Genomics and Life Sciences: Breakthrough Opportunities for Indian Agriculture and Public Health Sectors

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Introduction

Life Sciences is the study of living organisms that include bacteria, plants, animals, and human beings. The blueprint of life is written in the nucleotide sequence of an organism's genome, which determines its form and function. For example, each human cell contains 3.2 billion base pairs (3.2x10 9 bp) within its genome. Scientists estimate that the human genome codes for about 20, 000 different genes, which give an individual his/her form and function. The genes are distributed among 23 pairs of chromosomes and code for all the human body's proteins. While the functions of several human genes have been deciphered, many others are yet to be determined. The gene function, in many cases, can be inferred by how well the gene and its protein sequence are conserved across species, and if the gene function of one of the homologs is already known. The gene function can also be determined experimentally by knocking out (deleting) or mutating that specific gene individually in a cell andobserving the resulting phenotype. Mutations in the coding region of critical genes can lead to formationof abnormal proteins, result in disease phenotypes, premature death, or failure of an embryo to develop. Furthermore, mutations that affect the regulatory genes can result in aberrant gene expression within cells and give rise to cancer phenotypes.

Genetics is the study of individual genes, genetic variation, and their roles in inheritance. In contrast, genomics is the study of the complete set of an organism's genes, and their interaction with each other. High Throughput DNA sequencing techniques have enabled scientists to decipher complete nucleotide sequences of genomes of several organisms and species, which has led to the development of the field of genomics. Bioinformatics is used to assemble the genome from the nucleotide sequence information, annotate the genes, and analyze the function, structure, and evolution of entire genomes. Genomics has enabled discovery-based research as opposed to traditional hypothesis-driven research. Systems biology has facilitated the study of complex biological systems that were not approachable previously. Genomics addresses all genes and the inter relationships to identify their combined influence on growth and development of the organism. Examples of genetic inherited disorders include cystic fibrosis, Huntington's disease, and sickle-cell disease. Examples of complex diseases that have been studied by genomics include asthma, cancer, diabetes, and heart disease.

Thus, genomics is an interdisciplinary field of biology focusing on the structure, function, evolution, mapping, and editing of genomes.

Breakthrough Opportunities for Indian Agriculture and Public Health Sectors

India faces the critical challenge of producing sufficient food for a growing population living in a changing climate. Potential future crops derived through genome editing and synthetic biology include those that better withstand pests, those that are salt and drought tolerant, that have enhanced nutritional value, and that are able to grow on marginal lands. In many instances, crops with such traits will be created by altering only a few nucleotides among the billions that comprise the plant genomes. With the appropriate regulatory structures and oversight in place, crops created through genome editing and synthetic biology might prove to be more acceptable to the public than plants that carry foreign DNA in their genomes (GMO). Public perception and the performance of engineered crop varieties will determine the extent to which genome editing and synthetic biology contribute towards securing the world's food supply. It is critical for India to make substantial investments in these transformative technologies to makethem readily available to indigenous Indian scientists so that they can be part of the upcoming revolution in agriculture.

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Genome Editing (also known as Gene Editing)

Genome editing techniques - ZFNs, TALENs and CRISPR-Cas9 – all deliver a targeted chromosomal DSB within cells to stimulate recombination. Cells have evolved into two classes ofmechanisms to repair DSBs: 1) non-homologous end joining (NHEJ); and 2) homology directed repair (HDR). NHEJ is an error-prone repair mechanism that often results in a pool of mutants with variable lengths of nucleotide insertions and deletions, known as indels. Thus, NHEJ can be used to knock-out genes. HDR normally relies on recombination with homologous sequences of the undamaged sister chromatid, but cells can be tricked into using the investigator-provided donor DNA. HDR leads to the introduction of precise alteration to the genome that is specified by the donor DNA template. Thus, genome editing can be used to correct, introduce or delete almost any DNA sequence in many different types of cells and organisms. Genome editing seeks to modify genes of living organisms to understand

gene function and develop ways to use genes to treat genetic or acquired diseases. The 2020 Nobel Prize for Chemistry was awarded to Jennifer Doudna and Emmanuelle Charpentier for harnessing CRISPR- Cas9 system for genome editing and providing a simplified technique.

Opportunities for Indian Agriculture Sector

A few examples of genome editing applications in agriculture and animal husbandry are described below.

Anti-browning mushroom: Scientists at University of Pennsylvania reported engineering of the edible fungus – the common white button mushrooms – to resist browning. It was achieved by knocking out one of the six family of genes that encode polyphenol oxidase (PPO) enzymes. The targeted PPO gene was inactivated by deleting a few base pairs in the gene of the mushroom's genome using CRISPR-Cas9; this resulted in the reduction of PPO enzyme's activity by 30% in the anti-browning mushrooms. United States Department of Agriculture (USDA) has decided that it will not regulate a mushroom that has been modified genetically using CRISPR, making it the first CRISPR-edited organism to get approval from the US government. This means that the mushroom can be cultivated and sold without passing through the agency's regulatory process. Prior to this, several other plants have passed the USDA regulatory process that were made using ZFNs and TALENs. The gene edited mushrooms did not trigger USDA oversight because it did not contain any foreign DNA, such as virus or bacteria and therefore considered non-GMO. Similarly, gene editing has been used to generate anti-browning potatoes.

Changing flower color: Japanese scientists have used CRISPR-Cas9 to change flower color of the ornamental plant, Japanese morning glory, from violet to white. Japanese morning glory was chosen because its genome has been sequenced and DNA transfer protocols were well established to carry out gene editing. The researchers targeted a single gene, *dihydroflavonol-4-reductase-B (DFR-B)*, encoding an anthocyanin biosynthesis enzyme, which is responsible for the color of the plant's stems, leaves and flowers. CRISPR editing of the *DFR-B* gene inactivated the enzyme, resulting in an absence of the color pigment. Non-transformed plants with an active enzyme had violet stems and flowers.

Hornless dairy cattle: Over the last 10,000 years, humans selectively bred cattle to produce more meat or milk, selecting distinct genetic variation over non-domesticated cattle. Cattle have evolved horns to defend themselves against predators and gain reproductive advantage over competing peers by signifying health and vitality to females. Horns on dairy cattle can injure their handlers and other cattle.

Physical dehorning of cattle is widely practiced, which is a bloody and painful process that pits animal rights activists against it. Conventional selective breeding is a slow and expensive process, usually taking many generations before the trait is at a high frequency in the population. However, some cattle breeds, like the Angus beef breed, are hornless due to a natural genetic variation or allele that arose in the distant past.

Scientists have shown that the mutant allele, called *Polled*, prevents horn growth when an individual inherits one or two copies of the *Polled* gene from its parents, i.e., the *Polled* allele is dominant to the usual *Horn* allele, at one specific chromosomal locus of the cow genome. The complete DNA sequence ofthe cow genome including its genes is known from genomics. Dairy cattle do not carry the *Polled* allele and hence these animals have horns. Genome editing offered a simple and efficient way to transfer the small and natural *Polled* allele from beef cattle to dairy cattle, and quickly produce dairy cattle without horns and importantly while preserving the dairy cattle genetics of high yielding milk cows that were generated by classical breeding. Using TALENs, the researchers disrupted *Polled* locus and demonstrated the birth of two hornless male cattle in 2015, in reproductive crosses where the offspring normally should have been horned. These bulls then sired six hornless calves, which were born in 2017. Thus, genome editing technology is a safe and relatively fast way of transferring a small and naturally occurring genetic variant from one breed of cattle into another breed. Genome editing can speed up what humans have been doing for thousands of years using the very slow process of selective breeding for desirable traits.

Opportunities for Indian Public Health Sector

Genomics, through its impact on transformative technologies like genome editing and synthetic biology, has fostered applications in biotechnology, agriculture, and medicine. The “holy grail” of human gene therapy is how genes might someday be used, modified, or even changed to correct various human diseases. A few examples of genome editing applications in the public health sector are listed here.

Sickle Cell Disease: Mutations in hemoglobin can lead to two different disorders, namely sickle cell anemia and $\beta\beta$-thalassemia. Successful *ex vivo* genome editing of a sickle cell disease patient with her own CRISPR-edited stem cells to increase the normal hemoglobin level was successful. Three years into the treatment, the billions of genetically modified cells that were infused into her body appear to be alleviating virtually all complications of her sickle cell disease. However, the long-term safety andefficacy of the treatment need to be monitored before it can be considered curative. A similar strategy is being pursued to treat $\beta\beta$-thalassemia.

Hunter's Syndrome (MPS II): In 2017, Sangamo Therapeutics Inc. in the USA launched a Phase I clinical trial testing of ZFNs to correct Hunter's syndrome (MPS II) caused by iduronate-2-sulfatase enzyme deficiency, which is responsible for breaking down complex sugars in the body. Hunter's syndrome can cause abnormalities in the skeleton, heart, and respiratory systems. The clinical trial wasthe first for *in vivo* genome editing approach administered directly to patients, and the treatment was effective without side effects.

Chimeric Antigen Receptor (CAR) T-cell Therapy: Scientists are developing immunotherapy approaches to create 'off-the-shelf' universal donor T-cells that don't have to be developed for every cancer patient. This approach utilizes CAR T-cell therapies that use immune cells collected not from patients, but from healthy donors. The idea is to create so-called off-the-shelf CAR T-cell therapies that are immediately available for use and do not have to be manufactured for each patient. Cellectis (a French company) has launched a Phase I trial of its off-the-shelf CD19-targeted CAR T-cell product in the USA for patients with advanced acute myeloid leukemia (ALL). The company's product using patient's own cells has already been tested previously in Europe, in two infants with ALL who had exhausted all other options. Inboth cases, the treatment was effective. Other clinical trials that are underway include treatment for Leber Congenital Amaurosis (LCA), a common cause of inherited childhood blindness, and treatment for a rare protein-folding disease called hereditary transthyretin amyloidosis, a fatal disease caused by mutations in the gene TTR.

Synthetic Biology

Synthetic biology focuses on living systems and organisms. It applies engineering principles to develop new biological parts, devices, and systems by redesigning existing systems found in nature. Scientists use synthetic biology to design and construct novel biological modules and biological systems, or to redesign existing biological systems for useful purposes. Rapid advances in DNA sequencing and DNA synthesis techniques have made it possible to design and engineer viruses, biochemical pathways, assemble bacterial genomes, and synthetic yeast chromosomes.

Synthetic Biology Application in Medicine

Genomics has enabled important applications of synthetic biology such as rapid mRNA vaccines production. ***mRNA vaccine***: The goal of a vaccine is to stimulate the adaptive immune system to create antibodies that precisely target that pathogen. Traditional vaccines stimulate an antibody response by

injecting either a weakened virus, a dead virus, or a recombinant antigen-encoded (usually a viral coat protein) in a harmless carrier virus into the body. Antigens and viruses are prepared and grown outside the body. In contrast, mRNA vaccines introduce a short-lived synthetically created fragment of the RNA sequence of a virus into the individual being vaccinated. These mRNA fragments are taken up by immune cells (dendritic cells), which use their ribosomes to translate the mRNA into proteins (the viral antigens encoded by the mRNA). The mRNA is delivered by a co-formulation of the mRNA encapsulated in lipid nanoparticles that protect the mRNA strands and help their absorption into the cells.

The COVID-19 pandemic, and sequencing of the causative virus SARS-CoV-2 at the beginning of 2020, led to the rapid development of the approved mRNA vaccines. In December of 2020, BioNTech and Moderna, obtained approval for their mRNA-based COVID-19 vaccines. On 2 December, seven days after its final eight-week trial, the UK first approved an mRNA vaccine, granting emergency authorizationfor Pfizer-BioNTech's COVID-19 vaccine for widespread use. On 11 December, USA FDA gave emergency use authorization for the Pfizer–BioNTech COVID-19 vaccine and a week later issued similar approval for the Moderna COVID-19 vaccine.

The production advantage of mRNA vaccines is that they can be designed fast, manufactured quickly, and more cheaply using standardized protocols that are needed to improve responsiveness to serious outbreaks. Moderna designed their vaccine for COVID-19 in 2 days. Pfizer has optimized the manufacturing process for mass production to only 60 days, which is much faster than the traditional vaccines. The actual production time was only about 22 days: two weeks for cloning of DNA plasmids and purification of DNA, four days for DNA to RNA transcription and purification of mRNA, and four days to encapsulate mRNA in lipid nanoparticles. The rest were needed to perform rigorous quality control checks at each stage of the production run. Before 2020, no mRNA drug or vaccine had been authorized for use in humans, so there was a risk of unknown effects. The 2020 COVID-19 pandemic required faster production capability of mRNA vaccines and led to the emergency use authorization after the eight-week period of post-final human trials. Reactogenicity, the tendency of a vaccine to produce adverse reactions, was found to be like conventional non-RNA vaccines. People susceptible to autoimmunity may have an adverse reaction to messenger mRNA vaccines. The 2023 Nobel Prize in Physiology or Medicine was awarded to Katalin Kariko and Drew Weissman for the development of effective mRNA vaccines against COVID-19.

Early foresight and funding support for mRNA vaccine research came from the United States Research Agency, DARPA, which recognized the potential of nucleic acid technology for defense against

pandemics. It awarded a $25 million grant to Moderna that was critical to the development of the mRNA vaccine field. DARPA grant encouraged other government agencies and private entities to invest in mRNA technology. Large infusion of funds (~$2.3 billion in research and development) from US Government during COVID-19 pandemic, and faster FDA approvals of mRNA vaccines for emergency use authorization by United States Regulatory Agency, FDA, were also responsible for the rapid successof mRNA vaccines. In 2022, Moderna announced the development of mRNA vaccines for 15 diseases: Chikungunya, COVID-19, Crimean-Congo hemorrhagic fever, Dengue, Ebola, HIV, Malaria, Marburg virus, Lassa fever, MERS-CoV, Nipah, Henipaviral disease, Rift Valley fever, Severe fever with Thrombocytopenia, TB and Zika.

Finally, some thoughts about Indian Government's role in advancing these transformative technologies. Resistance to gene edited crops and GMOs need to be overcome by the Government and Indian population for successful use of genome editing for crop improvements. Exorbitant pricing of GMO seeds by commercial outfits to farmers may need regulation by the Government. Licensing of international patents and read through rights in the licensing agreements may also need to be examined carefully and regulated by the Government. Off-target effects with mutagenic consequences by genome editing technologies are still a major concern with medical applications. Continued improvements to reduce off- target effects are essential for patients' safety. Therefore, applications of genome editing technologies to human therapeutics will ultimately depend on risk versus benefit analysis and well- informed consent of patients. Also, affordability of these life-saving treatments may require regulation by Indian Government to benefit poor populations. One possible solution could be incorporating a common whole country-wide licensing for these transformative technologies at reasonable terms and pricing by the Government of India for use by Indian scientists for various applications.

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In summary, genome-editing technologies and synthetic biology have revolutionized life sciences. Their impact on biology, agriculture, and personalized medicine has been tremendous and will be felt for years to come. Nobody thought that rewriting life was possible before. Genome editing and synthetic biology have changed all that. These fields have enabled scientists to do precise genome surgery and create new organisms from scratch, respectively. It is a wonderful and exciting time for young undergraduate and graduate students, and medical professionals to enter these fields seeking a research career in biology or precision medicine.

Acknowledgement

This commentary was compiled using the information, data, and dates from articles previously published in RESONANCE from our lab, and those available from Wikipedia, listed under Suggested Readings.

Suggested Readings

(1) Singh AK, Ramalingam S, Rao DN and Chandrasegaran S (2021) Genome Editing Revolution in Life Sciences. **Resonance 26**: 971-998.

(2) Sambasivam V, Rao DN and Chandrasegaran S (2020) Rewriting the Genome of the Model Eukaryote *Saccharomyces cerevisiae*. **Resonance 25**: 801-816.

(3) Refer to topics mRNA vaccine and Synthetic Biology in **Wikipedia, The Free Encyclopedia.**

Feature Image Credit: Gene Solutions



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