

Editing the Code of Life

This BioCY News will highlight some of the researchers in GDCB at ISU who are working in the exciting and rapidly expanding area of directed gene editing. With the promise of better foods and permanent cures for disease, the power embedded in gene editing offers great hope for the future but will demand thoughtful development of a global usage policy.

CRISPR/cas: The New Gene Editing Paradigm

by Carla Mann

Researchers in GDCB are using cutting edge gene editing technology to improve agriculture and study human disease.

CRISPR/cas is the next stage in genome editing technology, following in the footsteps of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). All three technologies take advantage of natural mechanisms that DNA possesses to repair breaks in the double-stranded DNA helix, but the CRISPR/cas system is more cost-effective, efficient, and, critically, safer.

CRISPRs (clustered regularly interspaced short palindromic repeats) were first discovered in 1988 by a group working on *E. coli* at Osaka University in Japan. The scientists noticed odd clusters of repeats in the bacteria but it wasn't until 2005 that they got the first inklings of just what exactly those repeats were doing. Bacteria, just like larger organisms (including humans), can be infected and attacked by viruses. Some viruses, called bacteriophages, have evolved to specifically target and kill bacteria. In response to these invasions, some bacteria have developed sophisticated defense systems to protect them from these attacks. One such system is the CRISPR system. The short palindromic repeats the scientists at Osaka discovered are actually bits of viral genomes that were incorporated into the *E. coli* genome after it was infected with that particular viral strain. The bacterium creates RNAs complementary to the viral genomes (using the CRISPRs as a template), which then bind to foreign viral DNA and RNA. The Cas protein then cleaves the invading viral genetic material, rendering it harmless to the bacteria. In essence, the CRISPRs provide a mug shot of potential viral invaders, and the Cas protein kills viruses whose mugs match the shots.

Scientists quickly realized that they could hijack the CRISPR/cas system to edit genomes in other organisms by targeting and cleaving very specific DNA segments. The procedure can be used to remove defective genes, or to add in new genetic material as the situation demands.

One of the great promises of CRISPR/cas technology is that it offers the potential to revolutionize the way scientists and doctors treat diseases, especially inherited disorders. Genetic conditions that are currently a death sentence, like Huntington's disease or cystic fibrosis, could be completely cured in the future by editing the defective genes in embryos.

While scientists and policy makers debate the use of CRISPR/cas in humans, the system is actively being used at Iowa State to study disease and improve crops. The ISU Crop Bioengineering Consortium hosted a symposium on genome editing in April of 2015, and will host a [Plant CRISPR Workshop](#) in May of 2016.

This edition of the GDCB newsletter will showcase some of the new and exciting advances that GDCB scientists are making in CRISPR/cas.

CRISPR: A Gene Editing Power Tool

by Divita Mathur

Laura Schultz, a graduate student in the laboratory of Professor Maura McGrail, knows very well the responsibility that comes with the power to manipulate genes at will. She and her colleagues use the CRISPR/Cas9 toolkit to gain a better insight into genes in zebrafish (*Danio rerio*) that are potentially linked to central nervous system tumors. You can say that studying genes in association to diseases is standard genetic engineering, but with the emergence of the CRISPR/Cas9 system, which allows precise gene editing, Laura has been able to paint a detailed picture of how multiple genetic mutations play a role in brain cancer.



Professor Maura McGrail (above, left) and GDCB graduate student Laura Schultz (above, right) are using the CRISPR/cas gene editing system to study genes in zebrafish that are related to neurological disorders in humans.

In her primary target genes, which are potential suspects in brain tumors, she introduces different combinations of mutations and studies the resultant phenotype (or physical feature) in her modified zebrafish to understand the genetic interplay. A page in Laura's lab notebook details the instructions to order DNA strands for a variety of desirable mutations, a process as common to CRISPR scientists as is looking up a word in a dictionary for the layperson. These DNA strands are delivered overnight and added to zebrafish eggs, which soon grow into mature fish that express specific and targeted behaviors. Laura keeps a meticulous record mapping how the modified genes

interact with one another to cause tumors.

When asked about the complexity of the CRISPR/Cas9 system Laura smiles and says, "I just need a tank, a microscope, and glass needles." This simple response belies the power and responsibility of the new age genetic engineer.

Having performed genetic engineering the traditional way, which entails a tedious process of months of selective breeding of model animals, Laura, an alumna of the University of Michigan, has seen the old as well as the new science in the field.

"Labs would end up spending over \$30,000 to develop a single knock-out mouse line and sometimes still not get the desired genetically engineered mouse in the end." This number is excluding salaries of the scientists. Reflecting on the rate of evolution for this technology Laura states, "How much it has changed in 5 years...it blows your mind!"

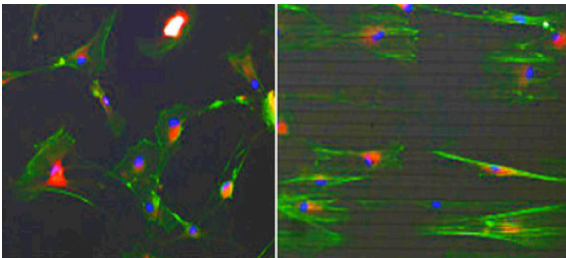
Now, with CRISPR/Cas9, Laura and others can perform multiple directed genetic mutations in a single embryo and exponentially accelerate their research while at the same time saving a great deal of taxpayers' money.

Reflecting frequently on awesome responsibility that is part and parcel of her awesome gene-altering capability Laura points out that benefits of this cutting-edge technology are certainly appreciated and understood by scientists who are curious to learn about the "book of life" written in DNA. But, she notes, there remains much to be learned from nature about the CRISPR/Cas9 genome-editing tool, its expansion, and its long ranging ramifications and these critical considerations have resulted in many discussions on the use of CRISPR/Cas9 for altering human genes.

Nerve Splicing: Neuroscience Meets Nanotechnology

by Zebulun Arendsee

Anup Dutt Sharma compares two photos of colorfully glowing cells on a black background. In one photo, the cells are haphazardly scattered blobs, in the other, they are aligned, elongated ovals. These are mesenchymal stem cells that have been chemically persuaded to differentiate into Schwann cells, which are required for healthy nerve function and repair. Sharma is pioneering the growth of oriented Schwann cells for use in nerve regeneration.



Naive stem cells (left) that have been persuaded to differentiate into a desired cell type (right).

Sharma left a lucrative career as a software engineer to pursue a future in regenerative medicine. He is now a fourth year PhD candidate working jointly under the GDCB neuroscientist Dr. Donald Sakaguchi and the chemist Dr. Surya Mallapragada.

Sharma's vision for regenerative medicine integrates three technologies. First, they are designing nanoparticles to administer

neurotrophic growth factors that signal neurons to grow. By adjusting the chemistry of the particles, they can fine tune the rate at which the factors are released.

Second, they are genetically engineering neural caretaker cells to release neurotrophic growth factors at a desired rate. When injected near an injury, these cells migrate to the damaged site and begin repair. Unlike nanoparticles, their effect is permanent. Sharma has developed a high throughput method to test the health of engineered cells, a prerequisite step for clinical application.

Finally, they are reprogramming mesenchymal stem cells into Schwann-like cells. Schwann cells are known to be powerful drivers of neural regeneration, but harvesting them from a patient requires the sacrifice of healthy nerves. Mesenchymal stems cells, however, are abundantly available in bone marrow and can be transmuted into Schwann cells. Yet when grown on smooth surfaces, they take on amoeboid forms of limited use. Sharma and colleagues have invented a method of optimizing their morphology by culturing them on biodegradable polymeric films patterned with microscopic ridges.

Sharma envisions a path to healing severed nerves without sacrificing healthy ones. Genetically modified Schwann cells can be grown on micro-patterned sheaths, rolled into tubes, and filled with a solution of fine-tuned, growth factor-releasing nanoparticles. These tubes may then be surgically implanted into a patient without risk of rejection and should stimulate fast regeneration, allowing better reattachment of severed nerves and recovery from certain types of paralysis. While Sharma's research focuses on repairing damage to the peripheral nervous system, his multi-cue approach may be extended to central nervous system diseases such as Parkinson's, Alzheimer's, or to traumatic brain injury.

Software to Geneware: An interview with Dr. Deepak Reyon

by Rahul Roy

Dr. Deepak Reyon has come a long way since receiving his Ph.D. in Bioinformatics from GDCB. His experience as a software programmer before joining the laboratory of Dr. Drena Dobbs facilitated his prolific contributions as a graduate student modeling DNA-protein binding dynamics of zinc finger nucleases. Deepak reminisces about how Dr. Dobbs instilled in him the passion for understanding how the dataset that they were analyzing was generated. Deepak's desire to fully grasp the "wetware" side of his science led him to intern with Dr. Keith Joung at Harvard Medical School. There he coupled his software skills with a deeper understanding of how experiments were conducted for collecting the data that he was so used to analyzing. This is where Deepak was introduced to the budding field of TALENs, proteins that specifically bind DNA base pairs (a protein-DNA binding code that was cracked by researchers at ISU). Deepak returned to Dr. Joung's lab after completing his Ph.D. at ISU and spent two years as a postdoc there. It was during this time he was introduced to the rapidly maturing gene editing CRISPR-Cas9 technology



Dr. Deepak Reyon (left) has translated his knowledge of DNA editing and bioinformatics, acquired as a GDCB student and postdoctoral researcher at Harvard Medical School, from academia to industry and is now a researcher with the gene editing startup Editas Medicine.

Following his postdoctoral studies Deepak moved to the biotechnology company Biogen where he was recruited to setup the genetically engineered models (GEM) center for generating cell-lines and animal models using CRISPR-Cas9 technology to support research operations. Deepak was then recruited to the startup company

Editas Medicine, a transformative genome editing company co-founded by his post-doctoral mentor Dr. Joung.

Deepak says he is excited to be working in a rapidly evolving area of genomic editing and biotechnology. His enthusiasm overflows as he discusses the promise of CRISPR in supplementing other therapeutics such as small molecules and antibody based drugs. Deepak states, "Gene editing will not be replacing a certain therapeutic, it will open up possibilities in situations where other therapeutics are not available". He also points out that there are challenges too, such as transitioning from an idea to an actual product that works. That said, he is confident that the field will progress.

With regard to Deepak's choice to transition from academia to industry he says, "I almost considered staying back in academia, investigating intricacies and mechanisms of the biological world that appealed to me. But then I realized, I was more interested in systematic, targeted research for discovering therapeutics. If the mechanism was interesting but not a potential route for therapeutic strategies, the level of intrigue was diminished for me".

At the end of the interview Deepak offered these words of advice to budding scientists, "Figure out where your passion lies. Figure out what kind of research you want to pursue. The academic and industrial worlds operate on different dynamics, but both are challenging and exciting in their own ways. It is crucial that you understand what your identity as a scientist is and then pursue it".

Scientists like Deepak are a source of inspiration for younger scholars like the author of this report and prove that taking risks and following your passion can pay enormous dividends, a sentiment strongly supported by GDCB.

Recent GDCB Awards and Accomplishments



GDCB faculty continue to participate in and have a leadership role in several [excellent interdepartmental graduate programs](#): Professor Diane Bassham (second from left) is beginning her term as Chair of the Plant Biology graduate program. Professor Carolyn Lawrence Dill (third from left) has been elected to serve as Associate Chair of Bioinformatics and Computational Biology. Professor Jeff Essner (left) and

Professor Tom Peterson (right) will continue their two-year terms as Chair and Associate Chair of the [Genetics and Genomics graduate program](#).



The GDCB research team led by Professor [Ling Li](#) and Professor [Eve Wurtele](#) has demonstrated that the QQS gene regulates the protein content in Arabidopsis seeds and leaves can increase protein content when introduced into staple crops, such as rice, corn, and soybeans. This study was published this week in the [Proceedings of the National Academy of Science](#). The research may have implications for agriculture in developing countries. View [ISU news release](#) to learn more.

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The GDCB Communications Team
Department of Genetics, Development, and Cell Biology
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Iowa State University
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