NUCLEAR SOLUTIONS TO A NUCLEIC PROBLEM

How a bacterium's DNA gives resistance to extreme radioation

Bacteria suffer from poor public relations. Ask any member of the public which bacterial species they know of and it'll read like the FBI's most wanted list: *E. coli, M. tuberculosis, V. cholerae*... villains left, right, and centre. But for every villain, there's a superhero.

Extremophiles are organisms that thrive in environments harsh enough to kill everything else. *Thermus aquaticus* proliferates in the hot springs of Yellowstone, shrugging off temperatures of 70°C, while *Chryseobacterium greenlandensis* can live in ice blocks 3000 metres below the surface. Just as Batman would seem out of place anywhere but shadowy Gotham, and Spiderman far more likely to be swinging through New York's skyscrapers than wandering through rural Texas, these bacteria's powers have evolved over millions of years to suit their environment perfectly. And then there's Superman, who is ridiculously powerful no matter where he is. Superman, otherwise known as *Deinococcus radiodurans.*

"CONVENTIONAL REPAIR ENZYMES ARE STILL FUNCTIONAL AFTER IRRADIATION"

Deinococcus radiodurans can easily survive an acute 5,000Gy dose of ionizing radiation. To put that into perspective, human cells will be killed by 5Gy. This is absolutely fascinating from a biochemical perspective. How on earth can a cell survive such an onslaught?

Radiation is the emission of energy. Ionizing radiation is that which has enough energy to liberate electrons from atoms. This can directly damage cell components, breaking the bonds that hold DNA and proteins together and altering their chemical structures. Luckily for DNA there exists a whole host of repair enzymes raring to patch it up, with different enzymes for each type of damage. This is hardly surprising given that genome stability and integrity is essential for survival. For instance, DNA ligases help repair breaks in DNA's sugar-phosphate backbone by catalysing formation of phosphodiester bonds. Ionizing radiation can prove particularly deadly if it causes double-strand breaks—breaking the sugar-phosphate backbone on *both* strands of the helix—because repairing them may lead to sections of the genome being moved to the wrong place.

At the levels of ionizing radiation that *D. radiodurans* can survive, it will have suffered so many of these double-strand breaks that its genome is essentially shattered. Hence it seems reasonable to think that *D. radiodurans* may have evolved a unique repair enzyme or biochemical pathway that allows it to repair DNA damage much more efficiently. Indeed multiple studies have shown DNA repair in our superhero to be startlingly efficient across a whole range of damage types. But whole genome comparison to other bacterial species showed that *D. radiodurans* possesses only the standard DNA repair enzymes. If the secret of extreme radio-resistance does not lie with unique DNA repair pathways, then where?

The key lies with how proteins, including the DNA repair enzymes, are protected from reactive oxygen species with unpaired electrons known as free radicals, which are generated by the action of ionizing radiation on water molecules. The inability of other organisms to protect their DNA repair enzymes from these reactive oxygen species is the root of their radio-sensitivity. Not only are free radicals highly reactive and damaging, oxidising the molecules they come in contact with, but in their destructive reactions they generate *more* reactive oxygen species in a cascade which only stops when they react with each other to form stable molecules where all electrons are paired. Proteins and DNA suffer immediate and unavoidable damage from free radicals generated in close proximity to them.

"FOR EVERY VILLAIN, THERE'S A SUPERHERO"

Rather than having a completely novel DNA repair system, *D. radiodurans* simply ensures that conventional repair enzymes are still functional after irradiation. Across different bacterial species, proteins in radio-sensitive species are much more vulnerable to free radical oxidation species than those of radio-resistant species. It simply doesn't matter how efficient your repair enzymes are if they have been rendered non-functional by an onslaught of oxidation. This is why only a few double-strand breaks are enough to kill bacterial and eukaryotic cells after irradiation—nothing is being repaired. However, extraction of *D. radiodurans* repair enzymes showed that the enzymes themselves are not resistant to oxidation. *D. radiodurans* must be using

another agent to protect its proteins.

"THIS RADIATION RESISTANCE IS A SIDE-EFFECT OF RESISTANCE TO PROLONGED DEHYDRATION"

Antioxidants are molecules that act as reducing agents to prevent other molecules becoming oxidised, and to remove reactive oxygen species. The vast majority of species rely on antioxidant enzymes and, under most circumstances, these enzymes are sufficient to remove any reactive oxygen species produced. However, the effects of high doses of ionizing radiation overwhelm the antioxidants, meaning that DNA repair enzymes cannot be protected. *D. radiodurans* avoids this issue altogether by using manganese complexed peptides as antioxidants.

Manganese is a transition metal that can exist in a number of stable oxidation states, from +2 to +7. In *D. radiodurans*' antioxidant complexes manganese is primarily in the +2 oxidation state, meaning that the complexes have great capacity for countering reactive oxygen species. The power of these complexes was demonstrated by adding them to solution containing the enzyme glutamine synthetase, which is normally destroyed by 150Gy of ionizing radiation. The manganese complexes offered dramatic protection, allowing glutamine synthetase to survive 50,000Gy. Another factor contributing to the extreme radio-resistance of *D. radiodurans* is its tightly packed chromosomes, facilitating repair of double-strand breaks by keeping DNA fragments within a small area, but this is simply a feature that allows the manganese complexes to better perform their function.

This superpower is not only interesting from a biochemical perspective, but also from an evolutionary one. How on Earth did *D. radiodurans* evolve such a degree of resistance, when the highest known background radiation level is only 260mGy per year? Such low levels of exposure would have never driven selection for traits that promote resistance to ionizing radiation. Mattimore and Battista (1995) have suggested that this radiation resistance is merely a side-effect of resistance to prolonged dehydration, since the damage caused by both is very similar. Areas of low water content are common enough for this theory to be plausible, even if it is a great deal more anticlimactic than the suggestion that *D. radiodurans* arrived to Earth on a meteorite.

But as interesting as this blue sky research is, does our knowledge of *Deinococcus radiodurans* help us? Extremophiles have been plundered for biochemical treasures before, notably *Thermus aquaticus*, the source of Taq polymerase, a heat-resistant enzyme used for the polymerase chain reaction which revolutionised the field of genetics. *D. radiodurans* could prove just as useful.

In 2003, American scientists showed that *D. radiodurans* could be used to store information that could survive a nuclear war. They translated the song 'It's a Small World' into DNA segments, and inserted this translation into the *D. radiodurans* genome. 100 bacterial generations later they could still recover the song without any errors. This would prove helpful in preserving information for future generations in a format that is resistant to the effects of radiation.

More importantly, extracts of *D. radiodurans* antioxidant complexes could be used to protect the antigens of bacteria and viruses when exposing them to radiation. The production of radiation-inactivated pathogens with their antigens perfectly preserved would provide us with potent vaccines.

"D. RADIODURANS COULD BE USED TO STORE INFORMATION THAT COULD SURVIVE A NUCLEAR WAR"

Genetically engineered strains of *D. radiodurans* have already been put into action, digesting solvents and heavy metals in radioactive waste. This was simply a matter of cloning the appropriate genes into the *D. radiodurans* genome. Alternatively, enabling other bacterial species to produce manganese-peptide antioxidant complexes could allow the use of more commonly used species to digest waste in radioactive sites.

Deinococcus radiodurans may not be able to leap tall buildings in a single bound, but the next time Superman faces a Kryptonite-wielding foe, he knows where to find back-up.

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