My name is Joe Bondy-Denomy and I discovered the first anti-CRISPR proteins, which suppress bacterial immune systems. Now my lab at UCSF is exploring how CRISPR works in bacteria, its "native habitat." AMA!

JoeBondy-Denomy [1] and r/Science AMAs[1]

[1]Affiliation not available

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R/SCIENCE JOEBONDY-DENOMY

ABSTRACT

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redditWinnower

This is great, thanks!

Inhibiting CRISPR-Cas systems may present a completely new drug target in the fight against antibiotic resistant pathogens

I'm not sure I see the link between CRISPR-Cas and antibiotic resistance. The CRISPR-Cas adaptive immunity system, as I understand it, provides bacteria a way to selectively destroy specific nucleic acid sequences. Do you anticipate blocking the CRISPR-Cas system may hinder the bacteria's ability to evade canonical antibiotics (e.g. penicillins, macrolides, tetracyclines, aminoglycosides etc.), and if so, how since these molecule classes don't seem like they would be subject to CRISPR-Cas mediated degradation?

SirT6

There are many ways I envision for CRISPR-Cas being a benefit in the long term battle against pathogens. I cite antibiotic resistant ones because they are currently our biggest hurdle, but I didn't mean to imply a direct link with antibiotic molecules.

1. A pathogen can't cause disease if it doesn't survive. Our bodies are full of phages that could destroy a pathogen. Maybe they use CRISPR-Cas to protect themselves long enough to invade to a tissue

where they do damage (i.e. blood, brain, etc.) If CRISPR-Cas could be inactivated perhaps that means the pathogen is less fit in the human body. (add to this a few extra phages delivered as a drug and I think we have a fighting chance!)

2. The idea of 'non-canonical' functions for CRISPR, i.e. regulating genes for the bacteria, would be another way that a drug that inactivates CRISPR (like an anti-CRISPR drug) could cripple a pathogen. There aren't very many examples of this yet but one great one came out in Nature in 2013: http://www.ncbi.nlm.nih.gov/pubmed/23584588 This paper basically showed that without CRISPR, the bacteria couldn't repress an important gene after infection. This helped the immune system 'find' it and destroy it.

Thank you for doing this AMA!

Recently, the magazine *Wired* published a lengthy article about CRISPR-Cas9 and the potential legislative hurdles it will face. Do you agree that we will need strong regulation of this technology to prevent improper usage in human trials? The article quoted how "researchers at Sun Yat-sen University in Guangzhou, China, announced they had used Crispr to edit human embryos" and discussed how this may open a can of worms we are not prepared for.

Edit: Here is the article: http://www.wired.com/2015/07/crispr-dna-editing-2/

NeverResting

I think this is a very important issue. Whenever a new scientific discovery or breakthrough comes along, it of course comes first, and following closely behind are ethical, moral, and legal issues to discuss. The idea of using this to edit human embryos is being widely discussed and scientists are requesting a moratorium on this until many things are worked out (see: http://www.nytimes.com/2015/03/20/science/biologists-call-for-halt-to-gene-editing-technique-in-humans.html?_r=0). There are many reasons people should listen to this. Unintended or off-target effects is still a problem, as is the inefficiency of homologous recombination in different cell types. Also, we actually don't have many examples in the biological world where one gene affects one condition (whether that is a disease or something like height or athleticism).

Assuming that people listen to these requests, the next issue is deciding what Cas9 should or shouldn't be used for and that is a huge debate. None of the people who initially developed this technology were doing this to generate designer babies. This has been widely adopted as a laboratory technique (i.e. making mutations in mice or human cells, or using Cas9 to repress/activate genes). This has already been a huge breakthrough. For the purposes of medicine, the Cas9 community (and I would put myself as a fringe member of that group, since I am not directly working on this) hopes that this could be used for correcting somatic mutations, i.e. correcting diseased alleles in an 'already born' human.

Can you share a bit the story of the initial discovery? What led you to the CRISPR-Cas system initially? Was it your initiative as a student with a supportive PI or an active project in the lab already? (Not to belittle your achievements at all! I didn't say it quite well, but I hope you understand.) I'm a big fan of the process behind these discoveries. I'm also a graduate student, so I like to hear how these discoveries were made on a day to day and datum to datum basis.

kenshin13850

Great question! In short, it was all a beautiful accident! and yes, lots of support from my PI. I did my PhD in a phage lab, so this is how I came into CRISPR, in a fairly organic and unintentional way. I was isolating phages that infect Pseudomonas aeruginosa (a human pathogen) because I was interested in the natural variation and genetics of this group. Around the time I was doing this work (2010), CRISPR

had been recently described (in 2007) as an immune system that targets phages. So naturally I wondered if the phages I had in my fridge were targeted by CRISPR. The surprising observation was that some were destroyed (as expected), but many weren't. Why aren't these phages being destroyed?? That was the question. The answer ended up being an incredible journey leading to anti-CRISPRs. In other words, the phages were producing proteins that were shutting down the CRISPR system, allowing the phages to 'win the battle.' That 'answer' probably took me about 3.5 years of incredibly hard (but exciting) work. And to the grad students out there, this was after ~2.5 years of grad school...before things really got exciting (and certainly before any papers were published). So these kinds of 'discoveries' are characterized by many experiments (mostly failures) and constant day-to-day planning, troubleshooting, and discussions. I had an incredibly supportive lab, community of labs and there is no doubt that my PI was instrumental to the success of this project. We had (and still have) an excellent relationship with ample communication (i.e. we would generally speak every day!). And I would say half of those conversations were about CRISPR and phage, while the other half were about the Toronto Blue Jays. :)

I appreciate the AMA; I've heard a lot about CRISPR and its potential, but come from a completely different background (law and engineering). I have a few questions below, feel free to answer any or none, thanks!

Do you or UCSF have any plan to commercialize this technology, i.e. make a start up company or license patents to one of the big players?

There seems to be a lot of investment flowing into CRISPR, do you think there is a CRISPR bubble or is this the real deal?

Team Doudna (Berkeley) versus Team Zhang (MIT), who do you choose?

How much of the CRISPR discoveries do you believe deserve to be eligible for patent protection (to what extent)?

windslashz

(disclaimer) Cas9 and the patent world is something I am not a part of, nor am I 'pulling' for any one side (so I'll skip question 3!)

1. Nothing yet... but time will tell!
2. Real deal. First, as a lab-based technology, this is an incredibly real thing. With a pretty rapid time scale (e.g. Cas9 was first described as a programmable nuclease in late 2012), this has become that technique that most labs working with eukaryotic systems to use to create mutations or regulate genes. It basically took two years for this to be widely adopted with great success. That is incredible. For medicine, that obviously takes more time, but I have no doubt that it will present viable cures/treatments for some diseases. I say some, because their are many hurdles (i.e. delivery to a given tissue, specific targeting of the gene of interest). Time will tell whether this truly changes the way we do medicine. Keep in mind that we are still limited by our knowledge of the fundamentals of human genetics. Many diseases are so complex that even with a perfect Cas9 system, we couldn't correct them (right now).
3. This is really a legal question when it comes to what is patentable from nature. I think it is clear that a technology like this one, which existed in prokaryotes and has been engineered to work in human cells is a large feat of engineering/biotechnology. This sounds patentable to me!

I've read many species of bacteria like E.coli don't have functional CRISPR systems. How do these

species resist phage, if at all? Do you think these anti-CRISPR proteins you have found could similarly work against vestigial CRISPR mechanisms?

From another perspective, is it possible to exploit a bacterium's native CRISPR-Cas pathway to destroy it's own DNA, i.e. delivering a crRNA that targets some essential protein coding gene?

gruhfuss

Restriction enzymes indeed! what a powerful and lovely example of something that we have learned and adopted from the phage-bacteria arms race. There are many other ways that a bacterium protects itself from infection. Here are a couple reviews from a fellow Canadian phage researcher (and my thesis external examiner!) http://www.ncbi.nlm.nih.gov/pubmed/20348932 outlines defenses against phages and http://www.ncbi.nlm.nih.gov/pubmed/23979432 outlines how phages fight back!

You are right though, only ~50% of bacteria have CRISPR (and like you said, some with CRISPR seem to be non-functional). Some bacteria live inside eukaryotic cells (obligate intracellular bacteria) so perhaps they never see phages. Others probably rely on a mechanism described above or others we haven't yet discovered. Keep in mind that CRISPR as an immune system was only functionally discovered in 2007, many more are likely to come.

Anti-CRISPRs wouldn't really 'need' to work against a vestigial CRISPR system, if it isn't active. Whether it would still interact with the CRISPR-Cas components would depend on the protein sequences of the Cas proteins and whether they have binding sites for anti-CRISPR proteins.

It is definitely possible to use a bacterium's own CRISPR-Cas against it. We do this in the lab all the time!.. it is handy for genetic screens (but I won't go into that here). There have been a few papers where researchers have basically used a phage to deliver a CRISPR system or even just a crRNA that will target the genomic DNA of a bacteria or a plasmid encoding antibiotic resistance genes, for example. (e.g. http://www.ncbi.nlm.nih.gov/pubmed/25282355)

Assuming this provides a completely new mechanism of action as an anti-biotic that is not effected by the existing adaptations allowing for anti-biotic resistant strains of bacteria to develop, what is the potential for bacteria to become resistant to this mechanism of action?

Is there anything particularly unique about this mechanism of action that would make it unusually difficult for bacteria to become resistant to it?

orangesunshine

Thats a good question. Based off of the anti-CRISPR proteins that I work on, I haven't yet done experiments to find mutations in the CRISPR-Cas system that escape anti-CRISPR function. I would expect they exist though, unless anti-CRISPRs are so 'perfect' that they bind to residues essential for CRISPR-Cas function.

As a drug target (i.e. a small molecule that would inactivate CRISPR) this is also sort of a hypothetical question right now, but it is an important consideration. Because CRISPR-Cas systems rely on multiple proteins and steps, perhaps a drug cocktail that inhibits multiple steps of CRISPR-Cas function (like the HIV drug cocktail) would be a good approach. This would reduce the chance of resistance.

What are your thoughts on the ethical/moral dilemma posed by using CRISPR-Cas to edit human genomes? Is this something that should be regulated by the countries of the world? Or will the general consensus of the scientific community dictate the practice for the foreseeable future?

shiruken

Due to the simplicity of CRISPR technology, it seems that it needs to be regulated. My understanding though, is that this is done on a country by country basis so it will likely be a combination of the wishes of the global science communities along with country specific rules. This isn't a new issue though. This has been dealt with (and is still being dealt with) for recombinant DNA and embryonic stem cells, for example.

If PCR is like the "printing press" of DNA, what is CRISPR?

ktool

I like programmable scissors. But I would like to emphsaize that CRISPR does more than just cut DNA, from an engineering standpoint. One can use Cas9 to recruit proteins of interest to a specific region of DNA. By tweaking Cas9 so that it doesn't cut anymore, researchers have been able to recruit transcriptional repressors, activators, DNA-modifying enzymes, RNA scaffolds, etc. to a specific site.

So maybe Cas9 more broadly is an in vivo sequence-specific molecular homing device. Not as enticing as programmable scissors but the applications are incredible

From what I understand, CRISPR is a mechanism to cut up phage genetic material during the second invasion of a virus or any invasion after that. But what allows the bacterium to survive the first invasion? And why can't it survive in that way for the subsequent invasions?

biocore1

This was also a mystery for the field. An important observation was that this was a fairly rare event. If you infect a million bacteria with a million phages, one bacterium might survive. And this would have a new CRISPR spacer. The thought is that this comes from a defective phage. Basically, one phage in this group will mess up somewhere, the replication will stall and the DNA will be a sitting duck. This gives CRISPR some time and prevents the bacterium from getting killed. It won't be so lucky next time, as the odds are, that it will get infected with a functional phage. Here is the paper: http://www.nature.com/ncomms/2014/140724/ncomms5399/full/ncomms5399.html

Are there any eukaryotic homologs of these anti-CRISPR genes which might be responsible for reducing the efficacy of CRISPR as a human gene therapy protocol?

We_Are_The_Romans

None that I have found so far. This is an idea I am very interested in though. Cas9 is a protein that doesn't naturally exist in eukaryotes, so one may argue that it would be unlikely for an anti-Cas9 protein to exist there. However, it isn't inconceivable that some cell types or organisms might somehow inhibit its function either directly (i.e. production of a protein antagonist) or indirectly (i.e. DNA modifications that prevent binding/cleavage, mark Cas9 for degradation). This may be the reason for some anecdotes that scientists I have spoken with telling me about Cas9 just not working for them. Some of these examples are very intriguing and your suggestion may be spot on.

Dr. Bondy-Denomy,

I work with Staph epi's III-A CRISPR/Cas system while I'm doing my master's, and yours describing the anti-CRISPR genes were easily some of the most exciting studies I've seen about CRISPR/Cas. The potential applications for combating the antibiotic resistance crisis are mind-boggling, and I'm very

excited to see what comes of it over the next few years.

Now that I'm done gushing, I'd like to know: how widespread do you think these anti-CRISPR proteins are among phage?

Have there been any/many homologues to these proteins found in more diverse phages (i.e. outside of P. aeruginosa or targeting systems other than Type I, which I believe all studies documenting anti-CRISPR proteins thus far have been in)?

What implications do you think this will have for CRISPR studies and applications in the future?

What do you think about Makarova and Koonin's papers on CRISPR as potentially being a toxin system to induce dormancy/suicide in infected cells (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3737325/)? To what extent do you believe this is what is occurring?

What ideas do you have for your lab to focus in on and pursue over the coming years? I imagine investigating anti-CRISPR will be among the aims, but is there anything else you're especially excited about?

Is there any work in any other labs you're especially interested in following and excited to see what comes of it? Personally, I'm fascinated by all of the non-canonical roles CRISPR/Cas plays, such as the scaRNA gene regulation that was described a few years ago.

Finally, on a more personal note, I'll be graduating with my master's in Spring 2017. Any chance you'll be looking for PhD students around that time?

An answer to any or all of these questions would be greatly appreciated! Thank you!

elcaminador

Thank you for your nice comments.

I think there are anti-CRISPRs all over the place. Hopefully we can find some more. We know that many phages have mechanisms to inhibit other bacterial defenses, why should CRISPR be any different?

Homologs of anti-CRISPRs have been tricky to fine because they are not just one protein. They are an incredibly diverse set. This is something that is still being worked on in the Davidson lab at U of T (where I did my PhD). So far, we just have Type I anti-CRISPRs and yes we have found them in plasmids, islands, prophages, and other mobile DNA.

I think I have addressed the implications elsewhere, but certainly more anti-CRISPRs will teach us new things about CRISPR-Cas function, their evolution (i.e. why are there so many diverse subtypes? because anti-CRISPR?) and even ways to fine tune the applications.

I haven't done any experiments on the toxin/antitoxin side of things so I can't really comment, but I generally am in awe of the amazing work done by Makarova and Koonin, so I would always be willing to listen to their ideas.

In the lab, we are exploring a lot of things related to anti-CRISPR mechanisms, finding new anti-CRISPRs and exploring non-canonical functions for CRISPR.

Good luck with your work! I look forward to hearing from you in the future, UCSF is a great place to be!

Great research! My question is a tad more general, I remember reading that bacteriophages were once thought of as an interesting therapeutic strategy and that they had been used in countries like Russia.

With the impending antibiotic resistance crisis and serious challenges to developing new anti-infective therapeutic modalities, do you think baceteriophages will have a role to play in the future to tackle this? What are the major obstacles to their use and what dampened interest in their use in Western countries after apparent initial interest?

do_you_smoke_paul

Yes I think phages will play a role in our future therapeutic arsenal. This is no longer just a thing that is going on in Russia. Clinical trials are underway for treating many organisms, both in North America and Europe. The NIH and FDA are also having serious discussions about how to fund this work, regulate the products, and ensure safety/efficacy. If I were on the verge of succumbing to an antibiotic resistant infection, I would gladly swallow a tube of phages. We have known for a long time that they work well (when targeted to the right strain and delivered in the right way) and that they pose very little risk to safety. Therefore, the more we know about CRISPR and anti-CRISPRs the better (in my opinion).

The major obstacles, like I eluded to are specificity, delivery, immunogenicity, and replication. Phages are incredibly specific in some cases and thus we need to deliver the right phage for the right strain (not just species) of bacterium. This is why antibiotics are so convenient, they are generally broader spectrum. Second, effective delivery could be tricky depending on the site of infection. Third, as has been mentioned by a few people, the human immune system could recognize these are foreign and try to get rid of them. This is being investigated, currently. Finally the fact that phages replicate is good and bad. Good because this means localized amplification of your drug, which sounds good to me... bad because people who regulate drugs don't like this sort of thing, where it is hard to know exactly how much 'drug' is being delivered if it can increase in the body. Given that phages have DNA and can mutate is also a concern to regulators. Lots of things to think about and work out but I think there is promise here. Some people are also exploring using phage-derived products such as enzymes that are toxic to bacteria.

Have you any idea yet what stage in the process anti-CRISPRs target? The most likely point of attack would be the recognition or nuclease steps, but is it possible they interfere with integration of viral DNA into the genome? If the anti-CRISPRs helped us understand that better we might have a new tool to edit bacterial genomes too.

altforscience

Your prediction is very accurate. Anti-CRISPRs function through multiple mechanisms to turn off CRISPR-Cas. Some of them block DNA recognition and another one blocks the recruitment of the nuclease protein. This was all described in my recent paper, which I have added a link to in the opening blurb.

Interestingly, new data suggests that they do also interfere with integration of viral DNA into the genome (i.e. new spacer acquisition), which definitely tells us something about how this process happens.

You guys should check out the Radiolab episode that they did about CRISPR, really interesting and informative as usual.

http://www.radiolab.org/story/antibodies-part-1-crispr/

TheDanilka99

agreed!

What were you trying to do when you discovered "anti-CRISPRs"?

hg57

I was trying to figure out how prophages (a phage that is integrated in the bacterial genome) modify the properties of the host bacteria. One property ended up being that prophages turned the CRISPR system off. weird, right?

Hi Joe! Are there observable effects on the host when native bacteria succumb to these viruses? Would it be possible for diseases which are currently classified as idiopathic to be the result of a 'sick' bacterial population?

carbsLAD

I assume you mean the host being a human or an animal? If I understand, you are asking about whether 'good' bacteria being killed by phages in the body could lead to a problem with the commensal flora? If I am right, then yes it is definitely possible. We are only starting to understand the commensal 'virome' in the human body. The microbiome is being heavily investigated but has mostly ignore the phage population. What phages can do to your good bacteria is a very interesting question, maybe this is why so many commensals have CRISPR-Cas?

Do you have a link to your study that isn't paywalled?

ngc5128

Happy to share with you ngc5128. Find me on ResearchGate

You said the bacterium can become resistant to the phage. Does that mean it can alter protein expression on its plasma membrane to keep phage "hooks" from latching on and lysozymic enzymes from allowing the plage from inserting it's dna|rna into the bacterium?

Vitaeamor

There are many ways that bacteria can become resistant to phage. Here is a good review: http://www.ncbi.nlm.nih.gov/pubmed/20348932 CRISPR and restriction are just two.

Thank you for your dedication!

What are you hoping to develop from your current research? And what have you learned that and been able to use so far that was completely unexpected?

Goats_vs_Aliens

Many goals, as described above. We are hoping to understand how this fascinating adaptive immune system makes its host bacterium stronger. Is this always through phage resistance or is it also performing some other adaptive functions. The most unexpected thing was certainly finding anti-CRISPRs, and if I could be more specific, finding that there are >10 different genes (so far) that encode anti-CRISPR activity was really the most shocking thing. So much diversity and yet, the same overall function.

One of the post docs in my lab is working on building CRISPR tools into Drosophila. Do you think this will be the way all genetic manipulations are handled in the near future for all animal models - replacing viral or lipid based transfection and GAL4-UAS systems?

I know there have been complications, such as complete homologous editing within a single generation, eliminating all heterozygotes from the breeding population. What further 'adjustments' need to happen to the tool?

lzawwlgood

I am not a eukaryotic biologist so I can't say for certain that it will work in every single model system, but it has gotten off to a good start I think. In short, yes, I think this will broadly take over as a mechanism for gene editing/manipulation. Still need good delivery methods, so the two that you mentioned are still important.

HDR has been inefficient in some cases but this is a work in progress. For example, the new Cpf1 (it leaves sticky ends instead of blunt) may be a game changer here. Here is a good summary of the finding: http://www.nature.com/news/alternative-crispr-system-could-improve-genome-editing-1.18432

Thank you for your AMA. I'm very interested in the CRISPR system and its role in bacteria. I have a couple of questions.

Do you think anti-CRISPR are widespread among phages that infect bacteria with CRISPR systems? And that perhaps much more anti-CRISPR proteins are waiting to be found?

The CRISPR-Cas type II system is found in many bacterial pathogens. Do you think that this CRISPR-Cas type also functions as an adaptive immune system against phages that infect those bacteria? Or that there might be different interactions going on.

Orussuss

Yes I think they are widespread and more are out there to be found (more detailed answer above).

Yes, I think the Type II system in those pathogens is acting as an immune system against phages. Other roles may exist in addition to this.

A potential advance in science and medicine this big is huge just like penicillin was. We're seeing resistance to that now. What concerns do you have of us creating something that can counteract any of the work that anti CRISPR can accomplish, and having that be out in the wild?

turbopro

That is certainly a concern. For therapy, targeting CRISPR in many ways, coupled with traditional antibiotics might do the trick. Keep in mind that although antibiotic resistance is a big problem (generated by their overuse and misuse), we haven't actually made pathogens better at causing disease. We have saved millions of lives along the way and now need new solutions. That is far better than not using something because we are worried about downstream resistance. Gotta take those basketball shoes out of the box and jump!

Do you think CRISPR/Cas9 is a potential magic bullet for HIV? Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells

PHealthy

What a great application for Cas9. If nothing else, this served as great proof that we can use Cas9 to cut out whatever we want. And what better element to cut out than a pesky HIV genome. Again, I think delivery becomes the issue, coupled with the potential for off target sites. I have no idea how many infected cells there are in the body on average, and then how many of those would need to be 'fixed' to have a positive impact? Could this be a cure? Tough questions to answer right now, but I can promise you it is being worked on.

The CRISPR locus is located in the bacterial chromosome, right? Is the acquired immunity inherited by the daughter bacteria, after the division? If so, why isn't the CRISPR locus enormous? After all, it should be accumulating new sequences over time.

elsoja

true. Spacers do get lost. Probably older spacers that the bacteria doesn't 'need' anymore. i.e. those phages are no longer in the same neighborhood

Is that true that a CRISPR lab can be set up for less than 2000 $?

http://www.businessinsider.com/how-to-genetically-modify-human-embryos-2015-4

If this is true it would mean a low to 0 entrance barrier for those who are interested in genetics .

Henrilloyd

I suppose it is possible in the sense of the genetic tools. You really don't need that much. Take this with a grain of salt though, the organism that one wants to edit will be limiting with that kind of budget. Could you mess with yeast to change the flavor of your beer with this kind of cash? perhaps. Could you modify embryos? Goodness no. Even working with human cell lines in a dish costs a large amount of money.

In the field of cancer genetics, could the CRISPR-Cas system be used to create next-generation treatments?

eisenreich

This is being done already in a couple of ways: 1) Using CRISPR-Cas9 to make mutations and regulate genes in the lab is already being used to discover many things about cancer (how are drugs blocked?, can we find new drugs?, what are the real mutations that drive cancer?). This is why Cas9 is already changing the world, before really being used to treat a patient

2) Could one deliver Cas9 loaded with gRNAs that will cut up a specific sequence that only exists in a tumor cell? In theory, definitely. This is being worked on. Again, delivery is the issue, but one could use viruses engineered to only infect a tumor cell, for example and deliver Cas9. CRISPR-Cas9 is a very exciting development for the cancer field, there is no question about it.

There was an episode of RadioLab about CRISPR a few months ago.

One guy described CRISPR saying, "I can use CRISPR to take a little dog and*poof* make it into a big

dog."

Is that a reasonable claim? Will we once day be able to take a pill to change our eye color? Will my friend finally be able to get a shot to make his penis grow?

Qxface

Many things to think about here. 1) are there just one or two genes that control these traits? do we know what they are? 2) I have a hard time imagining how you would effectively modify enough cells in a little dog to make a big dog. Perhaps at the embryonic stage, but this is what is being debated. 3) A localized change (I'll go for the eye color example) however, might be a possibility. Sounds like a risky thing to try though when there are contacts that do the same thing.

Hi Dr. Bondy-Denomy,

Thank you for doing this AMA. In one of your papers, you describe how bacteriophages infecting Pseudomonas aeruginosa have developed anti-CRISPR proteins to evade the host's CRISPR defenses. As you may know, Pseudomonas poses a particular challenge for patients in the hospital setting. It has over 50 resistence genes to antibiotics and even forms biofilms on medical devices which are nearly impervious to drugs. Aside from casuing urinary tract infections and deadly lung infections in patients with cystic fibrosis, pseudomonas poses a particular problem for burn victims and premature infants. What role do you think phage therapy has for intractable pseudomonas infections? If phages are genetically modified to have greater anti-CRISPR production, what possible consequences would there be on the evolution of pseudomonas in vivo? Is there a chance that pseudomonas will then develop greater or modified CRISPR activity, thus necessitating continual modification of phages in the same way we constantly come up with new antibiotics?

Eugene_Gu

Thank you for your question. This organism is such a pesky one and a problem for so many people. I imagine that the scenario you are painting would select for resistance to an anti-CRISPR. This is where it is great that we have discovered multiple anti-CRISPRs that target many different CRISPR-Cas proteins and therefore would be less likely to succumb to any one resistance mechanism. Using a phage cocktail plus antibiotics may be enough to wipe out this tricky organism.

I get the impression that the methods and success rates for using CRISPR/Cas are relatively easy to pick up, even for undergraduate students.

As an undergraduate student myself, what advice would you offer to interested students who would be keen to use CRISPR/Cas methods for their own prospective projects and/or interests?

And lastly, how do you feel about the movement toward hobby scientists, or so called bio-hackers, using CRISPR?

Cats_Like_Felix

The bio-hacker community is not something I am that familiar with. I think it is great for people to be interested in science like that though, even outside the traditional confines. I think the answer for them and for undergrads is that it is about the application. As a young researcher, what is the biology you want to investigate? What are you interested in? CRISPR could probably help you if your question has any genetic foundation. To actually get into it though, read papers, ask questions, talk to professors/other students about your ideas and get started. Most vectors and tools are free or low cost and are not an impediment for research purposes.

And for the bio-hackers, what are their goals? Is it modifying a plant to make a prettier flower, or modifying yeast to make their beer to contain more vitamin C? or is it something more sinister or risky. I think this is where some of these movements could be risky and need to be monitored, for both the safety of the hobby scientist and the public.

I have a feeling that the commercialization of CRISPR-cas technology is a bit rushed. As in, is the current rise in companies dealing with CRISPR going to slow down progress in actually understanding the technology- If the pioneering labs such as yours start patenting everything, will that slow down the quest to answer questions about "how they work and what they do" by the collective scientific community?

spredditing

The biology and mechanism behind CRISPR-Cas is not impeded by the patenting to any significant degree. Every lab that has developed Cas9-based technology has shared this information in publications and with groups like AddGene that distribute vectors at low cost. As scientists, we are evaluated on publications and contributions to a field. Even if Cas9 is protected for a medical or commercial use, labs can still work on it. Also, once a paper is published labs are required to share those reagents with others. So any paper you read about CRISPR-Cas9 means that those authors are consenting to sharing. Working in the field, I have been blown away by the openness and collaborative nature of most members.

I went to High School with you, Central Secondary School FTW!!

halloIT

golden ghosts!

Do you think the Jays will win the world series next year???

GreenDragonX

I sure hope so!

Last month I did a whole research paper on genetic modification and CRISPRs were my main topic. What sort of information can be pulled from CRISPR DNA? Can this be used to prevent and/or treat disease?

Provol0ne

I think this has been answered above, but I am not totally clear on the question. The CRISPR DNA itself (the repeat-spacer-repeat array) is to me, the most interesting stuff! This has the chronological record of past encounters with phage and essentially tells us the history of an organism. What an incredible tool. As far as the nucleases go, this has been addressed above with various applications discussed.

What other functions might CRISPR-Cas systems have?

I realize that this is something you're trying to answer in your research, but do you have any

hypotheses for this question? What other possible functions could something like this have that you're hoping to find?

valenaut

In short, regulating genes in the genome. Maybe even taking advantage of it in the way we do. By bringing a protein to a specific spot in the genome and enacting a function.

Could this have practical implications for inherited conditions?

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Cas9 definitely could. Correcting mutations is one of the highest ranking goals for this technology. Need to know what the mutation is though.

How concerned are you about the bioethical ramifications of genome editing that will inevitably be engendered by the discovery of CRISPR-Cas9 paradigm/mechanism?

hyperproliferative

I think it is definitely worth being concerned about. Lots written on that above and a couple of links that I posted. The 'good' from this discovery will be incredible though.

Do you do a lot of work with the DoD namely Navy and Army who are currently actively researching s. aureus phage and bacteriophage? I'd be interested in how the CRISPR-Cas system works alongside the products that are currently underway for testing pre-IND or if your system can work in combination with a phage product and if so, what are your plans on testing this?

1nVu

No I don't, but phage therapy has been in the sphere of the military (in general, not just US) for a long time. The more mechanisms we know about how bacteria resist phage (and how phages fight back), the better.