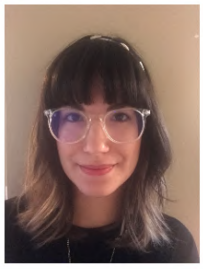
ASK AN EXPERT

Health Science Inquiry



DR. MARIA ROSALES GERPE is a post-doctoral scientist in Dr. Zhang's lab in the Department of Molecular and Cellular Biology at the University of Guelph. Her research interests include understanding the mechanisms that govern the cellular machinery and develop therapies to ameliorate diseases caused by dysregulation of these mechanisms. In the past, these interests have driven her to study viruses and virus-based gene therapy in the laboratories of Drs. Langlois and Wootton, and currently, under the mentorship of Dr. Zhang, the ubiquitin system to uncover additional important cellular mechanisms of the ubiquitin proteasome system that may lead to potential cancer therapies.

DR. WEI ZHANG has a unique training background in two disparate fields: DNA repair (PhD) and protein engineering (Postdoc). His PhD research with Dr. Daniel Durocher lab at the Lunenfeld Institute of Mount Sinai Hospital was centered on how cells differentiate natural ends (telomeres) from broken ends (DNA double-strand breaks, DSBs) and how spatial regulation of DNA repair is achieved. Meanwhile Dr. Zhang graduated from a two-year radiation medicine program (STARS21) in the Department of Radiation Oncology at University of Toronto. With a CIHR fellowship and later a Mitacs Elevate Fellowship Dr. Zhang further conducted postdoctoral work in the labs of Sachdev Sidhu and Jason Moffat in the Donnelly Centre at the University of Toronto to engineer ubiquitin for modulators of cell signalling. Currently, in his own laboratory Dr. Zhang proposes to leverage his protein engineering platform to probe and rewire DNA repair signaling with unprecedented precision to elucidate underlying molecular mechanisms and develop innovative cancer therapeutics.

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The Need for Ethical and Experimental Regulation in Genome Editing

Dr. Robert Edwards (Fig. 1A), the 2010 Nobel Prize in Physiology and Medicine recipient for the development of *in vitro* fertilization (IVF) research became infamous as the media labeled his work with the term *"test tube babies"*. IVF stirred panic and heated ethical debate, even prompting withdrawal of funds for Edwards' research [1]. Nevertheless, today IVF continues to help make parenthood a reality for many people worldwide [2] and its research led to multiple discoveries on the morphology and physiology of developmental diseases [2].

We are probably facing a similar predicament today as the term *"test tube babies"* transitioned to *"designer babies"* with developments in gene editing [3]. Gene editing refers to the manipulation of genes externally and incorporation of those genes into the genome of human somatic cells using viral-vector based delivery to correct rare genetic diseases. Gene editing is responsible for many breakthroughs including a blindness cure for those suffering from inherited retinal dystrophy [4]. However, most recently, the work of Dr. Jiankui He (Fig. 1B) has sparked further debate on the ethics of gene editing for the first human embryo experimentation involving CRISPR-Cas9 gene editing [5].

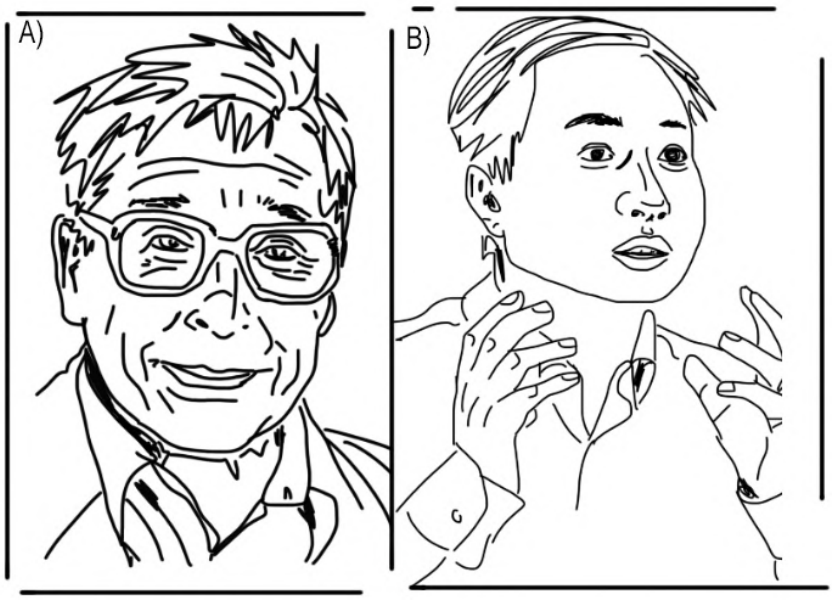

Figure 1: Illustrations of **A)** Dr. Robert Edwards and **B)** Dr. Jiankui He. Artwork by MCRG.

CRISPR-Cas Systems and Gene Editing

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats acquired by the bacterium from bacteriophages or mobile genetic elements (MGEs) [6]. CRISPR was discovered alongside Cas editing enzymes, which use CRISPR-transcribed RNA sequences as a guide for nicking the genome of pathogens and pathogenic MGEs (Fig. 2) [6]. With the discovery of CRISPR, researchers can now deliver a versatile and easy-to-use tool that can modify the genome at a single-base precision level, alleviating gene cloning capacity requirements in delivery vectors [6]. The most commonly used CRISPR-Cas system is CRISPR-Cas9 due to the small size of Cas9 compared to other Cas enzymes and the easily engineered guide RNA that Cas9 employs [6]. Currently, CRISPR-Cas9 technology is being researched for the treatment of several human diseases including multiple types of cancer, blood, respiratory and neurological diseases [7].

Developing CRISPR-Cas Modulators

Despite these advantages, the most critical setback of CRISPR-Cas9 is non-specific off-target editing [6]. Researchers have begun using the recently discovered anti-CRISPR proteins, the bacteriophage defense against the CRISPR-Cas system, as a natural off-switch. However, anti-CRISPR proteins have yet to be discovered for all CRISPR-Cas systems [8]. Protein engineering strategies such as directed evolution of natural anti-CRISPR proteins could be employed to generate novel anti-CRISPRs. Ubiquitin variants (UbVs) are an example of directed evolution developed to allosterically inhibit protein-protein interactions (Fig. 3) in the ubiquitin-proteasome system, important for many cellular functions and which can be exploited for cancer therapy, antiviral treatments, and gene therapy [9]. Most recently, UbVs have been used to modulate CRISPR's DNA repair pathway of choice, successfully increasing CRISPR-Cas9 gene editing efficiency [10]. Collectively, we believe that future work will identify artificial anti-CRISPRs that can improve the accuracy of CRISPR-Cas systems and act as an "off-switch" in CRISPR's potential clinical usage.

Conclusions

The scientific field should heed the verification of CRISPR-Cas systems' safety and efficacy because the impacts of germline manipulation are critical. Dr. Edwards' research showed that rigorous research and debate can have lasting beneficial impacts. In contrast, Dr. He's work was conducted without the knowledge of the scientific community and contains pitfalls that could have been avoided by thorough vetting prior to experimental execution. In summary, CRISPR-Cas-based gene editing can be extremely beneficial in treating rare diseases, especially those with genes too large to clone or deliver; however, CRISPR-Cas systems should be meticulously tested prior to use and modulators should be developed to improve safety prior to use in humans. We hope that native and synthetic anti-CRISPRs can help us learn about its mechanism while complementing future gene editing strategies.

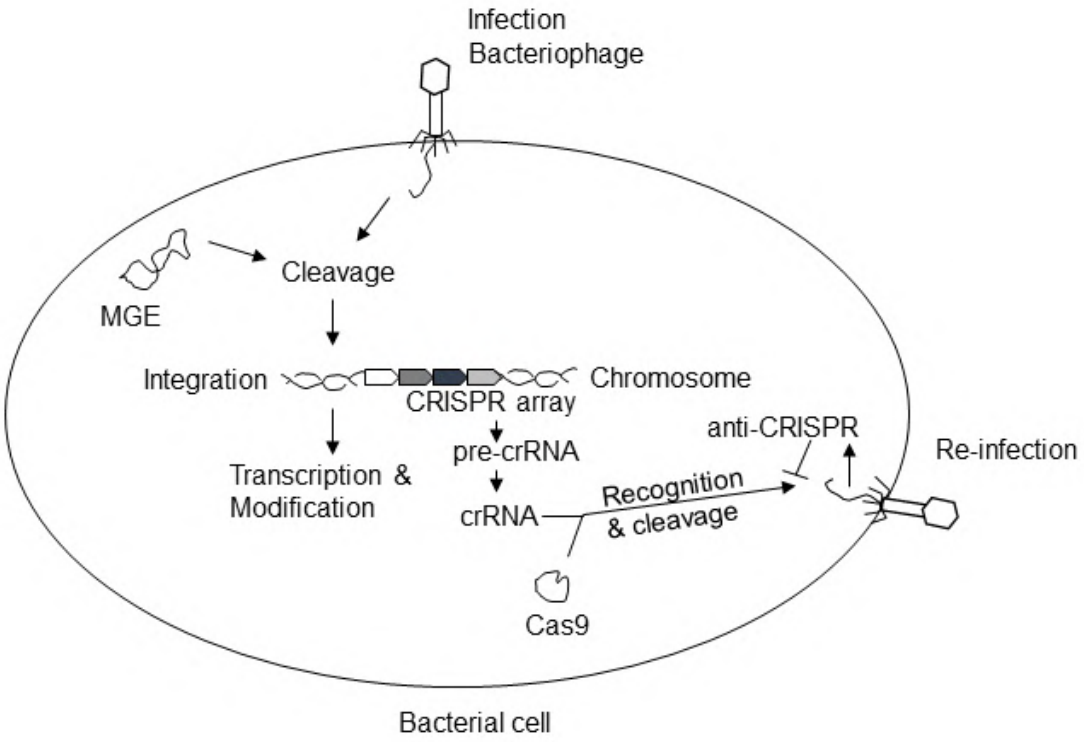

Figure 2: **CRISPR-Cas and Anti-CRISPRs.** Mobile genetic elements (MGE) and bacteriophage genome spacers are acquired upon entry by Cas proteins by cleavage and are then integrated into the CRISPR assay in the bacterial chromosome. Pre-CRISPR RNAs (pre-crRNA) are transcribed from this array and matured into crRNAs that can guide Cas enzymes like Cas9 to nick the genome in the areas complementing the crRNA. Anti-CRISPR proteins expressed from the bacteriophage genome target the CRISPR-Cas systems through a multitude of mechanisms, which include binding and activity inhibition. Illustration by MCRG.

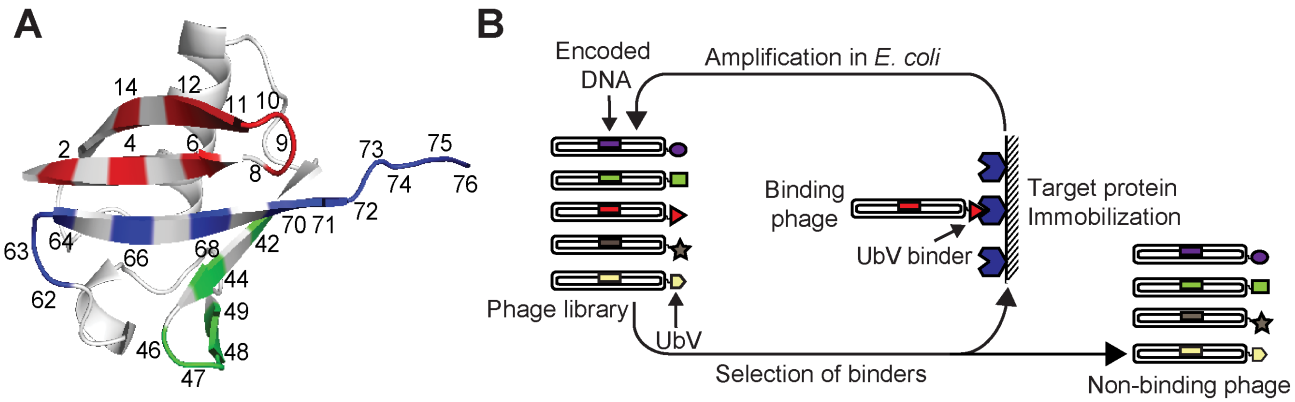

Figure 3: **Platform for generating anti-CRISPRs using ubiquitin variants (UbVs).** This platform employs phage display-based design, where UbVs are expressed by bacteriophages in their surface glycoproteins. **(A)** The Ub structure (PDB: 1UBQ) is shown as a ribbon (white) and soft-randomized (low-level mutations) residues are numbered and highlighted in red (region 1), green (region 2), and blue (region 3). **(B)** Phage display affinity maturation. Protein-bound M13 phages are amplified by infection of bacteria and further enriched through numerous selection rounds. Figure by WZ.

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