principle it should apply for a blood sample or a central CSF sample.

Michael: And the blood culture bottles will actually amplify the bacteria. I think the trick is you gotta get to 10,000 to begin to load up the lanes. That's why I came up with the grocery store, you have to get the full checkout line in order to be certain of your conclusion.

Vincent: Once you have to amplify that's no longer a 30 minute test, right.

Michael: No, and that takes it out of the point of care realm. The beauty of this is 10 minutes.

Vincent: Michael, what has to be done in terms of clinical testing to get this kind of a device into physician offices?

Michael: You effectively have to take it through the FDA process, and there's a specific protocol that you have to do, you have to validate it against the gold standard, the gold standard, Zarina can probably tell us, it's just diffusion assays and microdilution assays.

Michele: Which is a whole lot longer than 30 minutes.

Michael: Oh, it's often times days, because you have to wait for the first 24 hours to get a pure culture isolate before you can work on that machine.

Vincent: Do you think this is cool, Zarina?

Zarina: I think it is very cool. I had actually done a paper on antibiotic resistance my freshman year and there were papers from the 90s that were like this is a crisis, it's really bad, and I was like, oh, it's almost 2020 and we still haven't… I mean, there has been a lot more awareness about antibiotic stewardship, but I don't think there's been a device like this that would help us actively give doctors something so it's not like a 50-50 whether they should prescribe.

Elio: I consider this a brilliant case of thinking outside the box. This procedure, even though the microfluidic device was not available, you could think of adapting other devices to it, and one could have done this literally 100 years ago and nobody thought of it. This is a thought that was 100 years in the making. The fact that you don't need to grow a pure culture and then test it in the lab by the true and tried methods, all of that goes by the wayside, and it's just magnificent. I just think, by the way, it goes with another similar realization. We had the Polianos here once talking about a way of determining the mode of action of a putative antibiotic in two hours or less. This is by looking at the cell shape and form, so this is not related to this, but there are two examples here of something which you can break through the normal thought pattern of what it takes. It reduces microbiology to the single cell level.

Michele: And it's thanks to advances in microscopy and also the computational piece.

Michael: And if you think about it the clinical lab already has a lot of computation.

Elio: That's all true, but the basic idea that you don't have to wait 24 hours for the culture to grow out, that idea is so simple and so beautiful. I just salute these people.

Vincent: So Michael, even Maldi-Tof based methods require some growth, right?

Michael: They require a culture. They require a colony on a plate to go to it. But the clinical lab now has the computational power because of the Maldi-Tof that has become ubiquitous in our clinical labs. So the fast computers that are effectively interpreting the Maldi-Tof system, you know, big data computing has already made it to the clinical microbiology lab. So the way I look at this paper, this is point of care diagnostics is the wave of the future and it's both a future jobs maker and a future jobs disruptor.

Vincent: Of course. The clinical labs get disrupted, right, but on the other hand, you now hire people to work in physician offices to do this test and there will be others as well, right?

Michael: Yes.

Vincent: That's neat.

Elio: Beautiful. Nice job, Michael, I love your analogy to the checkout line.

Michael: Thank you.

Michele: Michael, I may have missed it, but did you name the journal and the authors at the beginning?

Michael: Yes, I did.

Michele: Okay.

Michael: It was Ozden Baltekin, Alexis Boucharin, Eva Tano, Dan Andersson, and Johan Elf, and they are at the Uppsala University in Sweden in the Department of Medical Sciences and the Department of Medical Biochemistry and Microbiology.

Michele: And this was published in PNAS.

Michael: PNAS, the Proceedings of the National Academy of Sciences. And it was communicated by Nancy Kleckner. Any of you who have ever worked on plasmids probably remember that name.

Vincent: Thank you, Michael.

Michael: Thank you.

Vincent: And now for our paper, we have a paper that Michele has been sitting on for a few, quite a few weeks now.

Michele: Since the Microbe meeting.

Vincent: Since Microbe. So Michele, now you have your chance.

Michele: Alright. The title is "Carbonate sensitive phytotransferin controls high affinity iron uptake in diatoms." And it is from Jeffrey McQuaid, Adam Kustka, Miroslav Obornik, Ales Horak, John McCrow, Bogumil Karas, Hong Zheng, Theodor Kindeberg, Andrease Andersson, Katherine Barbeau, and Andrew Allen. And they are at the Venter Institute for Microbial Environmental Genomics and the Scripps Institution of Oceanography of UC, both in La Jolla, California. Also Rutgers University's Department of Earth and Environmental Sciences in Newark, New Jersey, and the Biology Center and Institute of Parasitology and University of South Bohemia, which are both in the Czech Republic. It was published in March in Nature and was recommended to me by Jernej Turnsek

who I met last month at the Microbe meeting in Atlanta. So we were just out walking around the poster session happy hour and started chatting and led me to this fascinating work. So thank you, Jernej.

So this is a micro story with macro implications. It is set in the ocean and it stars diatoms. These are jewels of the microbial world. It features the metal iron and a simple ion, carbonate, RCO3, and an ancient protein, transferrin, or in this case, phytotransferrin, and the story plays out against a backdrop of what we know are rising levels of carbon dioxide in our atmosphere, acidification of our oceans, and our planet's oxygen supply. But let's start small and then we'll end macro. So diatoms are single cell eukaryotes that live in sea or fresh water, and they are part of what we call the phytoplankton, small microbes that float or bind to animals or plants in the sea. There are more than 50,000 species of these tiny diatoms and they are algae.

So that means that they are equipped for photosynthesis. They can capture sunlight and use that energy to convert carbon dioxide into the building blocks they need to make new cells including their remarkable cell walls which are actually made of silicon dioxide or glass. So they are tiny jewels and they are just beautiful, different shapes and sizes. But they are more than just a curiosity, these diatoms and other phytoplankton are a key component of the ocean food web. So they are food, of course, for larger organisms in the sea. But they also generate oxygen that's essential to many other life forms including humans.

So it's thought that diatoms account for almost a quarter of the global organic carbon fixation. They capture the carbon dioxide from the air, convert it into these glass shells, which then, when the organism dies, they will sink to the ocean floor and become a form of long term storage of carbon. These diatoms shells, incidentally, are also used commercially. They can be used as filtration devices for and abrasives in cleansers including toothpaste. Now to grow the diatoms not only need sunlight and carbon but also iron. So iron is a trace metal that drives many essential processes in many different cells from bacteria to archaea to plants to humans. It is key for oxygen transport, DNA synthesis, respiration metabolism, you name it.

So we all need iron, our cells need iron. So humans have a protein that floats in our blood and it can bind tightly to iron and then transfer the precious metal into cells by a process called receptor mediated endocytosis. So small vesicles take it up and deliver it. That protein is aptly named transferrin delivering iron but a lot of biochemical studies and molecular biology studies have established that transferrin cannot bind iron by itself. So we now know that the transferrin protein is folded such that one part of a protein forms a pocket with five different fingers. But those fingers cannot pick up an iron molecule by themselves, but if there is a carbonate molecule, a CO3 molecule around, the fingers can now grab on to both the iron and carbonate at the same time. And that design, that grasping of the iron and carbonate is so efficient that our transferrin can bind iron even when it is very scarce, like in the picomole range, 10^-12. So this is a very efficient system, and in our blood transferrin carries this metal and carbonate cargo into the cell.

Once the pH of the endosome drops slightly, a free hydrogen latches on to the carbonate ion, forming bicarbonate, and that is enough to kick off the iron. So the iron is now free and it can be put to work elsewhere in the cell driving these enzymatic processes. So in this lovely example carbonate then acts as both what we call the coordination ion, it aids iron binding to transferrin, but it also forms this pH sensitive trigger so that when the pH drops a bit it will release its iron cargo. So keep that in mind as we go back to the microbiology and then think about this in the context of the ocean and climate. So back to these diatoms. They are floating or riding around on marine organisms in water where nutrients and iron is scarce.

So how do they get their iron? Recently on this group and others had found a diatom protein that can bind and transport iron and they named it ISIP2A, I'm gonna call it ISIP 2A, but when the computers looked at the primary sequence of this protein it looked like an orphan. The computer algorithms couldn't match it to anything that had been characterized previously by scientists. So it was kind of a mystery about how this could work. So what they did was apply in this paper bioinformatics, phylogenetics, molecular classical genetics, and

also some cell based assays to show convincingly that from the same ancestral protein transferrin and this phytotransferrin protein of diatoms independently evolved to exploit this five finger domain to grasp iron and carbonate and deliver it into the diatom and support its growth. So the they, in this case, is Jeff McQuaid, he is the PhD student in Andrew Allen's group that led this work, and Vincent, maybe you want to tell us about this puzzle about the sequence of the ISIP2A protein.

Vincent: So Jeff, this started with his PhD thesis where he had taken part in a marine metagenomic survey of East Antarctica and had lots of information and wanted to study this and use the PhD program to do that. He writes that the growth of, this is important because the growth of marine microorganisms can influence atmospheric CO2 and it is controlled by the ability of phytoplankton to access dissolved iron. And so of all the genes that he pulled out from this survey, an iron stress protein ISIP2, which you have heard about, kept showing up in every marine metagenomic sample, and in every major phylogenetic group of organisms. So his dissertation became trying to understand at what the gene and the protein was doing.

And as he puts it, a key a ha! Moment came when he connected this ISIP2 gene to the transferrin family. It had been known that they were marine algal transferrin or phytotransferrin since 1982, but it had never been found in the environment, the proteins had been known for a decade but as Michele said they're not similar to any known protein family so making the connection required going old school and stepped away from the algorithms in the homology tools which said there is 8% homology between ISIP2 and the transferrin family which is basically no homology. And they printed out the data and looked at it using their eyes and their hands.

Michael: Very Woesian, that's very Carl Woese.

Vincent: Hand alignment. He says when you stare long enough at alignments, there are weird strings of amino acids and repeats that just seem too similar to be mere coincidence, and your eye can pick these out better than a computer. And then of course that is what led to the experiments that Michele is gonna tell you about. By eye and hand, isn't that great? (laughs)

Michele: It is and what tenacity.

Vincent: Humans can be useful.

Michael: Well this is why they invented beer.

Michele: Or coffee, or coffee.

Michael: You need lots of coffee.

Michele: So Jeff had the hypothesis then the model that perhaps this phytotransferrin was acting similarly to the transferrin molecule that had been previously studied in detail from human blood. So to test this idea, they aligned many of these different phytotransferrin to transferrin and sure enough, they found that one motif that was common in the transferrin was lacking in a common progenitor that was known to be able to bind anions but did not bind iron. So they had some reason to believe that they had identified by eye a domain that could allow this protein to use its fingertips to pick up both the iron and the carbonate ion. So to test this idea they used genetics. They generated a diatom mutant that lacked the ISIP2A phytotransferrin.

But I'm thinking, how in the world do you do that? These diatoms have this glassy shell, I don't know how you do that. But there are techniques. So in 1999 Bowler's group in Italy worked out what they call microparticle bombardment which is a way you can force plasmid DNA into diatoms. So they have antibiotic resistance cassettes, they identified promoters and they used again a technique called transcription activator like factor

genome editing which allowed them to go in and specifically by homologous recombination replace the gene with an antibiotic cassette. So something that we take for granted in E. coli but they actually do these methods in diatoms as well. So they generated a mutant and compared it to their wildtype and put each of them into a low iron broth and found that the mutant grew much more slowly when the iron was scarce. And then they did they used radio labeled iron and measured the uptake rate and compared the wildtype to the mutant and were I'm sure thrilled to see that the mutant took up iron at about ten percent the rate that the wildtype did and that is shown in figure 2B and extended figure 4B.

But of course they had just bombarded diatoms with plasmid DNA oligos, homologous recombination enzymes, so they had to go back and really verify that the phenotype that they had spotted in the lab actually mapped back to the gene of interest, the ISIP2A. So of course they used classical genetic complimentation. So when they put the wild type gene back in and expressed it in trans they indeed restored uptake of iron rates equivalent to the wild type. But then they took a bold next step and they asked can the human transferrin also substitute and function in their diatom growth?

So to do that experiment they first studied the amino acid sequence and they appreciated that the single celled algae actually dock their phytotransferrin on to the membrane. So it has got a membrane spanning domain which is lacking in human transferrin. So they put the human transferrin in either N-terminal or C-terminal domains after their signal sequence to make sure that it got to the outer membrane and then they followed it by the membrane spanning domains so it would stay attached to the diatom protein and then repeated their iron uptake and both the C-terminus and N-terminal transferrin domain motifs were able to complement and restore iron uptake. Not completely to wild type levels but really quite striking. So that must have been a great day in the lab.

But to test this idea even more rigorously we pointed out that there are highly conserved residues in this motif that is known to coordinate the carbonate and iron binding and so that mutated two of the highly conserved tyrosines in the diatom protein and then repeated their iron uptake assays and found indeed that each single mutation did as predicted reduce iron uptake by about 50% and the double mutant was more severe than either single. So I think they felt confident that the biochemistry was meeting their predictions. But they took it another step, they actually did some microscopy. So they generated a fusion between their phytotransferrin gene and red fluorescence protein, added that exogenously to a culture of diatoms and then looked at the cells and they could see that the fluorescent signal was bound to the surface and was also present in little, what look like vacuoles inside the cytosol, consistent with the protein being taken up by endocytosis.

So having established those bases, they wanted to look in more detail and see whether carbonate was an essential component of this binding reaction as it is known to be for human transferrin. So to do this they repeated their iron uptake experiments with their wildtype and mutant diatoms using either low or very very low iron concentrations, but now they also varied the carbonate concentration and that is shown in figure 3. They discover that indeed the rate of iron uptake depended not only on the iron concentration but also the carbonate concentration. And for the biochemists in our audience I will tell you that it exhibited beautiful second order rate dependency that we can deduce to mean that the iron and the carbonate have to bind simultaneously in order for the transferrin to latch on to the molecules.

The other key point is the concentrations that they were using for the experiments where those that are typical of sea water so this looked to be physiological. They also did some specificity controls, they asked if other simple organic acids could substitute for the carbonate, so they tried formate, acetate, for example, and they found it could not. So this was very specific to carbonate ions. And then they stepped back and thought about these results in the context of where these diatoms live, in the context of the ocean, where the carbonate comes from, thinking about atmospheric changes.

So we know that an increase in the atmospheric carbon dioxide reduces the oceans pH, and this is a broad concern now as we burn more and more fossil fuels, release that carbon into the environment. We know that it can acidify the ocean's pH. Once the CO2 gets into the water, hydrogen ions latch on to the carbonate, generating carbonic acid and we remember from the transferrin in our human cells that once the pH is reduced slightly then the iron will be liberated from human transferrin So the possibility that they wanted to test was whether slight acidification of the ocean could interfere with the capacity of phytotransferrin to bind and scavenge iron from the waters and support growth of the diatoms.

Elio: Michele, does it sound a little paradoxical? More CO2 leads to less activity because more CO2 leads to acidification, I had to wrap my head around that one.

Michele: Yeah, and the authors address that paradox in their paper, too, and try to take us through the kind of spaghetti thinking, but so what they did is in their lab setup they have their sea water and they injected CO2 into the sea water and found that that in fact altered the pH. So they could generate a pH range of interest which was between 8.7 and 7.2. So we're not talking Coca Cola acid, we're talking fairly neutral range. But what they found, and this is in figure 4A, the uptake of iron via this ISIP2A protein was sensitive not only to iron but also to pH so that it elevated CO2 and therefore reduced pH of the sea water, the diatoms took up less iron, and the process was sensitive to the concentration of carbonate. So they did really a beautiful concentration series for both iron and for carbonate and could demonstrate that that relationship held.

Michael: And they're measuring attomoles of iron.

Michele: Yeah. So we are talking very scarce. (laughs)

Michael: The issue is is you have to scrub your glassware in water so well to get rid of any contamination in order to do this because iron is everywhere. It principally comes out of our water distribution system because of the iron pipes that much water goes through in major cities. So these experiments are just absolutely incredible.

Michele: Yeah, and Jeff pointed out that they actually had to set up essentially a clean room, much like where you would do engineering of computer chips because even dust will have sufficient iron to goof up their experiments. So this was done under really tightly controlled conditions. Technically very demanding but when you look at the paper it looks so elegant. So they also found that when the diatoms were grown in low iron conditions they actually made more of the ISIP2A protein, so that diatoms sense the iron starvation and they compensate by making more of this protein that can then bind and sequester the iron. So, lovely biology. So the experiments then show that the capacity of diatoms, these jewels out in the sea, to grow, requires iron and the ability to take up that iron is exquisitely sensitive to carbonate whose concentration is dictated by pH of the water because if there is more hydrogen ions around, the carbonate will be shifted to carbonic acid, and therefore that anion will not be available to act as the coordinating iron and help the phytotransferrin bind to the iron.

Vincent: That explains Elio's conundrum of before.

Michael: Right.

Michele: Right. Right, right. We also know that the pH of the water is in turn sensitive to the carbon dioxide in the atmosphere and so this is where we go from this really beautiful microbiology and thinking about now on a global macro scale what the implications are for ecology on our planet. So again, by their phylogenetic analysis, they found that a wide variety of phytoplankton use this phytotransferrin mechanism and it allows them to scavenge this rare metal iron from nutrient poor environments in the ocean. We know that iron is essential for

life and growth of the phytoplankton. We also know that the phytoplankton itself is vital to the ocean's food chains. It provides food but it also fixes and sequesters atmospheric carbon and it generates oxygen for us to breathe.

So the question is, what will the impact of our gradual acidification of the oceans and life in the oceans and on Earth mean? Surely it is going to disrupt the ecology and disrupt the ability of the diatoms and other phytoplankton to thrive, but how soon will we see compensatory mechanisms, how disruptive will this be? When we are thinking about climate change and the acidification of the ocean, we are often talking about bleaching of the corals, etc, but even on a micro scale this could have profound effects. So this study is now a mechanistic description of what the chemistry is and gives us insight to monitoring that disruption.

Michael: Michele, do you think that this could be a molecular chronometer to effectively monitor climate change by looking for mutations or looking for changes in the systems associated with iron regulation in the phytoplankton?

Michele: This is exquisitely sensitive to acidification because of the carbonate so it would be a readout. I think that does seem realistic. I don't know how difficult these assays are, so it's hard for me to know whether this translates as well as your technology, Michael, for antibiotic susceptibility.

Michael: Well, no, I'm thinking of linking it to some of what Sharon Peacock said at Microbe when she was talking about sequencing and big data. Could we use a sequencing approach to effectively monitor the genome, specifically over the regulatory components that are controlling the phytoplankton and the suite of genes, to ask the question are there mutations that are accumulating akin to what we see in antibiotic resistance to effectively be able to model that the organism is experiencing selective pressure because of the increased CO2 concentration in the atmosphere while dropping the carbonate concentration in the water, which is going to have a systemic effect on the phytoplankton and how it regulates its genome, and just do something as simple as transcriptomics asking, okay, here we have a point in time in 2018, what's actually going on in the genome in 2019, to maybe inform how serious of an issue this is.

Vincent: It would be interesting just to see if you could make a phytotransferrin that doesn't require carbonate, right?

Michael: Yes.

Vincent: Alter the protein and if you could that would suggest that with enough pressure of high CO2 then it could arise, right, unless it had some other issue.

Michele: Yeah. Although, it seems like nature has been doing that experiment for a couple hundred million years and…

Michael: Billion, probably.

Michele: Yeah. Their extended figure 1 shows how many different phylogenetic tree have all independently evolved this same mechanism.

Vincent: Maybe there weren't low acidic conditions for most of that time. Maybe this is an unprecedented situation.

Michele: The authors do point out that the diatoms have other mechanisms for taking up iron but they are slower and more expensive, economically expensive for the cell, so that they would not support the same growth rate that the diatoms now enjoy because of their ability to scavenge this rare iron.

Vincent: I think probably there was high CO2 at other times in Earth's history, so maybe you're right that this has happened before and maybe it can't, maybe this protein doesn't work without carbonate.

Michele: And some of the background reading that Jeff provided, they do say that some of those events due to, I think during the volcanic period there was a lot of release of CO2 into the atmosphere and it corresponded with some major die offs including, I believe, in the ocean. So it has happened before and it wasn't pretty.

Vincent: I should point out 70% of oxygen is made by marine plants so this could have a big impact.

Michael: We're gonna have to start holding our breath.

Vincent: Or go to another planet.

Michael: That is unlikely.

Michele: Or hope that microbes adapt and we get variants that can find a new way.

Vincent: Yeah, maybe.

Michael: So the take home is, are we going to run out of sand?

Michele: Sand.

Michael: Phytoplankton actually becomes sand, it becomes an abrasive.

Michele: Silica.

Michael: Yeah, silica, but it's in the sand, too.

Michele: Yeah.

Michael: It's pretty scary.

Vincent: So let me tell you a little bit more about Jeff. Jeff says he is a fan of education via the installment plan. He got a BS in biology in his twenties, an MS in environmental microbiology in his thirties, and his PhD in his forties. And that's when he started looking at these data sets as we said earlier. He says, by way of advice, I encourage students and young researchers to occasionally step back from the computer and look at their data. As data sets get larger and statistical packages and gene crunchers more powerful, we tend to get disconnected from what we are actually looking at. We can miss things. It is important to get a feel for the numbers we are churning out and I think that we as humans can see trends and numeric relationships that a computer can miss. And who knows, maybe we will keep ahead of the machines that way.

Michael: Truer words have never been uttered.

Vincent: I think it is important because it is tempting to put everything in your computer and wait, but you can still look at things and get patterns that you wouldn't get otherwise.

Michele: This also points out places where we need to improve our algorithms because clearly they are not catching everything.

Vincent: Zarina, do you think that is a good bit of advice? Are you taught that in college to look at your data, not just use a computer?

Zarina: Well, because when you are first working in labs, you are working with such small data sets you kind of have to. (laughter)

Vincent: That's a good point. Yeah. Wait until you get to graduate school.

Zarina: Although, it was interesting because last, we were at the Hampton Scholars, there were like ten different sites, we were taken to the Amgen facilities out in Thousand Oaks, California, and one of the people who was giving the tour of the facility, he asked all the students if we had ever held a pipette before, and of course all the students said yeah, we had, and then he said how many of you know how to work software? And only one student raised their hand.

Vincent: Interesting.

Zarina: So I think probably a lot more students are aware of how to do things by hand than the other way around. But looking at numbers, actually getting a feel for numbers, that's different, though. A lot of students kind of focus on learning the skills of like, how do I make a chart on Excel, versus like, actually analyzing what implications numbers have.

Vincent: So you're an Amgen scholar? This is a program of putting undergraduates into labs for the summer.

Zarina: Yes.

Vincent: Six week programs?

Zarina: Ten weeks.

Vincent: Nice. It's a competitive program, right?

Zarina: Yes.

Michele: Wow. Ten weeks goes fast.

Zarina: It does, it goes by so fast.

Vincent: Is it almost over?

Zarina: Yeah, next week is my last week.

Michael: Aww.

Vincent: Back to New Jersey, huh.

Zarina: Yeah, across the river.

Vincent: It's not too far, I know.

Michael: So no discussion of iron would be appropriate without the Kipling quote from the poem "Cold Iron."

Vincent: Go ahead.

Michael: "Gold is for the mistress, silver for the maid, copper for the craftsmen, cunning at his trade/ Good, said the baron, sitting in his hall. But iron, cold iron, is master of them all."

Vincent: That's great.

Michael: The poem goes on longer than that, but that's the gist, that's the best part.

Vincent: Iron is the master.

Michael: Iron is indeed the master of them all.

Vincent: I think that was a title of a TWIM once, right?

Michael: I don't know if we ever used iron is master of them all.

Vincent: We could find out.

Michele: It's familiar, I know it has been referenced before.

Vincent: Thank you, Michele.

Michele: My pleasure.

Vincent: What a great paper. Thanks to whoever recommended it to you.

Michele: Yeah, Jernej, he, as I said, we just started chatting at the Microbe meeting. So all of the people that I met at the meeting, when they come up to me and talked about TWIM, I said send me your ideas! We don't have that many people that can spend that much time searching all the literature and we know that microbial sciences are vast and fascinating, so feel free to send us some suggestions.

Vincent: Alright. Let's read a couple of emails before we finish. The first one is from Brian:

Dear Vincent and the rest of the TWiX crew,

Thank you so much for the TWiX podcasts. Although I'm a science dilettante, you make the materials clear enough that I can understand much of the discussions.

I recently discovered the podcasts via a typical 21st century route. I was taking a wonderful lifelong learning class I Contain Multitudes – Bacteria on and Around Us, which had inspired me to google various topics. One of those led to "Small Things Considered", where I saw a reference from Elio to one of your podcasts.

I listened to that TWiM podcast and was hooked.

As I was listening to a TWiM podcast, you mentioned a "TWiV". (I actually went back in the podcast to make sure I hadn't misheard a TWiM reference.) I happily added TWiV to my podcast app and started listening to those too. Then you made a reference to parasites and a TWiP episode. Well – who doesn't love learning more about parasites? – so I knew I had to subscribe to that too. This time, I figured there might be more hidden podcast wealth out there, did some online searching and found the full microbe.tv show listing. My podcast app show list is much larger now …

I'll end with the apparently obligatory weather observation: It's mid-June and Ann Arbor Michigan is delightfully sunny and warm.

thanks – Brian

Right there in Ann Arbor, Michele, you have a fan of our work.

Michele: That's great.

Vincent: Michele, can you read George's?

Michele: I sure will.

Hello All, I'm currently working my way though your back-catalogue having only recently stumbled across your podcast and realised that I'll have absolutely no chance of winning any books this way. So, I try to listen to them as released and supplement with older episodes that take my fancy.

I'm hoping to qualify as a Clinical Scientist in infection sciences (a protected title in the UK for scientists working in pathology for the NHS) in the coming months (assuming I pass my final practical assessments) and this book would be incredibly useful for my future career ambitions, as my background is in applied microbiology but has shifted to clinical microbiology.

Keep up the excellent work and thank you for your efforts.

Dr Ryan George

Manchester University NHS Foundation Trust

So which book is he angling for, there, Vincent?

Vincent: Every now and then we give away a book and today, all the others have been given and there's nothing today, but next episode George and everyone else, I have a book to give away and we'll do that, so stay tuned. Michael, can you take that last one?

Michael: I will.

Dear TwiM hosts,

My name is Teresa De la Mora and I'm a biology faculty at Normandale Community College in Twin Cities, MN. I stumbled upon your podcasts while reading other ASM sources and I want to thank you for your wonderful work in putting this show together. It has helped me a lot in obtaining material for my microbiology class for health science majors. I was wondering if I could ask for your expertise in recommending a good CRISPR review? I'm starting to prepare for my fall class and I'll like to be able to explain this system to my students.

Thank you for your kind information and for all your work on this show. I haven't stopped listening during my commute, even my photographer husband got into the show!

Michele: Alright!

Vincent: So I have two suggestions. There is a review called the "CRISPR Toolkit for Genome Editing and Beyond", this was published in Nature Communications just this past May, it's a really good review, and there is a brand new book out by Jennifer Doudna and Sam Sternberg, it is called "A Crack in Creation", which is a really nice personal history of the development of CRISPR and it helps understand what is going on, as well. Michael, you had a suggestion, right?

Michael: I had one, as well. There was a review that appeared in Biochemistry and Molecular Biology Education in the March/April edition by Deborah Thurtle-Schmidt and Te-Wen Lo, from UCSF/Davison College where Dr. Thurtle-Schmidt is, and Dr. Lo is from Ithaca College in Ithaca, New York, and it is entitled "Molecular Biology at the Cutting Edge: A Review on CRISPR-Cas9 Editing for Undergraduates". And it is an open access piece and that will be in the show notes. And then of course there is the authoritative source of all things relevant that might be appropriate to introduce an undergraduate class to and that is of course the YouTube video by John Oliver of HBO fame, and I've put into the show notes his diatribe on gene editing using current movie events. But he was pretty well factual in his description of gene editing. It's an interesting piece.

Elio: If that's not enough you can simply Google CRISPR reviews and you will get several dozen.

Vincent: You do, but Theresa--

Michele: We've curated it.

Vincent: Wanted a curated list from the experts, alright.

Michele: Theresa, I use our podcasts for the first year class that I teach called Current Topics in Microbiology so these kids are essentially right out of high school. I have them listen to a podcast first and in class, we kind of flip the classroom and groups of maybe 3 will each study one figure or one panel and I will walk around the room and help them understand any questions they might have about it and then they take turns presenting their figure to the rest of the class. In that way we build up the paper. So it gives them a sense for how science, what we understand about the world, can be learned through experimental science. I enjoy it, I think the students do, too.

Vincent: I love that, it's a great idea. Alright, that will do it for TWIM 182, you can find it at asm.org or Apple Podcasts, in fact any app you use to listen to podcasts on your mobile device, phone, or tablet, just search for TWIM, This Week in Microbiology, please subscribe so you get every episode twice a month as we release them. If you really like what we do, consider contributing financially. You can go to microbe.tv/contribute for the various ways you can do that. Of course, send your questions and comments to twim@microbe.tv. Michele Swanson is at the University of Michigan, thank you Michele.

Michele: Thank you.

Vincent: When do the undergrads come back, a couple of weeks? Like a month, right?

Michele: Yeah, not till after Labor Day.

Vincent: Wow, that's pretty late. But then you go late, too.

Michele: Most of my teaching is at the medical school, so I'm not that familiar with the undergrad, but yeah.

Vincent: Zarina, when do you go back?

Zarina: I go back on the 26th.

Vincent: My son goes back on the 26th as well. Elio Schaechter is at Small Things Considered, thank you Elio.

Elio: My pleasure, of course.

Vincent: Michael Schmidt is at the Medical University of South Carolina. Thank you, Michael.

Michael: Thanks, everyone.

Vincent: And Zarina Akbary is about to go back to Drew University. Thanks for stopping by.

Zarina: Thank you so much for having me.

Vincent: One day you'll be on podcasts as a microbiologist. What do you think about podcasts in general, do you like them?

Zarina: I love podcasts. Yeah, I actually commute to Drew, so it's very helpful. It was actually interesting, while I was sitting here I was like huh, I'm not driving. (laughter)

Michael: Now you must know how we feel, if we're not driving.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. I want to thank the American Society for Microbiology for their support of TWIM and Ray Ortega for production. Music on TWIM is by Ronald Jenkees, ronaldjenkees.com. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(music)

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