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BIOLOGICAL SCIENCES

Discovery and Redemption emerge from a scientific mistake

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We were confused and perplexed. Our team in the laboratory of Pamela Ronald at the University of California, Davis could not reproduce previously published results. Instead of building on the reported discovery that a microbial signal triggered the plant innate immune response, we were thrust into trying to figure out what went wrong. It was the most difficult time in our careers. Then, we made a remarkable new discovery.

The story began in the 1970s, when Professor Gurdev Khush and colleagues demonstrated that a wild species of rice was immune to most strains of a serious bacterial disease. This was exciting because such broad-spectrum resistance had not previously been identified. Such traits are agronomically important because farmers can plant resistant varieties rather than spraying pesticides. In 1995, Pam's lab reported that the broad spectrum resistance was controlled by a single gene, called *Xa21*, predicted to encode a receptor that senses the microbe and then activates the rice immune response.

In 2009, Pam and her former team members reported the discovery of such a molecule. However, in 2012, while trying to build on those findings, we discovered major errors in this work. Pam contacted the editors to inform them of the identified problems. She also decided to notify the scientific community of the issues at an international research symposium. Ben still remembers the day when Pam announced the errors in front of so many distinguished colleagues, many whom Pam had known her entire career. "It was remarkable that Pam mustered all this courage to inform the community at this top research symposium. Some in the audience buried their heads in their hands and were clearly uncomfortable but still more people were awed and expressed their support." Sophien Kamoun, a leader in the field of plant biology, displayed his respect in a tweet:

.@pcronald RESPECT for clearly explaining issues with Ax21. This is all part of science. Great lesson for everybody... #KSplant

- Sophien Kamoun (@KamounLab) April 8, 2013

Finally, in October 2013 we had accumulated sufficient new experimental evidence definitively proving that key aspects of the study were incorrect, Pam and her former colleagues that co-authored the 2009 study retracted the original Science paper.

Pam and the several of us in the lab lost many nights of sleep as we racked our brains to figure out what went wrong. We had many intense discussions on how and why this could have happened. As

Ofir remarked, "Pam was on sabbatical in France when we discovered the problem and so we had these electrifying video conference calls to bring everyone on the same page." It took time and persistence to break down the problems one at a time as we were working backwards identifying what were solid results we could build and what we could not. One day Ofir and Ben were having their morning coffee outside in the Californian sun when Ofir announced that he had found that some of the bacterial strains used in the previous study were mixed up. Rory still remembers this day vividly, "Even though this was not happy news, we were *so* happy, we finally had a partial explanation of what went wrong." From that day onwards we were getting back to solid ground. The scientific process had pulled us through and pointed us in the right direction. Our team discussed the mistakes and corrections in lectures, blog posts and in a scientific publication. The process with which we addressed the problems was highlighted in an article in Nature magazine and in Retraction Watch, a blog that reports on retractions of scientific papers, as " Doing the right thing". In a dubious claim to fame, the 2009 retraction was included in the top 10 retractions of 2013.

While we were notifying our colleagues, discarding old strains and correcting the literature - an essential part of science so that others do not waste (more) time trying to build on fatally flawed work- we were also working hard to discover the microbial partner of XA21. In the mist of all the fog Rory identified a new bacterial mutant strain that was able to infect XA21 rice plants. We were excited but cautious at the same time. Could this really be the correct mutant lacking the microbial partner of XA21? The 15+ year lab veteran Mashweng was especially skeptical as he had lived through the whole story form the beginning. He often suggested critical controls and reminded us to be extra critical with our own results. A superb team of collaborations from around the world allowed us to incorporate additional experiments and independent controls perform in former critics' laboratories. After many independent tests we were certain of our results. In mid 2015, we reported the identification of this long sought after molecule that activates XA21-mediated immunity in Science Advances.

We named this small microbial protein RaxX. We found that bacteria that lack RaxX are able to evade detection by the rice XA21 immune system. Bacterial strains found in farmer's fields in India, which express alternate versions of RaxX can cause disease on XA21 rice plants.

Wrestling with trying to reproduce flawed experiments and discovering the new molecule in rapid succession was an enormous challenge. In our view, the key point in straightening out such complicated and delicate situation was the persistence and collaboration of our laboratory team. Pam worked hard to keep the team together. We were a dedicated crew of senior scientists, technicians, postdocs and graduate students (as well as former team members who shared their records with us because they also wanted to know where they had made mistakes) motivated by a supervisor determined to get to the bottom of the situation. Looking back, we find it stunning how this all worked out. "It's amazing what can happen if you are doing science with a set of respectful and likeminded people. We were focused on setting the record straight rather than blaming others. It clicked. We were on a run", Ofir still remembers to this day. We felt our careers were on the line. We realized that there was no moving forward without first going backwards. We did not give up even though at times we wished we could. Working *together* on this daunting challenge buoyed all of our spirits. "Back then, I [Ben] was living in this lovely trailer with a trellis outside. We had 'poker-nights', when everyone came along to talk and be jolly. At times it felt like 'group therapy' with drinks."

We were impressed by the supportive and kind response from the scientific community, our editors, and funding agencies including NIH, BSF, and HFSP. We received many letters of encouragement - even from complete strangers. These conversations helped keep us going. We even had new scientists join Pam's lab in midst of the mess. Anna Joe, a postdoc who joined the lab at the time of the retraction remarked, "Their open mind and transparency attracted me to the lab."

Pam tells us that there are still hills to climb, "Some scientists may be extra skeptical of results from my lab for a long time to come." For example, in a critique of our submission, one reviewer asked, "how do we know the strains weren't mixed up again this time?"

We all know errors are part of the process of scientific discovery (although most of us don't see *ourselves* as making these mistakes). The key is to track them down as fast and efficiently as possible, ideally *before* publication. To this end Pam has improved and instituted new laboratory practices: generating duplicate stocks of key strains (validated and maintained by the lab manager), mandating electronic notebooks for each lab member and requiring that all new assays be independently validated by three independent researchers before publication.

After all this stress and uncertainty, it is still invigorating when a colleague comes by after one of our talks and says, "It is nice to see how that you handled and communicated all the errors, corrections, retractions, recoveries, and discoveries. These stories are so important to tell as they are part of doing science."

The new finding of RaxX as activator of rice immunity has opened many new research directions. We all are excited to figure out the biological function of this potent novel molecule. We are moving on.

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Additional reading:
Retraction Watch, What do you do after painful retractions?

Nature News, Rice researchers redress retraction

The Tree of Life, A Phoenix Rises from the Ashes