In 2018 on Weds 11th April we went to a workshop on whether CRISPR should join New Zealand's 'toolbox' for healthcare and for pest control. These are questions that we sent after the workshop to the five scientists involved on the panel, and the answers we received from Dr Marc Rands in return:

Dear Nicola and students

Here are the answers to the student's questions:

Great Questions!!

Q: HOW DO YOU DELIVER CRISPR TO HUMANS OR DOES IT DIFFER DEPENDING ON THE DISEASE HOPING TO BE TREATED? E.G. SICKLE CELL IS OUTSIDE THE BODY BUT POTENTIALLY HOW WOULD IT BE DELIVERED TO THE LIVER? VIA A VIRUS?

A: For cells **outside** the body you can use viruses engineered for the purpose but increasingly people are using nanoliposomes – tiny particles that can penetrate cells. For delivery to cells **inside** the body, viruses are the key tools. They can **target a certain tissue to some extent** (called tropism) but it is not fool-proof and this type of delivery problem is slowing down many medical advances. Specific targeting of many drugs – not just CRISPR - is a real challenge.

Q: WHAT ABOUT IN RATS - IS IT ALWAYS DELIVERED TO THE EMBRYOS OF RATS? HOW WOULD YOU TARGET IT TO SPECIFIC ORGANS? WHAT'S THE MECHANISM?

A: It depends very much on what you are trying to achieve. If you want to make a germline modification, or one that is present in all the tissues of a rat, then making your edits in early embryos (1 cell stage) is much easier than trying to modify every cell individually later on when there's more of them. You can target some organs - the liver for example is relatively easy to target in mammals because the whole blood volume goes through it, and cells there seem able to pick up molecules like RNA and proteins. Targeting to other specific organisation is really only achievable with things like viruses that target specific sorts of cells. For example HIV targets T cells, other virus have other targets.

Q: HAVE YOU EVER THOUGHT ABOUT THE POSSIBILITY OF CRISPR LEADING TO A WORLDWIDE PANDEMIC? WHAT COULD BE PUT IN PLACE TO DEFEND AGAINST IT / PREVENT IT?

A: I suppose it is possible but not any more so than any other engineered toxin that could be delivered by a virus of some kind. The major inhibition is the virus – it needs to spread

quickly amongst a vulnerable population with next to no immunity being established. Few viruses fit the bill. So the dangerous element is not so much CRISPR as the virus that it would need to be packaged in.

Q: HOW COULD YOU USE GENE DRIVES TO CONTROL THE GENDER SPLIT?

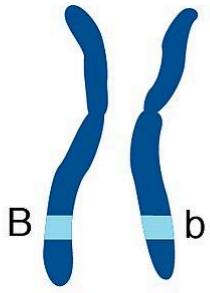
A: You need to know the mechanisms by which gender is specified in your target organism. In most mammals 'maleness' is specified by the gene sry, which lives on the Y chromosome. If your gene drive targeted that gene and knocked it out, making it ineffective, then even if your organism had a Y chromosome, it would still develop as female (as shown by mutants). In insects things are a bit more complicated, but specific genes that trigger male or female development could be targeted. The key is to know the mechanism you are targeting, we don't really know, for example, how gender is specified in a possum.

Q: HOW DOES A GENE DRIVE ACTUALLY WORK? HOW DOES IT CAUSE THE GENE TO ALWAYS BE PASSED ON - YOU SAID THAT IT COPIES THE GENE FROM ONE OF THE CHROMOSOMES IN THE PAIR TO BEING ON BOTH. WHAT GENE EDITING TOOLS HELP TO DO THAT? HOW DO THEY WORK?

A: The attached figure might help you out. Cas9 cuts DNA. Where it cuts DNA is specified by the guide RNA - if you get cas9 and the guide RNA in a cell, then the DNA gets cut.

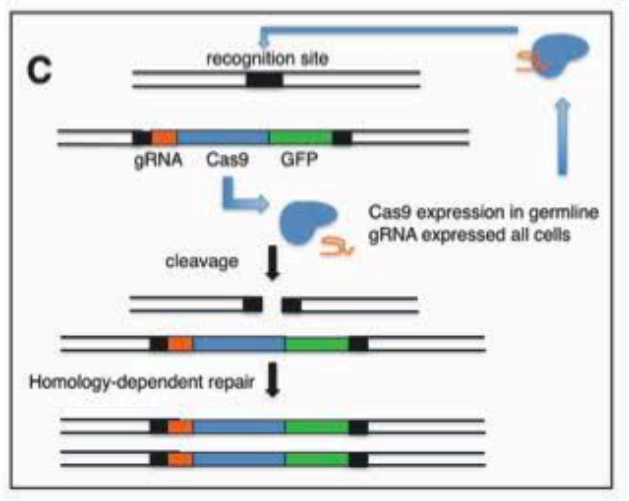
The gene drive mechanism is to put the cas9 gene and the guide RNA on a piece of DNA you put into a particular site in the genome - the SAME site that would be CUT by the cas9 guide RNA you put into the DNA.

Many organisms are diploid, so think about a heterozygous cell, which has one copy of the site with the DNA inserted, and one 'empty' copy.

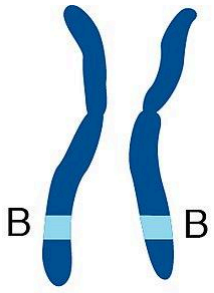


Now if the gene for the cas9 and guide RNA are turned on, they will only cut the 'empty' site on the other chromosome - in the same location on the other chromosome but without the piece of DNA you put in it.

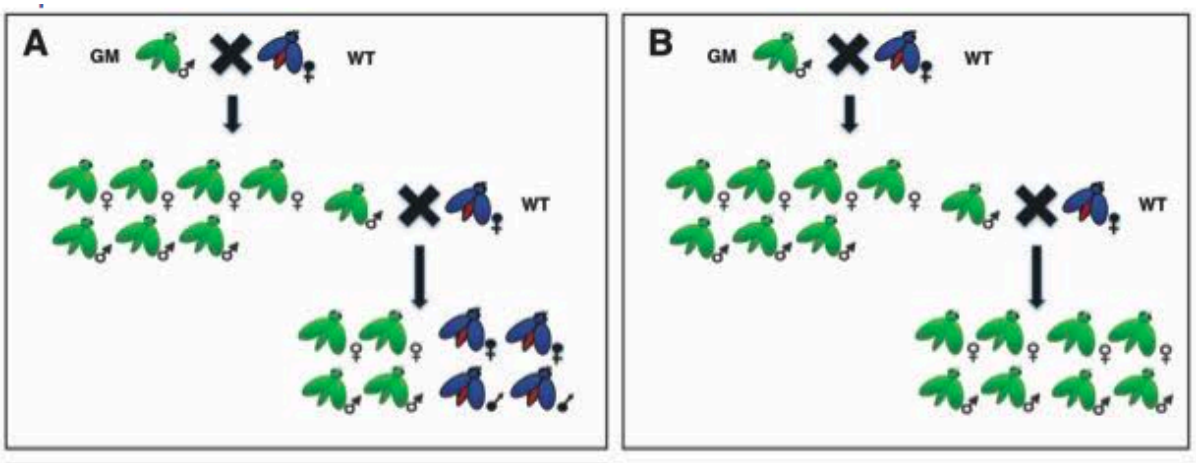
When the cas9 and guide RNA are activated they will cut the ‘empty’ copy of the DNA on the other chromosome. Now this is the important bit: when a cell detects a cut in its DNA it panics a bit, and looks to see if it can find any similar bits of DNA. In this case it will find the other copy of that broken DNA on the other chromosome which contains the DNA insertion. To fix the hole, the cell will copy that piece of DNA into the hole where Cas9 cut.



This will convert the empty site into one containing the insertion; making your heterozygous cell homozygous.



Now you can see if we activate the cas9 in the **germline** when **making gametes**, instead of a heterozygous animal passing on the transgene to 50% of its offspring, all the cells will be converted to homozygotes and all the offspring will carry the transgene. In the next generation this will happen again, and so any heterozygous animal will produce more heterozygous animals, spreading the gene through the population.



I hope that helps!

Dr Marc Rands
Senior Researcher