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Thu, Oct 12, 2023 11:52AM 56:55

SUMMARY KEYWORDS

gene, crispr, soybean, plant, gotcha, gene editing, edits, sequence, cast, promoter, genome, people, work, viruses, system, cell, protein, herbicide, steve, spacer

SPEAKERS

Ed Zaworski, Brandon Kleinke

B Brandon Kleinke 00:00

I see that plants podcast shares the stories of people in plants, pests and pathogens and the conflicts among them. Join us as we speak to the folks who are helping the rest of us with healthier, more productive lives and pest management research. We strive to make science accessible. I see that plants are created by the Crop Protection Network and hosted by Ed Zaworski. The Crop Protection Network is a product of Land Grant University.

E Ed Zaworski 00:21

Welcome back to the ice dads plants podcast. I'm your host, as always, Ed Zaworski. And today, I'm joined by a colleague of mine, Dr. Steve Widom, and we're going to be talking about something a little bit different today, not necessarily a single passed, per se, we're going to be talking about the CRISPR cast nine system. And the paper that we're going to be talking about is Steve's paper that Steve is part of, and it's called The CRISPR cast nine based gene editing using egg cell specific promoter in arabidopsis and soybean. It's a mouthful, as most titles for scientific publications are, but we're going to do a lot of background information. Today we're going to break down what CRISPR casts nine is, and even a little bit further than that, I think but before before I do that, I'll just give Steve a quick introduction here. He got his bachelor's here at Iowa State University in agricultural biochemistry, got his master's and PhD in plant pathology at UC Berkeley. Probably a little bit nicer weather over there than over here,

01:33

typically. Although it's cold in the summer, sometimes.

E Ed Zaworski 01:36

Yeah. And you know, before we get started, I just I have to tell a story about Steve. We were both over at dinner at a colleague of ours, Darren Mueller, our soybean pathologist here at ISU,

and we had dinner and we were playing some card games. I think we're what we're playing, we're playing cribbage I think I think we're the champ cribbage with Darren son Nolan, who I think was about 15 or 16 at the time. And he were playing the three of us are playing cutthroat Kirby, Jim Nolan says, he asked me he says, Hey, so who's the most famous person you know? And I said, I don't know at all. And, you know, I'm trying to think through my head it, you know, as far as people I've met, and he says, oh, it's Steve. It turned out that he had, I think, you know, Darren had had him doing some busy work, he had to bring them into the office one day, and he had him. I don't know what he had them doing, looking at people's lists of publications or CVS or something. And he noticed Steve had the most vocations out of anybody that you could find. And so by that, he said, Steve is the most famous person he knows. And the most famous person I know, which I thought was pretty cute. And also probably true. Let's be honest, I don't know a lot of famous people. So

02:58

I don't know about that.

Ed Zaworski 03:01

Well, certainly, Steve, you are definitely one of the smartest people I know. And one of the most also down to earth researchers that I know. So I picked you to do this because I thought it'd be a good fit to kind of educate people on a really complicated topic, in my opinion. Maybe you don't think so? But I definitely do. So let's do a little bit of background. As I said, before we we get into the research where we're talking about using this system on a rabid abscess and soybean, but what what is CRISPR? Stephen, your most basic explanation? You know, pretend you're talking I always tell people, you know, pretend I pretend I'm talking to my mom lives in a she's a nurse, she lives in a high rise in Chicago. What is your most basic explanation of CRISPR?

03:48

Yeah, so CRISPR is basically and CRISPR itself is an acronym, right? So it stands for Clustered Regularly Interspaced Short Palindromic Repeats, which is quite a mouthful. So we'd rather say crisper than, than all that, right? But it's a palindrome means that, you know, it's, it's a sequence of letters or numbers that that are the same, either forward or backward, right. And this is one of the features of these of CRISPR, when it was first discovered, that kind of gave it away that hey, this is something interesting that we should look at. And people found these sequences in bacterial genomes. And this became possible because of sequencing technologies. And, you know, in the early, early, late 1990s, early 2000s, it became possible through advances in DNA sequencing technologies to sequence entire genomes. And so, as people were sequencing bacterial genomes, they started finding these interesting genetic features in these genomes, which they hadn't found before. And they gave them the name CRISPR. And then people did some additional work the Like, what do these things do? Or what are they doing in these bacterial genomes? And they found that. So we have these, they have what's called these conserved sequences, which are the palindromes, the Palindromic Repeats. But in between each of the palindromic sequences, there was then a unique sequence that's about typically on the order of 18 to 20 DNA bases, or 24, DNA bases. And these were, these

were unique, and they called them spacers. So, the spacer sequences, they eventually figured out few different groups that were working on this figured out that the spacer sequences actually correspond to viruses that attack bacteria. Gotcha. Yeah. So. So what what they figured out that these CRISPR, they're called CRISPR loci, if you will, what they figured out these CRISPR loci are is that bacteria get invaded by viruses. They then they then capture these viral sequences, just a little bit of it, like 18 to 20, bases, DNA bases of a virus, they capture that bit, and then they insert it into their genome at this at this CRISPR locus. And, and so you have these spacer sequences now that that basically tell you that there was a virus that had this sequence that tried to attack this bacterium, this bacterium kind of fought back, acquired a piece of the virus, and now it uses that piece of that virus to defend itself against viruses that try to infect it, that correspond to that sequence. And so these CRISPR loci, you can kind of see the history of different viruses that have attacked the bacteria. So, so the bacteria store this information about these different viruses at CRISPR loci. So some, some bacteria can have dozens of these sequences. So you can see that in the, in the long history of this bacterium. of its ancestors, there have been dozens or hundreds of viruses even that have tried to attack the bacterium, and it successfully defended itself. And it's kept this little piece of it in its in its genome.

E Ed Zaworski 07:19

Gotcha. Yeah. And I suppose there's a lot of back and forth, you know, the, you know, the as the as the both of them evolve, right? They, probably a lot. Yeah. I was gonna ask you, if they had lots and lots of these, what was it? What was the low sigh?

07:34

The CRISPR? Low, super low. So, yeah, yeah. Yeah. So, so you can find, it depends on the back, it depends on not just the bacterial species, but even the lineage of, of a particular bacterial species. You know, what, what viruses did that? The predecessor of the current bacterium, right? What, what viruses did it see, which could be different from, you know, its cousin over here, which may have seen different viruses or not as many or something like that. So Right. So it's not even a species specific thing, necessarily, how many of these spacer sequences and how big their CRISPR loci are, but it's, it's more of, it's more of an indication of all the ancestors of that particular bacterial cell or whatever that you're looking at now, what have they encountered over over time, but a given a given species will have the same mechanism for requiring spacers and for deploying them against viruses, but they can have a different complement of spacers in their in their genomes?

E Ed Zaworski 08:40

It's an interesting interpretation, a different kind of history. Yeah, exactly.

08:44

Yeah, you can basically see a history of all the different viruses that this bacteria was able to successfully defend itself against.

E Ed Zaworski 08:53

Gotcha. Yeah. So that's generally what, what CRISPR is and what a CRISPR low sigh is, folks. So then the other part of the CRISPR caste nine system would be the, the caste nine, it's a protein right? Correct, right.

09:07

Yeah. So, caste stands for CRISPR associated protein, okay. And there are different forms of cast proteins. So, you know, in, in biotechnology, we use primarily cast nine, but there are many different versions of cast proteins from different bacterial species. So there are cast twelves and okay casts elevens and all this. And there, there are different cast proteins that also have different kinds of functions in the CRISPR system. So, in in bacterial genomes, there are cast proteins that are responsible for cutting that cutting that spacer sequence out of the virus and inserting that at the CRISPR locus. Okay, and then there are other casts proteins which are involved in now, they're the ones that actually are like what we call effector proteins. So, and that's what cast nine is. So cast nine will take the take that spacer sequence, yep. And it will use that spacer sequence as a guide to tell it, okay, any any DNA sequence that enters this cell, it should be destroyed. So, so that's the function of caste nine, basically, in a bacterial cell that uses this all the spacer sequences that are encoded in the CRISPR locus, gotcha. It uses those to basically be a surveillance system. So that as a new virus tries to enter the cell, if if that spacer sequences matches that virus, then that cast nine protein is going to cut it, and the virus is going to be destroyed, and the bacterial cell is protected.

E Ed Zaworski 10:52

Gotcha. What? What's special about cas9? You mentioned there's other cast proteins. What's special about cast nine in this situation? Well, or maybe, maybe we want to wait till we get into it. But yeah, well, I'll leave that up to you.

11:09

Maybe just for now, we'll say that cast nine is kind of the first one that was really discovered and well, well characterized, okay. So the original, the original work, where it was demonstrated that we could harness these bacterial systems for really useful purposes and biotechnology that originally involved the cas9 systems. And, and since then, and kind of a caste nine locus was one of the first ones to be really well understood and described as well. But since since that time, the people who work in this area of discovering new CRISPR systems have discovered many, many, many new new ones. And Gotcha. Every time a new bacterial species is discovered, that's one of the things they do is look at the type of CRISPR system does it have? And okay, and yeah,

E Ed Zaworski 12:04

gotcha, gotcha. That's how it all works in bacteria. So as far right, as far as I gather, so how do

you achieve gene editing? And then I'm sure this is gonna be complicated, but bear with us, folks,

12:19

right? So, so I guess at the most basic level, how we have attained gene editing, is the really, really the key, the key aspects to it are this, this, the spacer sequence that, that which formed part of what we call a guide RNA. So the thing that guides the cast protein to cut, the viral DNA that enters a bacterial cell can basically be directed against any DNA sequence, whether it's an animal cell or a plant, so or a fungal cell. So, so the key is then that there, you know, it's programmable, right? So yeah, so I can I can engineer a spacer sequence to basically correspond to any gene in a plant cell, for example, in my lab, and then that, that engineer, that that spacer sequence targeting and gene can be combined with cast nine. Yep. And that allows cas9 to go to that gene, and cause changes to the coding sequence of that of that gene.

Ed Zaworski 13:32

Gotcha. So can it so you're basically, let me say make sure I understand. You can basically cut out gene from, like you said, a plant or an animal genome that maybe is undesirable?

13:48

No, we don't. Well, you can, you can use it to cut out undesirable genes, but, but you can also use it to create sequence variation in in genes that that are of interest as well. So. So what the cast so cas9, as I said before, cuts viral DNA when it enters the cell, right, right. So if we program it against to recognize a plant gene, yep. Now the cas9 will cut that gene, but it's basically just a it's just a slice. Right?

Ed Zaworski 14:23

Gotcha. So it's not necessarily removing it. Yeah, making a cut. Yeah,

14:26

yeah, it makes a cut. And your chromosomes have two DNA strands, right, that wind around each other and helix. And so cas9 cuts both of the strands. So what that does is it makes a break in the in the chromosome. And then our, you know, cells have have ways to prepare break repair brakes and chromosomes, because brakes and chromosomes are not tolerated. Right, right. So that's that would be the death of the cell. So, so there's a natural repair pathway that takes over. And it will rejoin the two DNA strands. And the process is called non homologous end joining. And when that happens, the repair isn't always perfect. And so frequently, you'll get a small mistake that happens, either a few bases at the cut site will be deleted, okay, or a base or two might be added. So you can get a small insertion or a small deletion. Yep, at the site where you've targeted the cas9 system to, to do its thing on the on the gene you're interested in modifying. And so what what happens, then, you know, you can

inactivate a gene if you do that. And typically, that's, that's what will happen. But you also could create some diversity in that gene as well, that might, that might allow it to be had some improved property that you're that you're looking for. Gotcha. But you also said deletion earlier. Yeah, you you can delete genes as well. And, and there's been several demonstrations of that, where, instead of supplying one guide RNA sequence, to cause cas9, to cut only in one position, yeah. Which will cause what I just described, which is the small insertions or small deletions, yep. of DNA. You can give it say to guide RNAs. Yep, that might flank a gene might be on one side of the gene and others on the other side of the gene. So if that DNA gets cut at the same time, yeah, you can actually remove a gene that you don't want.

E Ed Zaworski 16:45

Gotcha. So that would be like I was saying, like, right, if you want to remove Yeah, yes, some kind of dis Advent, like, you know, yeah. I don't even know what that would be off top my head. Well,

16:56

there, there are many cases where you'd want to do that. So there's, there's a very common problem in plant breeding. That's called linkage drag. And what Lincoln's drag is, is you have a, let's say, you have a disease resistance trait that's very valuable. And it protects a plant against a really important, say, a fungal pathogen. Sure. And frequently, these genes come from other, they might even come from other species of plants, but we've brought them in naturally through through the breeding process. So when you when you do plant breeding, you don't always just bring in the gene that you want, right. But you bring in other genes that are close to it on on chromosome segments, and so. So a lot of times, what's very desirable is you brought in a useful trait, but you also brought in this other thing that might tastes might make something tastes bad, or reduces the yield. Right. Okay. And so to get to help to solve this issue of linkage drag, then you could use CRISPR cast to, to drop out these genes that might be having a deleterious effect. Gotcha. So that's a potential future application of this. I mean, people have demonstrated in concept that, that this works, yeah, we did

E Ed Zaworski 18:18

also work on the flip side to add things in a whole gene, per se, or

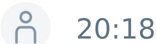
18:25

Yeah. So that that's more complicated. So, so making the changes that I described to the simple insertions and deletions or even making this larger deletion? Yep. Those are relatively simple. And I'll use simple in quotations. Right? Yeah, simple applications of, of CRISPR casts. But, you know, there, there is also research being done to use CRISPR systems to actually bring genes in and to insert them at a specific position in the genome. That's harder, because now you have to supply what we call a template, which is the thing that's going to be incorporated. So delivering both the cas9 system and delivering this, this template, yeah, to bring in the new gene that will add some important advantage maybe to the to a plant variety or something

that's very desirable, but it's much more technically challenging. People have people have demonstrated that it's possible, okay. It uses a different repair mechanism than the one that I described before. It uses something called homology directed repair, okay. Which plants also have this mechanism? It's not as active as the the other repair mechanisms are non homologous end joining mechanism, but they can do homologous homology directed repair.

Ed Zaworski 19:57

Okay. Gotcha. Yeah, know that that's, that's really good to know, from my standpoint, I think, you know, my basic knowledge of CRISPR. Before I had done a reading for this podcast or anything was that I that was my understanding of it was, oh, yeah, you're, it's adding in genes. But so so it has the capability, but that's not necessarily the most basic

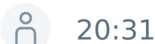


20:18

form of it. Yeah, that's a more advanced form of Yeah.

Ed Zaworski 20:22

Okay. Yeah, gotcha. So what? Give me some examples of like, things that we've used some basic stuff that we can use CRISPR for so far.



20:31

So maybe the, I'll just give you an example for for sleeping. Yeah. So well, is that CRISPR? There's different types of gene editing technologies. And so one, that one that people are are working on is to modify the oil composition of soybean, right, so, so that you don't have to go through certain industrial processes to get certain types of oil and things like that, right. So. So we can we can, we can use CRISPR and other gene editing technologies to modify the genes that encode the different oils that are produced in the soybean, soybean seeds, so you can modify the oil composition. And some of those are actually going on the market now, like from okay, this company called calyx, right, so. So it's a high high Lake oil, then we have other things that are of interest would be to be producing soybeans that they might have resistance to herbicides, for example. So, so herbicide resistance genes, you know, in the past, like for roundup resistance, yep. Or what's the Liberty glufosinate resistance? We brought genes in from other other organisms, like bacterial genes or something to add to, that detoxify the herbicide and allow the soybean plant to to be resistant to the herbicide but not the, not the weeds? Right. Right. But we also know that in weed populations, we see that weeds become resistant to to herbicides, right. And when you start looking at what's going on there, we see that the gene setting code, the proteins that are targeted by the herbicides are have been have, let's say evolved, yeah, so that the plants are now resistant to do the herbicide. So so people know, we know that DNA bases we need to change in in plants to make them resistant to things like like glyphosate. So and course, sulfur on self. So sulfonylurea types herbicides? Yeah. So so we can use gene editing. And instead of needing to bring, say, a bacterial gene into soybean plant, yep. We can use gene editing to simply modify the gene that's a target for

glyphosate or for Song sulfonylurea herbicide. Gotcha, it's a very small change, you can just change one, you can just change one DNA base that causes a one amino acid change in the protein that makes it resistant to to the herbicide.

E Ed Zaworski 23:33

Gotcha. Yeah. Yeah. And I mean, that's, that's kind of a good example. And the fact that like, I think weeds and herbicide resistance are probably, I mean, obviously, that's not the only problem that we have with resistant to pesticides. But it's, it's one that's very important right now. And there's a lot of herbicide resistance weeds. So I can imagine having the ability to do this kind of just adds to the toolkit of plant breeders, as far as you know, right? Winning this arms race? Yeah,

24:05

absolutely. Like, you know, if, for some, for some herbicides, there may not be an alternative, like maybe there's not a gene you can find in another organism to bring it in to protect against that herbicide. Yeah, but if we know the target of the herbicide, and we can use something like gene editing to tweak the Tweak the target a little bit so that the herbicide no longer is able to, to effect soybean or corn. Yeah, then that definitely gives a new a new tool to for the for the toolbox. Yeah.

E Ed Zaworski 24:38

So that that's, that's a good example, I think of a couple of advantages of CRISPR. Are there any pitfalls or challenges with using CRISPR?

24:54

Yeah, I think some of the well there are many challenges certainly Like,

E Ed Zaworski 25:00

yeah, yeah, yeah.

25:03

So, like in a for a public, for public researchers, there's a big challenge in, you know, corn and soy. So to get these things into corn or soybean plant, yeah, we have to go through a process called transformation, which means we have to introduce the CRISPR gene, you know, the spacer sequence and, and we have to introduce the caste nine protein into the, into the plant cells, yep, we have to then induce the edits that we want to happen. And we have to screen screen for them, make sure we got get the ones back that we want. And then we have to regenerate a plant that has all the characteristics we want, in addition to the in addition to the

Edit, so, so that process is is a complicated process that requires very skilled people that can do things like tissue culture and, and know how to work with plants in, in this tissue culture setting and then and then regenerate the plants. And it's a pretty time consuming process as well. So it takes several, you know, probably takes eight or eight or nine months to, to go from thinking about a gene you want to target to actually having a plant that now has a gene that's that's targeted. So

E Ed Zaworski 26:29

you don't just inject some you don't Yeah, you don't,

26:31

it's not. You don't just inject something willy nilly, and it's gonna happen. It's a, it's a really rigorous process that you have to go through to, to get to the end, where you have a successful gene editing event, we call it so

E Ed Zaworski 26:47

gotcha, gotcha. I'd say that's pretty good background on CRISPR. Okay, the cast, what do you think I think I think we can go ahead and move forward to the research and sounds like they will probably encounter some more background information and terminology that we'll have to further explain in there. But we can do, I think it's easier to do that as we go. So, yeah. Again, the publication we're going to talk about here is CRISPR, cast nine based gene editing using egg cell specific promoters and arabidopsis and soybean. So Steve, I know, I know, this is a hard thing to do. But can you give us you know, an overview of what you guys were trying to do and how you did it with that research? Right.

27:33

So as I just mentioned, there are challenges with deploying these CRISPR cast systems in, in, in our case, it's soybean, right? So. And one of the things that we were interested in knowing or, you know, one of the ways we're interested in proving improving the system was to try to have a system that induces edits more, that we know are going to be better, more likely to be edits that we know are going to be inherited into the next generation. Yep. Because those are the ones you're really interested in. And, and prior, you know, prior to this work, at least in soybean, people were using CES, the using the CRISPR caste system. But they were using systems that were the CRISPR cast was always on. So it basically was on in every cell. Okay. So what that means is that you're, you're producing edits. Yep. But not all those edits get passed to the next generation. Right? In fact, so most of them don't. So the

E Ed Zaworski 28:47

plant that you're working on that you're doing tissue culture for would have this edit, but then, you know, may or may she make seed and plant it. Okay, gotcha

28:57

a or may not be there. So, those are, those are what we call somatic edits. So that means that they happen in cells that don't give rise to the, to the next generation. So somatic edits, in some settings, they can be useful. But if you're interested in plant breeding a somatic edit is not not very useful. So, so we wanted to see if if we used a different type of way to drive the expression of the CRISPR caste system, where it was expressed specifically in cells that gave rise to the next generation. Could, could those give us a higher probability of inducing gene edits that, that we would have more confidence that we'd be passed on to the next next generation? So that was, that was kind of our goal also, we were looking for ways to possibly improve the efficiency of, of gene editing and And possibly reduced some of the, some of the negative effects of, of sometimes if you, if you try to knock a gene out or silence it, it can have detrimental effects on the plant. Sure. And so if we, if we got to a later generation, we may have fewer problems with with that song.

Ed Zaworski 30:23

Gotcha. So you use you did experiments with arabidopsis and soybean, right? So, is that because a rabid abscess is easier to work with to kind of test some of this stuff out first and then move on to soybean or

30:39

totally? Yes. So rabbit opsis is a model. It's a model plant.

Ed Zaworski 30:44

Oh, yeah, we should celebrate talking about what arabidopsis is first. So route

30:48

rabbit ops. This is a it's a it's a it's a weed. Yep. But it is a model of plant species. And, and so many of the things that we currently understand about plant biology, were first discovered in arabidopsis and then and then we've we've taken information from a rabbit opsis. And we've translated it to crops that we're interested in. So, yeah, so arabidopsis is really simple to work with. It's super small. Yeah, I can probably grow, I don't know. Yeah, I can grow 1500 or 2000, rabbit opsis plants in the same space that I can grow one soybean plant to maturity, I

Ed Zaworski 31:32

don't know, they grow really quickly, right,

31:34

they grow very fast. So in, in about, I can go from i can go I can make a transgenic. Or I can do a transformation experiment with the rabbit opsis In about two months, okay, versus, like, eight or so eight or nine months with, with soybean, you get back 1000s of 1000s of seeds, you know, from Yeah, from a single plant. They're super small, but you get 1000s of them. So you have a lot more material to work with than you do with with soybean as well.

Ed Zaworski 32:05

It's kind of like the equivalent of the fruit fly. Right.

32:10

Right. Yeah, it's the fruit fly of the plant world because

Ed Zaworski 32:13

yeah, fruit fly are the same way, folks, they reproduce really quickly, and you can really quickly see phenotypic or specific changes. So the the appearance or the appearance of the flyer, its genetic differences, right. So phenotypic and genotypic Gotcha. So, so use the Arabidopsis to kind of speed up the process. See, you know, how that might translate. So what are the so obviously, soybean takes eight months, or epitaphs. This takes a couple of months. And part of that is because of how the plant grows. But are there what are the other challenges with soybean versus are avid apsis? In this case,

32:52

in this case, probably another one would be soybean. Soybean has a more complicated and larger genome. Rabid abscess genome is very compact and, and yeah,

Ed Zaworski 33:07

simpler. Simpler. Yeah, yeah, we, we talked about this a little bit before we started, because I was quizzing Steve on terms that I didn't know, but we were talking about that soybean is paleo. Was it paleo? polyploid? Paleo polyploid? Yeah. And that its most general sense. I think you gave me a good explanation earlier, but give me

33:30

I'll see if I can reproduce. Yeah, right. Yeah, so. So basically, Paleo polyploid means that there's an ancestor of of soybean, where all the genes were were duplicated. So so in a, you know, normally in a diploid setting, you have two copies of every gene. Yep. in a, in a polyploid, you have to extra, you know, in a in a, in a, in a polyploid, you have like two extra copies, right. So,

so the genome at some point, the genome underwent a duplication, and so in an ancestor of soybean, there were four copies of each gene, right. And so what that means is that instead of having one, I'm repeating myself now. That's okay. Instead of two copies of every gene, you now you know, how for Yeah, and so and these genes have similar functions. So it creates some challenges when you try to study what the functions of an individual gene are. A lot of times we use techniques where we want to want to knock a gene out to study what the effect of was of knocking a gene out was, but in soybean, you might have to knock out four copies of a gene. Gotcha. So that really really complicates things. In rabid opposites, there are some duplicated genes, but there's it's much less of a problem than in soybean.

E Ed Zaworski 35:07

Gotcha. Um,

35:10

I guess the short answer is polyploid means more than diploid, typically, right? So you have 468 copies of genes.

E Ed Zaworski 35:19

Makes more makes sense to me. It's it in a very basic sense. To me, it seems like alright, it's much more complicated genome here. So it's much more challenging to get what you want out of it. So yeah, so So yeah, that makes sense. And then yeah, it's harder to get it to pass on the traits as you want in further plant breeding outside of this, so that makes sense. So, yeah, here, let's take a step back. My mind is racing right now. So, so we use the arrived apsis to, you know, basically find the simpler method, then you tried to duplicate this same transfer transformation, right? into soybeans. And yeah, so what was the what was the outcome then? So were you able to do this? And, and what what happened? What were some of the things that happened along the way? I guess? Yeah.

36:29

So. So we had these, so we designed these different versions of their CRISPR caste system, we will use what's called an egg cell specific promoter to drive the expression of the caste nine protein. What what that means is that was that the caste nine should only be expressed in egg cells? Okay, so these are female, basically, the female germline cells, right? Eggs,

E Ed Zaworski 37:03

because these are the genes that are going to be passed on to the next generation. Yeah. Okay.

37:07

37:07

Yeah. So. So that was kind of our reasoning is that if we express this in Excel, which is a germline cell, yep, then we have a higher probability of inducing edits that will be heritable in subsequent generations.

E Ed Zaworski 37:22

Can you explain just generally, what a promoter is. And so we're talking about exile promoters here, what what is a promoter doing for you,

37:31

so a promoter? So all of our genes, your 20, some 1000 genes in your body? They have to have instructions on when to be active and when not to be active. Gotcha. All right. So so in some ways, you can think of a promoter as being a switch that or a rheostat, maybe a rheostat, might be even a better a better analogy. But the promoter, basically is telling the gene, that it's, it's time to be active. And, and how active it should be. So. So it's, it's not necessarily an on or off switch? Yeah, it can be. But it can, it also tells you tell us a gene, how much level the expression is, as well. So So promoters, basically, are the instructions to tell a gene when and where it should be expressed.

E Ed Zaworski 38:35

Let me take a stab at this really quick. You tell me how wrong I am. So what a promoter, you know, you've got your whole genome. Yep. It, but would it be right? To say that all right, if if a human is developing, right, promoter might be saying, all right, these cells need to be producing teeth. And the cells need to be producing eyes and right, is that generally well?

39:00

Yeah, I mean, generally,

E Ed Zaworski 39:02

okay. Okay. I'll take generally I knew I wasn't gonna be spot on, but yeah. Well, hey, okay. Yeah, go ahead. I'll elaborate on that a little bit, though.

39:13

Yeah. They, I think, maybe a different way to look at it could be that, you know, it's enough not necessarily to be producing eyes. Yeah, whatever. But, but a lot of things that are important are transitions. Okay. And, and so as, as an organism is developing, it goes through different trend transitions. Yep. You know, everything starts off at one cell. Yep. Cells divide. Yep. And then and then different gene. Different programs for genes start taking taking over. And as as

cells continue to divide, they start Developing into different different groups of cells or lineages that then give rise to different tissues and organs and things like that. Yeah. And all that's determined by not only promoters, but but the genes, the gene action, when they're expressed, how much they're expressed. When they get turned off, all those things are happening in a very coordinated way, right, as an organism develops to eventually result in the final final form, which is has the teeth in the eyes?

E Ed Zaworski 40:37

Yes, I'm oversimplifying. But so the promoter is part of that. It's not the sole driving factor,

40:46

right? It's not part of it. Not the sole thing, but it is super important. Yeah.

E Ed Zaworski 40:49

All right. So we've got our egg cell promoters that you are you trying to use as a driving force still to pass on these genes that you've edited with? CRISPR? Right?

41:02

Yeah. So So basically, the egg cell promoter is telling the cast nine protein to be on in the eggs. Right? So So if in the in the egg cells now, we have our CRISPR RNA, yep, that's gonna guide cast nine to make a break in the DNA. And in those eggs, that break is going to be repaired. All in all likelihood, there's going to be a sort, small mistake made when it gets repaired. Yep, we're gonna have an insertion or a deletion. And then that little mistake is going to get passed on to, to the progeny. Gotcha, because we had the, the cast nine CRISPR, cast nine system was on in those in those egg cells. Gotcha.

E Ed Zaworski 41:49

So what happened?

41:52

Well, it worked. It worked. Yeah. So. So. So we tested the system in arabidopsis, as we discussed in the model organism, so we were able to rapidly rapidly test in in arabidopsis, we tested I think two different two different genes. And we saw that some not all of the egg cell promoters could could induce edits that are then passed on to to a rabid opsis seedlings. So after demonstrating that, then we wanted to see if we could get the system to work in, in soybean. Yep. And so we, we take the same set of of CRISPR cast nine constructs we call them with the different Excel promoters and we put those into soybean Yep. And then we generate the the plants that express that carry the CRISPR cast nine CRISPR cast nine genes. We grow

them to maturity. Yep. And then we look in the next generation, what we call the we call it the T one generation to see if any of these carry the edits in the expected expected genes. Yeah. And, and we found that there was one particular promote, so we tested four different egg cell specific promoters, to or from originally from arabidopsis and to originally from from soybean. Interestingly, we found that there was one rabbit opsis XL specific promoter that seemed to work really pretty well at causing the or inducing gene edits in, in soybean that were that were so seen in the in this T one generation. Gotcha. Yeah.

Ed Zaworski 43:42

Gotcha. So, did it work 100% of the time and all of the progeny then or?

43:50

No, so, so that's, that's one one of the challenges that we're still kind of working on. So yeah, it's not, it's not 100% efficient. Yep. It's about I can't remember the numbers. Exactly. That's okay. It's, it's efficient enough that it's interesting. Yeah, yeah. But it's not it's not 100% efficient. So every, like every plant that we put this, this cas9 construct into, not every plant is going to produce progeny that that have edits. Gotcha. So you still, you still have to, you know, you still have to screen multiple different lines. Yep. To find those that that have multiple progeny with with edits, and then those are the ones you continue to work with.

Ed Zaworski 44:39

Okay, so you've got like a lot of different would it be different gene edits or different lines of soybeans? Is that what you mean they're

44:48

different. So when we when we do when we make the transgenic plants, we don't just make one transgenic plant, right. We make multiple transgenic plants. So each one of those represents a line. And they're they're unique. They're unique transgenic events are not the same. They carry the same DNA that we put in. Yeah, but they there are some some differences between them. And so we grow those to maturity. And then we look in the progeny of each each one of those. And so, in some, in some lines, we don't see any edits, but in some we do. And there's probably different reasons for why that happens. But we

Ed Zaworski 45:36

Yeah, it's, we won't get that we won't go that deep. It is

45:40

it's a bit frustrating. And it's one of the reasons why we have to make multiple lines. So this is a

very common thing that happens. Some, in some lines, when you make a transgenic line, the gene that you put in works well, in some, in some the they don't. And so you just have to select the ones that that work well.

E Ed Zaworski 46:00

Gotcha. So you're Yeah, so you're picking from the ones from the many lines that you created? You're picking the ones that did work? Well,

46:07

yeah. But I should say that, you know, as I said, we tested four different egg cell specific promoters arriving cas9. And there was really only one that worked. So So you know, we had multiple lines for each of the, of the Excel specific promoters. But there was only one for which we we had lines that we could recover gene edits in the next next generation. So that that one's the most interesting one to carry forward. Gotcha. In future studies. Yeah.

E Ed Zaworski 46:38

So that's, yeah, it's a good stepping stone. Like you weren't expecting it to be all right now we made 100%. No, no viable. Right. So if I, if I can speculate with you a little bit here, like, what, you know, move moving forward. If you're a soybean breeder, obviously, it's not 100% there yet, but like, what? What do you see for the future of this? You know, do you think eventually, you know, soybean breeders are able to, you know, produce large batches of seed with these gene edits? Are we are we really far away from that? Or are we close to it? What do you think?

47:22

Well, there are, there are many genes in in the soybean genome that we could, that we could try to make changes to the to to improve soybean in a variety of different ways. Right. So Right. So we already mentioned the the oil traits, for example, right?

E Ed Zaworski 47:44

Oh, yeah. So I should let me rephrase this. Yeah. So there's already genes that you that have been edited? Yeah. And they're heritable, right? They can be passed on so. So what's different about about this system, where we're looking at the egg cell promoters specifically to pass stuff on? Are we tired? Are you targeting different traits? Are you

48:07

the types of edit? Yeah, the types of traits or edits is not really different? It's the way in which we deliver the CRISPR caste system. Okay. So our goal is to try to optimize the delivery of the technology to get more more efficient. Gotcha. But it doesn't really change the types of genes

that we can target or the types of changes that we can make. But

E Ed Zaworski 48:35

is it the heritability, then? The success rate, then? Yeah, it's,

48:38

I would say it's this success rate. Yeah. Gotcha. Yeah. And, and the useful edits is maybe another way to look at it, the ones that okay, the ones that will carry forward into the next most likely to carry forward into the next generation.

E Ed Zaworski 48:55

Gotcha. Yeah. So it's a it's an improvement on some things that have already been done. Yes, it's an improvement and heritability and Okay, yeah. Gotcha. What does this mean? What do you think this means? These are the hard questions here towards the end. What do you think this means for growers? Like if you're a grower, if you, you know, put yourself in their shoes, if they're listening to this? What is it mean? I think eventually, maybe not right now. But what do you think it eventually means for a grower? If you're out there planting seeds.

49:33

So CRISPR cast gene editing tools are, are really powerful. Yeah, I think. And I think, you know, we're still in the infancy of really using them to their full capabilities. Yeah. And so I think, you know, this is a step towards that. towards, you know, enhancing their utility. And, and, you know, I'll also say that, you know, some some research goes directly into into plant breeding. But there's other research that happens in the, you know, happens in the background. And yeah, and it helps to build tools and resources and, and things that kind of help the overall research community to, to move forward faster and to do new things. So, so I think we might, we might place this research in that second category is something that something that doesn't necessarily have a direct, you know, you won't necessarily see it directly used in the, in the field, but for like a farmer, but it's something that if we look at, you know, helping out other researchers to, to push to push the envelope to, to maybe study some genes or more, more quickly edit genes that they they are interested in, that might have important agronomic consequences if we can figure out what they do, and then how we can, how we can change their functions. Right. That's more where I see this research making a contribution that I see that will eventually help.

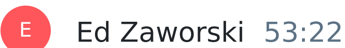
E Ed Zaworski 51:25

Well, rather than, you know, you're targeting a specific problem that soybean growers are having you're trying to, you're adding a building block and further developing just the understanding of gene editing and CRISPR. In general overall, right? That's right. Well, so we've already my final question always, is how does this affect, you know, the average person in the

general public? My mom in Chicago? But that, I mean, what you just said? I think, I mean, maybe you want to build on it even more, but I mean, yeah, helping out the general scientific community, and, yeah, and our understanding.

52:08

So I don't know, I think, you know, a more broader way to think about this is that we have, there's a lot of challenges ahead of us for, you know, for agriculture, production, right. So yeah. Yes, we have climate issues, water issues, disease diseases, there's always new new diseases coming along. So so all these things are challenges for sustainably producing crops. So. So, you know, I think the more the more tools that we have that allow plant breeders to develop the kinds of crops that we need to address our future challenges that I think, you know, the better, the better off we are. And this, this could go to, you know, food security kinds of kinds of issues, right, which I think are food safety and food security issues, which I think are of interest to, to everybody. So,


Ed Zaworski 53:22

yeah. I couldn't have said it better. I saw I think they're the future is bright, especially with guys like Steve at the helm of this gene editing stuff. I mean, come on the most famous person I know.

53:37

Okay. I can't believe well, I need to get out more.

Ed Zaworski 53:45

I need to meet more people. Yeah, with that, Steve, I can't thank you enough for doing this. I know this is it is kind of a hard topic to dissect it. But I think you did a great job of, of doing so. And I hope that the listeners of this podcast learn a lot from it. You know, with that, Steve, you have the floor for any things. How do people get a hold? Or maybe not get a hold here, but how do they find your research? How do they learn more about what your lab is doing? And any things that you want to that you want to give out there? Go for

54:20

it. Oh, well, thanks for thanks for the opportunity, Ed to talk about this this work today. Yeah, if people want to find out more, I don't know what my Wow. We have a loud web page. You know if they Google Yeah, well, if you Google Steve, Steve Widom. That's why t h a m?

Ed Zaworski 54:42

Yeah, so I was gonna say you guys, because I'd always

54:46

Yeah, if you Google that. I think the top hits will be a lot of our papers and things like that maybe our lab website or maybe my, my, my profile on our department webpage and then you can link to our webpage and, and from there you can see kind of a synopsis of the different kinds of research projects we have. Gene editing is just one. Right one of the things that we're, we're doing right now and you can link to our other papers and things like that as well. So perfect. Yeah. Oh, also I should give a shout out to the ISU crop bioengineering center. So if you Google that as well, you can go to the ISU crop bioengineering Center webpage and kind of see in on a bigger scale and of what's happening on our ISU campus and the different faculty that are involved in, in research and other issues related to, to gene editing and in crop plants.

E Ed Zaworski 55:50

Perfect, folks. I'll put some links in the description in the show notes as well. But check out the most famous man I know Steve Widom. Steve, thanks again for joining us and, folks, we'll see you on the next one.

B Brandon Kleinke 56:10

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