Behold the Revolution Heralder: CRISPR-Cas9

Jennifer Doudna (left) and Emmanuelle Charpentier (right) share the 2020 Nobel chemistry prize for their discovery of the game-changing gene-editing technique CRISPR-Cas9 system.

Precision, Accuracy, Speed

The 2020 Nobel prize in Chemistry was awarded to Jennifer Doudna and Emmanuelle Charpentier for the wonderful gene-editing tool- CRISPR-Cas9. CRISPR is actually a natural system in archaea and bacteria which helps fight invaders like viruses by chopping up their DNA and storing that DNA snippet as a part of the host's own genome in the Locus of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR).

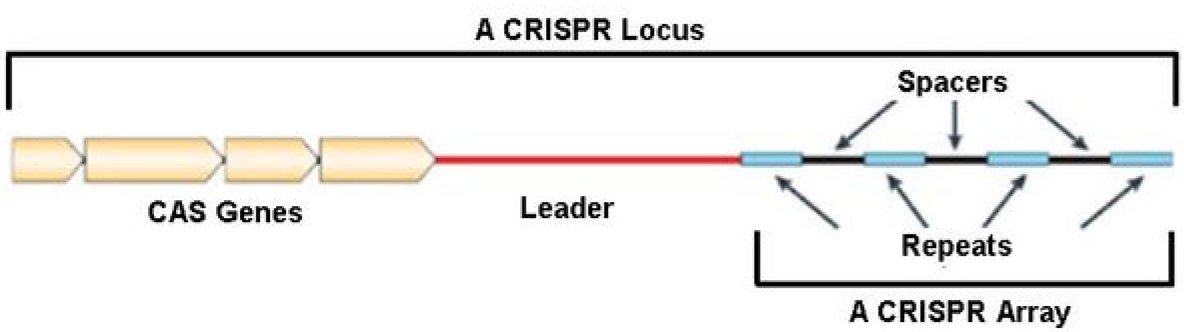
Let's start with the basics. The base foundation of DNA (DeoxyriboNucleic Acid) is a sugar base like ribose attached to a phosphate group and a nucleotide. Adenine(A), Thymine(T), Guanine(G) and Cytosine(C) are four options for nucleotides that form the crux of the entire living world.

Now let's discuss some basic chemistry. A hydrogen bond is formed between Hydrogen and an electronegative element(loves to gain electrons) like Fluorine, Oxygen or Nitrogen, wherein these elements try to seize Hydrogen's electron, which brings those groups closer as a result of partial delocalization, thereby stabilizing the overall complex. Adenine binds to Thymine via two Hydrogen bonds, while Guanine binds to Cytosine via three Hydrogen bonds. This is known as Watson-Crick base pairing.

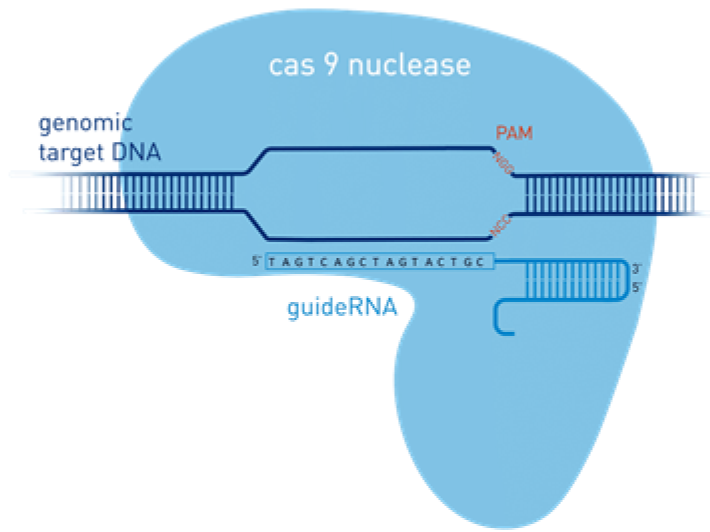
Now, what exactly is a palindromic sequence? It is a nucleic acid sequence in a double-stranded DNA or RNA(RiboNucleic Acid) molecule wherein reading in a certain direction on one strand matches the sequence reading on the complementary strand.

For example, GAATTC is a palindromic sequence because if we look sequentially from both the endpoints, G is complementary to C, A to T and again A to T. This means that this sequence can base pair with itself, forming a kind of hairpin loop-like structure.

The CRISPR locus has several short palindromic sequences known as CRISPR spacers which are regularly separated with DNA sequences known as CRISPR repeats. When transcribed, i.e. when those DNA sequences are converted to RNA, which is the first step towards protein formation, these viral, chemical mugshots are encapsulated as RNA (range varies, usually 17 to 21 base pair long) in CAS (Crispr ASsociating) proteins which verify the complementarity of those 'guide' RNA sequences with any DNA matter lying around. After successful verification, these molecular scissors chop up precisely at a particular sequence point. The chopping is done upstream, i.e., towards the 5' end, from a region known as Protospacer Adjacent Motif or PAM consisting of three nucleotides- N(any nucleotide)GG. The presence of this sequence tells the bacteria that this is some foreign DNA as the bacteria's own genome is devoid of it. This way, the second time the bacteria encounters a similar strain of invader, it would recognize it via the CAS proteins that would then proceed to chop up the invader's genome.



An overview of a typical CRISPR locus



CRISPR spacers are the places where viral DNA are stored. They are typically 32 base pair long. CRISPR repeats separate CRISPR spacers and are typically 21 base pair long.

Cellular Swiss Army Knife

CRISPR is so much more than just molecular scissors. Numerous applications for CRISPR have come forward in recent times.

Some of them are listed here:

Neutralizing antibiotic resistance

The Cas protein receives a specific signal to facilitate the chopping up of the bacteria's own genome so that it cleaves the antibiotic resistance genes of bacteria with precision. This special signal in the form of DNA is stored in a plasmid (extrachromosomal self-replicating DNA) which is introduced through a bacteriophage (bacteria-targeting viruses). As soon as the relevant genes are knocked off, the antibiotics can be administered, which effectively eliminate the bacteria. This way, CRISPR can be used to counter the menace of the ever-evolving antibiotic resistance to our drugs , which constitutes a huge problem while treating bacterial diseases in both plants and animals.

Quick and efficient testing

Cas proteins are of different types. Some target just the specified region of DNA while some chop the same along with any other regions of DNA localized near the site. These destructive Cas proteins cause collateral cleavage. These can be used to quickly and efficiently report whether a particular section of disease-causing DNA like that of a virus or bacteria is inserted in one's genome after infection. After getting one's DNA sample via nasal swab or a spit sample, PCR(Polymerase Chain Reaction) is carried out. PCR helps to amplify the desired DNA fragment. Subsequently, destructive Cas proteins along with a reporter gene are added to the PCR product mix. A reporter gene fluoresces when any of its parts is cleaved. So, if the Cas proteins have been trained to cut the infectious DNA part with collateral cleavage, they will cleave the adjoining DNA fragments of the reporter genes, too and thus cause the entire test tube to fluoresce. This forms a quick yet very easy test to identify whether someone has an infection of any sort.

Xenotransplantation

Xenotransplantation is the method of transplanting living tissues, cells or organs from one species into another. Pigs are currently thought to be the best candidates for organ donations. Retroviruses found in the DNA of animals like pigs tend to insert into the host genome, which can cause potential cancerous mutations and myriad immunity issues. Thus, programming the CRISPR system to cut specific portions of the pig DNA results in a much lesser probability of those retroviral portions affecting us humans after accepting the transplant. Embryos created from these modified cells and placed into surrogates create ideal donor animals with inactive viruses.

Ethical concerns and challenges

CRISPR Cas9 is an evolving technology with limitless scope. Over 5000 papers were published on CRISPR in the year 2020 alone! Cas9 is a type of nuclease which cuts the regions where the guide RNA tells it to. This means that methods to play around with genes have become more precise and reliable than ever before. But if not within ethical constraints, any promising technology can quickly become a controversy—and CRISPR is no exception.

The Controversial Germ-line designer babies

He Jiankui, a scientist at the Southern University of Science and Technology, Shenzhen, China, used CRISPR to disable the CCR5 gene in human embryos during IVF (In-Vitro Fertilization). HIV (Human Immunodeficiency Virus) uses the CCR5 gene to allow itself access into the human body. After disabling this gene, He aimed to create HIV resistant human babies.

In November 2018, the first gene-edited twin girls- Lulu and Nana, were born. The father of the girls was HIV positive, and so the couple agreed to this experiment. He’s intentions were undoubtedly good though the approach used was considered a bit too extreme and premature. CRISPR isn’t perfect yet. A lot more research needs to be done before we can perfect embryo editing. Even if we could, there are many ethical questions regarding germ-line editing- affecting directly the course of human evolution. He Jiankui couldn’t reproduce the required mutations to their entirety, and now the two girls are stuck with unknown CCR5 mutations for the rest of their lives. The ideal motto should be to make people better, not better people. If not controlled within limits, this juxtaposition of unnatural selection with random off-site mutations could prove fatal for our future.

All these points just depict one thing—CRISPR-Cas9 is an extremely new technology. We don't yet have enough research done to predict the consequences or effects of a particular insertion or deletion accurately, especially in the case of germ-line cells. There's this media hype about CRISPR being an easy and cheap technology. Well, the CRISPR-Cas9 system is definitely cheaper than the previously existing gene-editing techniques like Zinc Finger nucleases, TALENS or mega nucleases but unknown consequences due to mutations and off-target effects outweigh this point to a far greater extent. The regulations haven't caught up with the technology yet and so a moratorium, i.e. a temporary suspension of activity in germline editing has been called forth by the leaders of this revolution in order to take some time and discuss its future usage. The parameters to be considered are as follows:

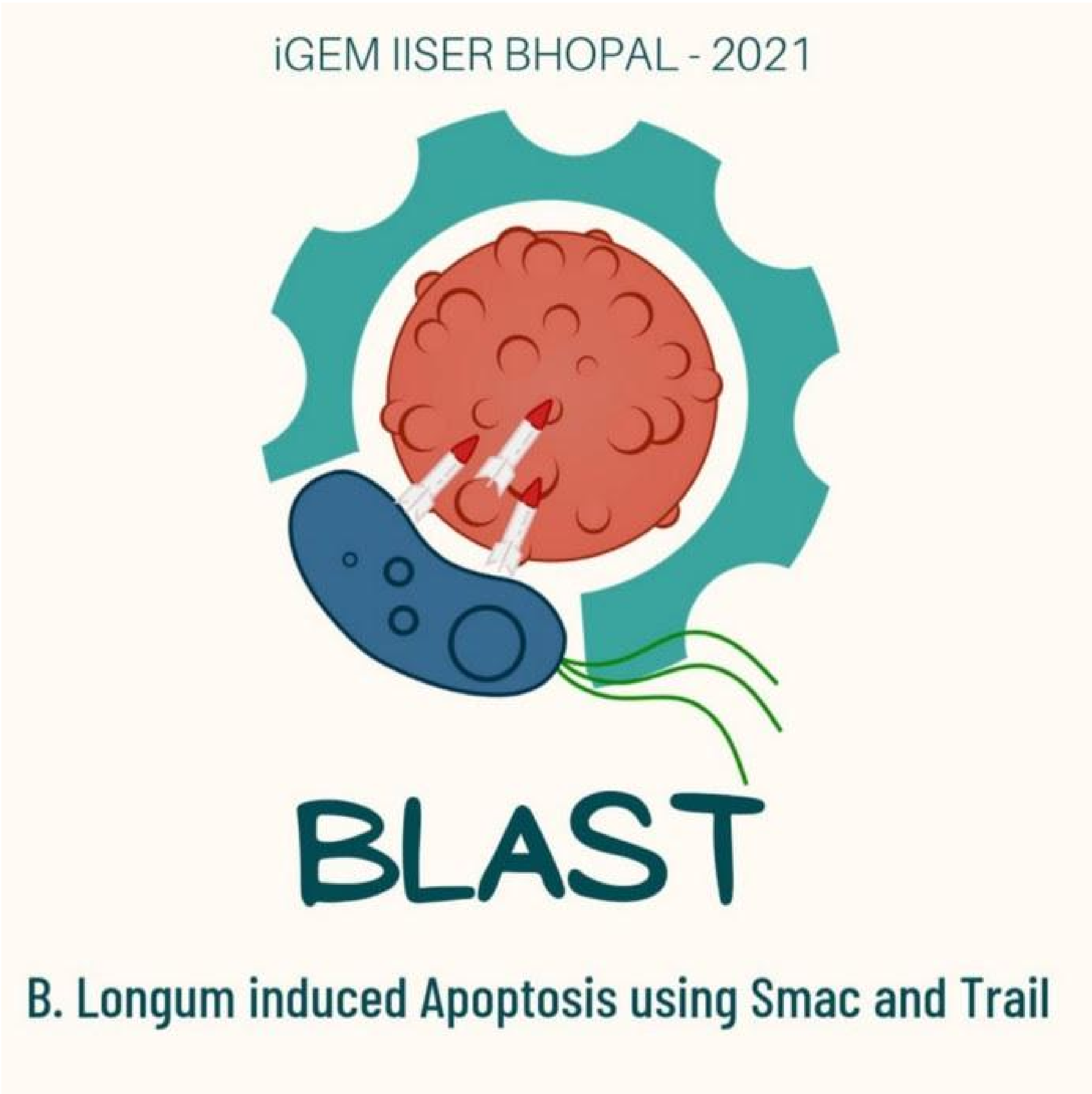
- First and foremost: The consent of the yet unborn baby.
- Social class affordability.
- The possibility of designer babies with enhancements and favourable traits leading to the creation of super-soldiers, or any indirect destructive use of this technology, almost manifesting science fiction horror in real life.
- The most important aspect is that- just because we can, should we?

Concluding Remarks

Genetic engineering will inevitably dominate in the coming times, unleashing unthinkable possibilities for us. CRISPR-Cas9 promising to be a frontrunner in the prospective advancements in this field. As we do have a revolution in our hands now, what we do now will directly impact our future. This powerful weapon can pose many issues if not yielded in the right and controlled way.

Here are some of the videos I referred to while writing this introductory bit:
https://youtube.com/playlist?list=PLT50hfWenVPkUkMlfGVd3gq9FyCJsTU47

Synthetic Biology: Re-designing Life



iGEM IISER Bhopal- 2021 project

This is a talk by an iGEM participant from IISER Bhopal who’s crazy passionate about science and wants to inspire young high school students to pursue scientific research. Amey will be briefly introducing them to the emerging field of Synthetic biology and give them a glimpse of how the practice of synbio is making our lives better.

Amey: Hello everyone, how are you all doing? Today, I am going to take you through the exciting field of Synthetic biology. By the end of this, I hope you all are able to design some aspects of synbio in your minds.

To start up, what do you think synthetic biology is?

I can give you a hint; it's equivalent to renovating your home. Now, what could it be in terms of biology?

Student_1: Judging by the topic of the talk, I think it's got something to do with making useful substances using the biology of cells.

Amey: Damn right you are! Now let me start by asking you another question; mind you, it's relevant. Have you ever played with legos?

confused faces with huge smiles

Amey: Huh, my bad, who hasn't played with legos. Now, what if I say that synthetic biology is similar to a game of legos. You create meaningful objects using legos and re-design the same object by adding or removing legos to form something else. This is exactly what happens in synthetic biology. You add DNA sequences in the genome of different organisms to produce useful substances for yourself.

Synthetic biology is an area of research where you create new biological systems or re-design systems that already exist in nature by engineering cells to produce useful products. Each piece of DNA stores information for different proteins. All you need to do in synthetic biology is identify the DNA piece of interest, incorporate it into a growing biological system, and let the system produce the product for you.

Synthetic biology involves the use of methodologies from various branches, including biotechnology, genetic engineering, molecular biology, and whatnot. Advancements in genetic engineering and genetic sequencing have further boosted the scope of this field in identifying crucial pieces of information for genetic manipulation. In addition, improvements in the cost and speed of DNA synthesis have enabled scientists to design and manipulate biological systems for human benefit.

Student_2: Does synthetic biology also involve crazy scientists making glowing animals?

giggles all around

Amey: Indeed! Crazy scientists perform crazy experiments in their laboratories, and engineering glowing animals is one of them. All you need is the genetic sequence for bioluminescence and the organism that you intend to glow. Bioluminescent organisms offer a treasure trove of light-emitting enzymes that have huge applications in the areas of bioengineering, from biosensors to medical science. However, it does not mean that you will go around making glowing animals; it is impractical and restricted. Remember that synthetic biology is a potent tool that has to be used very carefully.

Student_3: If the genetic material of organisms can be manipulated, does it also mean that coronavirus could be human-made?

Amey: Well, let's hope it's not! Benefits come at a cost. While synbio is making life much easier by providing valuable substances, it is also being exploited to produce biological warfares. Although bioweapons have been used in war for many centuries, a recent surge in genetic understanding, as well as a rapid growth in computational power, has allowed genetic engineering to play a more prominent role in the development of new bioweapons.

Now, you can engineer new deadly pathogens with increased virulence, drug resistivity, and infectivity. While the positive societal implications of improved synthetic biology are apparent, the "black biology" of bioweapons development may be one of the gravest threats to humankind and the environment. However, experiments concerning bioweapons are strictly prohibited in various countries, so you all are safe. Also, no evidence has been found suggesting that coronavirus is human-made, which means that you don't need to worry. But still, stay safe and wear masks.

fear and relief on faces

Amey: I didn't mean to fear you all. Let's talk about some positive aspects of synbio, shall we?

Over the years, synthetic biology has proven to be a tremendous helping hand in improving areas such as agriculture and medicine. For example, synbio has been used to develop bio-factories by engineering bacterias with desired genes to produce medicines, proteins, and other biofuels. It has also been exploited widely to produce nutrient-rich crops such as vitamin-A enriched rice. Other variety of applications ranges from drug delivery to ending the Covid-19 pandemic, and of course, including designing some glowing animals.

Student_4: If synthetic biology can create new organisms by altering their genetic material, can it also be used to bring back extinct species?

Amey: A Jurassic world fan, it seems! Indeed, you can bring back extinct species with the aid of synthetic biology. At present, the synthetic biology and conservation communities are largely strangers to one another, even though they both share many of the same concerns and goals.

However, synthetic biology can have a potential impact in the field of conservation. For example, you can take a preserved cell from a recently extinct animal, extract and swap its nucleus into an egg cell from the animal's closest living relative, and implant the egg into a surrogate host. Another option is by using genetic engineering tools, you can swap relevant genes from the extinct animal into the living species and implant the hybrid genome into a surrogate. Cloning can also be used to make identical genetic copies of extinct species for increasing their population.

As cool as it might be to find woolly mammoth and sabre-toothed tigers in the wild, the real world can't have a Jurassic park as there is more to do with conserving these animals than any other reason. The main goal in conserving selective species is to restore the ecological balance and its function. While there are a lot of ethical and technical issues involved in applying synthetic biology for conservation, it definitely provides tremendous potential for the restoration of ecology.

Student_5: Can we increase the life expectancy of humans or maybe, create immortal humans?

Amey: Well, someone here doesn't want to see wrinkles on their face. But yes, synthetic biology can indeed be used for increasing the lifespan of an individual or, in fact, prevent ageing.

See it this way, as you age, you face a number of age-related diseases. The treatment requires a huge variety of health care items, such as drugs that are manufactured using the methodologies of synthetic biology. Health care items aid an individual to fight off diseases, thereby leading to an increased lifespan.

Another way of increasing a healthier lifespan is by literally delaying the process of ageing. You start ageing when your cellular machinery is no longer able to cope up with the body's requirements, causing cell senescence. What if I say that you can use synthetic biology to alter the cellular repair machinery and boost its ability to repair any damages within the body. Isn't that amazing?

There is a whole new emerging area dedicated to ageing research that tries to do precisely the same. So immortality can be achieved, but it ain't happening any time soon as it involves lots of ethical issues and decades of research that might age you.

* another hand raises *

* and then another *

Amey: Hold on! You all seem really passionate about synthetic biology. But the time is ticking, so let's call it a day. I am sure that you'll find your answers about the immense field of synbio on @igemiiserb. It was an absolute pleasure talking to you guys. Till then, take care and keep playing legos.

clapping all around

[The Gut Tales: Synthetic Biology Takes Over]

Introduction

Representative Image of Gut Microbiota

Human Gut Microbiota is a term used to indicate the various microorganisms (bacteria, archaea and fungi) that reside in the human intestinal tract. Especially in the large intestine's "pocket" -caecum, while maintaining either a non-harmful coexistence or a mutualistic relationship with its human host. In recent years, the gut microbiota has been thrown into the limelight after discovering its significance in overall human health and well-being. It plays a pivotal role in our immune response and also in controlling blood sugar levels. The most important of the lot is the microbiota's effect on gut health itself.

With all of these positive effects, the microbiome now is popularly referred to as every human's personal treasure of good bacteria!

With the current lifestyle, proper knowledge of one's microbiome has become a necessity and not an option. Hence, synthetic biology and its application in the betterment of gut health have become a matter of colossal importance. Here, a summary is provided on the recent Gut related developments in the field of synthetic biology and a curtain-raiser on food trends of Kombucha and Kimchi that are getting increasingly famous and all for the right reasons!

I will be asking the questions and, well, answering them myself. Feel free to give yourself a point if you could answer the questions too!

What are Probiotics?

Now all of us are acquainted with the gut microbiota, so the next big question is how do we make sure we take care of the microbiome and thus ensure good gut health.

We can do this by making probiotics a part of our diet!

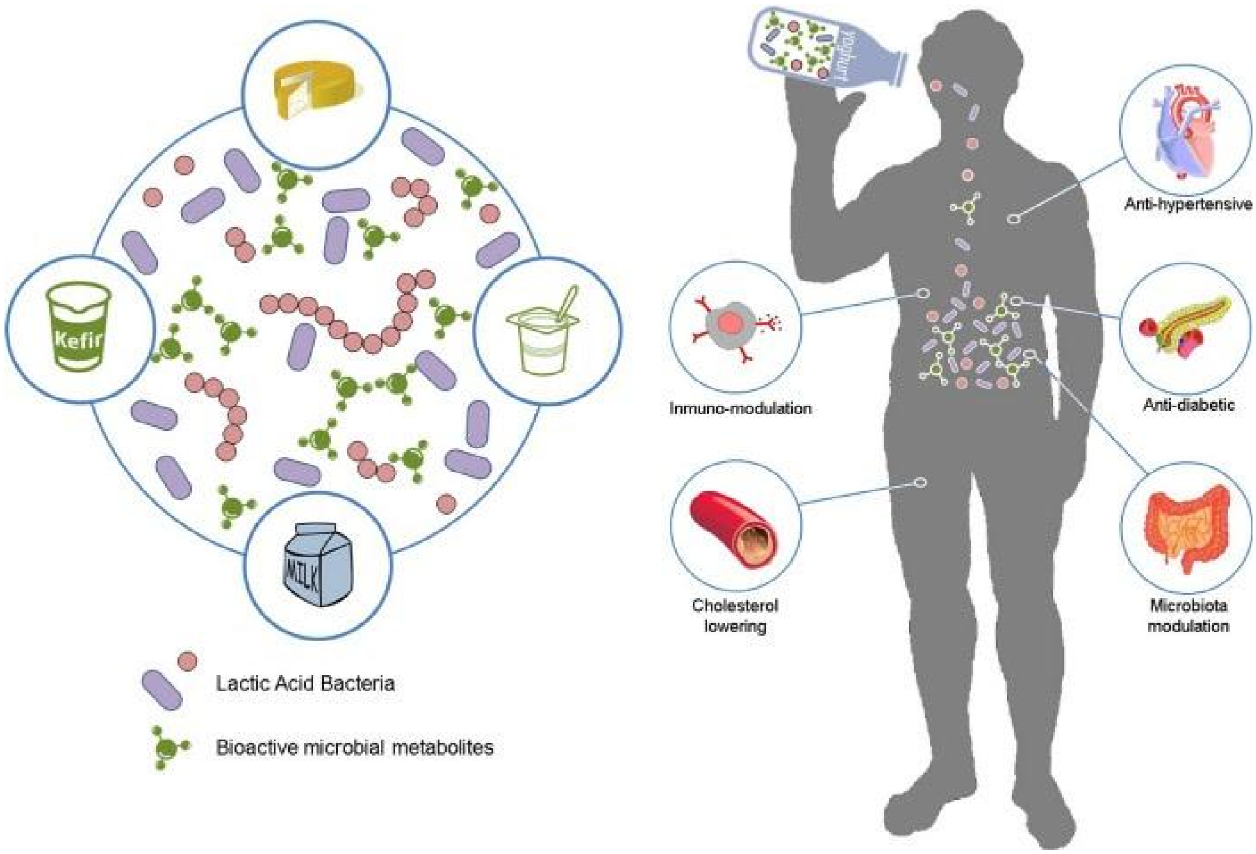
Probiotics are live microorganisms that have health benefits when consumed because they help maintain our gut microbiome's composition. All of us have been unknowingly consuming probiotics right from birth — Yoghurt and Curd are nothing but probiotics. There are many other foodstuffs that are natural probiotics and are a part of our diet, but in today's times, dietary probiotics are just not enough; we need supplementary, synthetically enhanced probiotics too.

So, what is so special about the present time that this need arises?

The most relevant answer is the wide and frequent use of antibiotics. Antibiotics are the go-to prescription for illnesses. After an antibiotic regime, it has been noticed that they get rid of a big part of our good gut microbiota along with the harmful ones. In such cases, synthetic probiotics with enhanced functions are prescribed as a post-antibiotic regime to restore good bacteria in the gut. **Engineered bacteria can also potentially monitor our health status, detoxify by-products, and deliver therapies in a precise manner.**

Can Probiotics be used as 'Living' Medicine and Live Therapeutics?

YES!



Lactic Acid Bacteria And Their Benefits

In Gut Tales, we can't miss out on talking about probiotics as medicines to replace antibiotics themselves in some cases. When the accumulation of a toxic metabolite in the gut is causing a disease, a modified bacteria can break down this metabolite and act as a form of treatment. The patient can consume this engineered probiotic bacterium and be promptly relieved from any pertaining symptoms.

Another example of using probiotics as live therapeutics is that of Lactic Acid Bacteria (LAB) — used for centuries in fermentation and preservation of food.

LAB has a long history of safe and health-promoting interactions with the human gut, making them ideal candidates to engineer as a synthetic probiotic by enhancing their functions.

This is made possible by numerous genome-engineering tools which have enabled the engineering of more complex functions in the bacteria. I can go on and on about probiotics as therapeutic agents, but these two examples suffice as an insight into the future of synthetic biology in engineering medicines in the form of probiotics.

Can Probiotics be used to improve our Lifestyle? (Kombucha lovers, you know it well!)

A Refreshing Glass Of Kombucha. Photo by [Tim-Oliver Metz] on [Unsplash]

Many current probiotics are being marketed as nutritional supplements, and both Millennials and Gen-Z are all up for it!

From Kombucha to Kimchi, awareness of the effects of probiotics and how they can be part of a healthy lifestyle and act as nutrition supplements have travelled far and wide. Adding these probiotics to our diet is again a means of improving the gut microbiome, and the success these probiotics have earned will pave the way towards normalizing probiotics as a part of our diet.

This is where Synthetic Biology makes its entry.

SynBio researchers have come up with solutions to use engineered probiotic dietary supplements to treat conditions like lactose intolerance and hangovers.

In 2019, *ZBiotics* launched the first engineered probiotic on the market. The product acts efficiently as a hangover cure, where an engineered microbe breaks down acetaldehyde (a toxic by-product of alcohol breakdown by the body after its consumption). Another spin-off comes in the form of improving the performance of athletes by the use of engineered gut bacteria. Researchers discovered that some bacteria are more prevalent in the gut of marathon runners. *FitBiomics* then reached a licensing agreement with Harvard's Office of Technology Development, aiming to explore and commercialize research towards using probiotics to enhance athletic performance.

Where Do We Go From Here?

The only way is forward! From ordinary probiotics to synthetic, live therapeutics, innovations have led to ground-breaking solutions. The key issues must continually be addressed to further understand mechanisms of action for future innovations in monitoring, understanding and developing healthy gut microbiomes. It's high time we raise the curtain that shields us from probiotic usage and gut health awareness. This will surely bring about radical upliftment in our overall quality of life and be key to a better lifestyle.

P.S. Could you answer all the questions before reading up the explanations? If you got a 4 on 4, congratulations, my friend! Please accept a virtual Kombucha and lots of validation from my side.

Edible Genesis

The Rise Of Genetically Modified Crops

In this era of global warming and climate change, we need crops that can produce higher yields and are drought resistant to sustain the ever-increasing population.

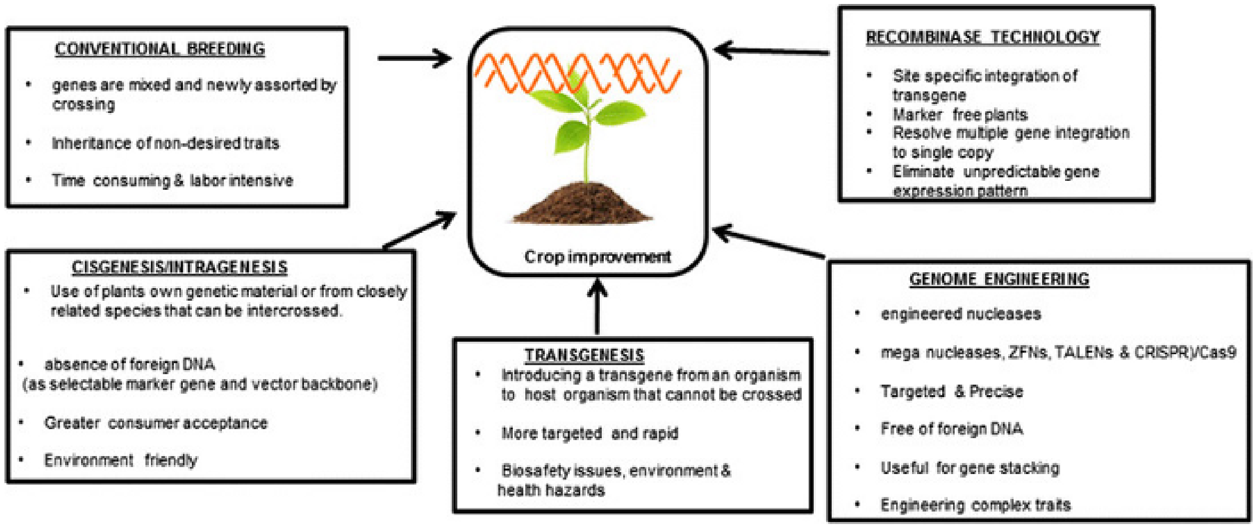
Photo by www.zanda. photography on Unsplash

Here, **Genetically Modified(GM) crops** come to our service.

GM crops refer to the crops produced from seeds whose genetic makeup is changed in the lab to get higher yields and other advantageous traits.

Producing GM crops involves making mutations, i.e. altering the gene structure to get an altered or a new gene product. This is done to induce or halt, increase or decrease the expression of certain genes to get the desired crop variety. These crops can be modified to grow in any environment, like environments with high salinity, high temperature, or water deficiency. We can even procure pest and virus-resistant crops. Apart from stress tolerance, we can obtain crops with high nutritional values by genetically modifying them.

GM crops can be produced by a variety of methods which are highlighted in the following image.



Various Approaches For Crop Improvement

Though there can be a variety of modified crops, the main focus of this article would be to discuss GM crops that are drought-resistant and nutritionally enriched.

Drought Tolerance

Drought results in increasing the reactive oxygen species (ROS) inside the cell, which in turn alters the concentration of various solutes in the cell and leads to the malfunction of the cell machinery.

To counterattack these changes and survive under these strenuous conditions, plants activate certain transcriptional factors (proteins that regulate gene expression) that lead to the activation of certain genes known as 'stress genes'. In GM crops, we modify these transcription factors to enhance the expression levels of the said stress genes.

Genetic engineering has been extensively used to insert certain genes from distantly related species into plants to make stress-tolerant varieties. ***Arabidopsis thaliana*** **is the model plant chassis on which synthetic biology experiments are performed*.*** The results then are extended to improve other crop varieties.

Crops can be made drought-resistant mainly in two ways: altering the signalling pathways involved or changing the expression levels of several transcriptional factors involved in regulating plant stress hormones.

Examples:

- Transgenic rice seedlings (seedlings produced by insertion of genes from other species), in which a transcriptional factor named OsWRKY11 was expressed, showed longer survival and less water loss when exposed to stress from drought-like conditions.
- LEA (Late Embryogenesis Abundant) genes have been manipulated in many plants to increase drought resistance. When expressed, these genes prevent the aggregation of proteins and also prevent the plant from collapsing due to desiccation.

Genetical engineering can target the expression of genes that are involved in water and ion uptake and transport to make the crops more drought-resistant. A wax layer around the plants protects them from pest and fungal attacks and helps prevent excessive water loss. Modifying transcriptional factors regulating the production of wax layers can help us thicken the layer and make the plant more drought resistant.

Advanced engineering techniques, one of them being CRISPR/CAS, are required to change multiple genes simultaneously and produce the required variety. Altering the signalling pathways using genetic engineering provides one main advantage over manipulation of transcriptional factors: the manipulation of signalling factors can control many other functions and thus provide tolerance for multiple aspects. Another advantage is that the manipulated signalling factors can be activated in certain stress conditions while inactivated in others. Thus they can work as a molecular switch (which is only switched on when required) and won't show any adverse effects under non-stress or normal conditions. This is very important when considering the crop yield because many transgenic crops can show growth retardation and alterations in basic metabolism if these stress response signals are on even in normal conditions.

Nutritional improvement

Genetic engineering can be used to improve the nutritional values of crops either by increasing the content of existing nutrient or by introducing a new nutrient into the crop by inserting genes from other closely related species.

Some Examples:

- **'Golden rice' is a genetically engineered crop that contains an additional compound- beta-carotene, a precursor to vitamin A**, while normal rice does not contain beta-carotene.
- **A potato Tuber highly enriched with carbohydrates can be genetically modified to produce a potato rich in protein content as well.** Introduction of certain genes from other species can help us to achieve that.
- **Nutritional value can also be improved by blocking the expression of anti-nutrient factors (ANF), which plants produce as a defence strategy against pests and herbivores.** Their expression needs to be blocked so that humans can consume that

plant even though not all of the ANFs are harmful to animals and humans. Again, genetic engineering can help us achieve that.

- Vegetable like grass peas are an important source of proteins, but they also contain an anti-nutrient, Oxalic acid. **Excess intake of oxalic acid can lead to kidney-related problems. Thus, this anti-nutrient expression should be decreased to make the grass pea edible, which can be done by introducing a gene encoding an enzyme capable of degrading Oxalic Acid.** Similar strategies could be used to reduce the content of food allergens in the crops and make them suitable for consumption.

Some other advantages of GM crops

- We have often observed fruits getting soft if kept exposed for many days. Excessive softening can lead to hindrance in both- climacteric (ripening controlled by the production of ethylene compound) and non-climacteric (ripening independent of the production of ethylene) pathways. Genetic engineering can be used to increase fruit life even after harvesting.
- Another domain of genetic engineering is creating disease-resistant varieties of crops. Scientists are also attempting to synthesize nitrogen-fixing pathways of legume plants in staple crops like wheat and thus develop a self-fertilizing cereal.
- Crops can be engineered to produce insecticidal proteins, which will keep the insects far from the crops and thus, the use of insecticides and pesticides can be reduced drastically.

Disadvantages of GM crops

- The gene of interest in the transgenic GM crop can come from bacteria, virus or any other distantly related species. We might encounter a case when the plant does not fully accept the gene, or the gene might interfere with the plant's basic metabolism. This may lead to allergies or other infections in humans when eaten. Thus, overall this can be quite detrimental to the plant's nutritional value.
- Plants engineered to have one specific trait can result in the deprivation of some other important trait.
- Some pest-resistant crops can release toxins in the soil, which can be harmful to the essential soil microbes.

Advances in engineering techniques are being developed to ameliorate these disadvantages associated with GM crops.

Summary

Benefits of GMOs	Risks of GMOs
Nutritional value of foods could be improved (e.g. by introducing proteins, vitamins or vaccines)	New traits could cause adverse health reactions (e.g. new proteins may cause allergic responses)
Crops can be produced that lack known allergens	Removal of traits could have unknown effects
Crops can grow in arid conditions for better yield (e.g. by introducing drought resistant genes)	Crops may limit biodiversity of local environment (increased competition with native species)
GM crops can produce herbicides to kill pests	Cross pollination could lead to 'super weeds'
Improve food supply / agriculture in poor countries (GM crops can be engineered for improved yields)	Patents restrict farmers from accessing GM seeds (biotech companies hold monopolies over crop use)
GM crops may have longer shelf lives (less spoil)	Foods with GM components may not be labeled
Reduces economic costs and carbon footprint – less need for land clearing and pesticide usage	Different governments may have conflicting regulatory standards concerning safe usage

Benefits and risks of using GMOs

Conclusion

GM crops can serve a wide range of purposes. They help in increasing yield even in adverse conditions. They also reduce environmental pollution caused due to chemicals that are otherwise used while cultivating crops. Furthermore, they can help curb food shortage and malnutrition due to enhanced yield and increased nutritional values. A variety of pathways can be changed, and the desired varieties of crops can be curated accordingly. The concept of cisgenesis(use of genetic material from same species) and intragenesis (use of genetic material from closely related species) has been introduced to meet the growing public concerns and biosafety issues associated with transgenesis. Advancements in molecular biology have allowed scientists to modify genes at specific locations without the incorporation of foreign DNA, which increases the acceptance of GM crops even in the countries where GM crops are frowned upon. These crops ensure farmers and merchants improvement in the quality of food in an effective and efficient way. However, still, a lot of questions remain unanswered and even more research is needed to understand the entire gamut of this seemingly limitless field.

Creating Synthetic Life

An Overview

Introduction

The creation of life is indeed one of the most remarkable process.

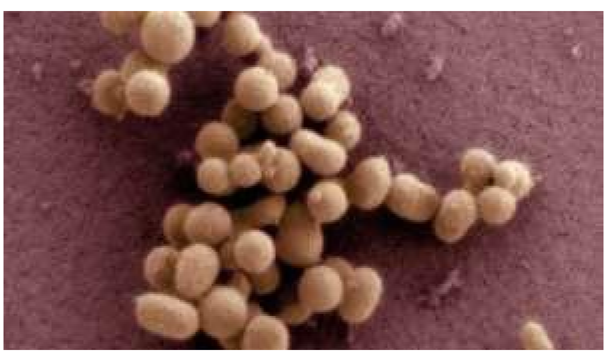
> **The formation of synthetic life, as the name suggests, is an attempt by scientists to replicate this process in-vitro, i.e. in test tubes outside the bodies of living organisms.**

As exciting and ambitious it may sound, it has its own fair share of challenges that need to be overcome.

This article aims to shed some light on some of the obstacles faced in synthetic biology.

Inception

The concept of synthetic life dates back to the theory of spontaneous generation proposed by Aristotle, which elaborated on the advent of living organisms from non-living matter. But this theory was soon refuted and it was universally accepted that living organisms could originate only from living beings. This, however, could not bring down the spirits of other scientists working on finding methods to create synthetic cells.



Mycoplasma laboratorium

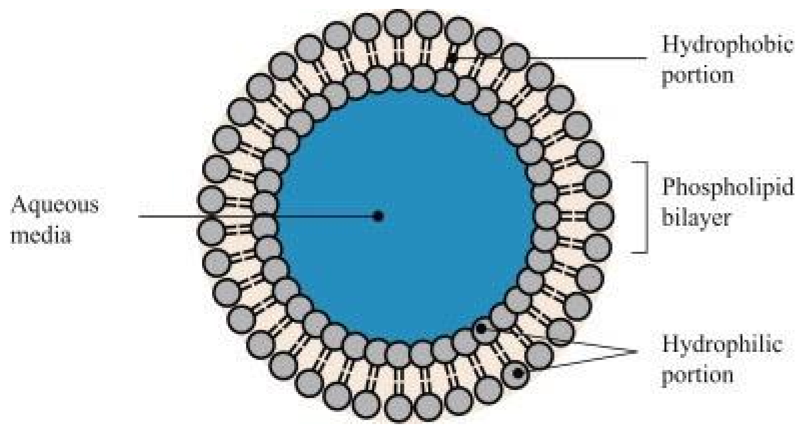
Craig Venter, for example, made an invaluable contribution to this field by developing a genetically engineered bacterial strain- Mycoplasma laboratorium, also called ‘Synthia’.

An artificially expanded genetic code was produced in 2014. Finally, in 2017, researchers collaborated for the **'Building A Synthetic Cell' program-** which aimed towards building a synthetic living cell within ten years. Many factors have to be kept in mind while going about this herculean project.

Pre-requisites

Numerous complex phenomena make "life" possible, and these need to be taken care of while designing synthetic cells.

The most underrated yet the most important feature of living cells is the presence of a membrane that helps in the compartmentalization of biological moieties inside the cell.



Liposome

This indicates the vital requirement of membranes for synthetic cells too, which could be fulfilled by constructing **liposomes** which are spherical vesicles having structures similar to that of cell membranes. For certain molecules to be brought together and kept apart, it is essential to know about the function of each component which could be achieved by evolutionary analysis or isolating individual genes and studying them.

Secondly, living cells require a constant source of energy to function and this requirement is fulfilled by the mitochondria-popularly known as the powerhouse of the cell.

It is believed that synthetic cells too should have their own inbuilt energy supply either through the use of enzymes like ATP synthase which could help in generating ATP or by setting up metabolic pathways in these synthetic cells that could produce molecules like glucose. The produced glucose could then be broken down to produce energy.

Synthetic cells also need to be somewhat imperfect and possess the need for evolution as the development of new modified functionalities every now and then ensures long term survival of the gene pool.

Efforts ought to be made to estimate the minimum number of genes vital for the growth and replication of these cells. Lastly, the ethical consequences of releasing synthetic organisms in the biosphere should be thought upon. Efficient biocontainment and stringent measures would ensure that the available technological benefits are not being misused.

Conclusion

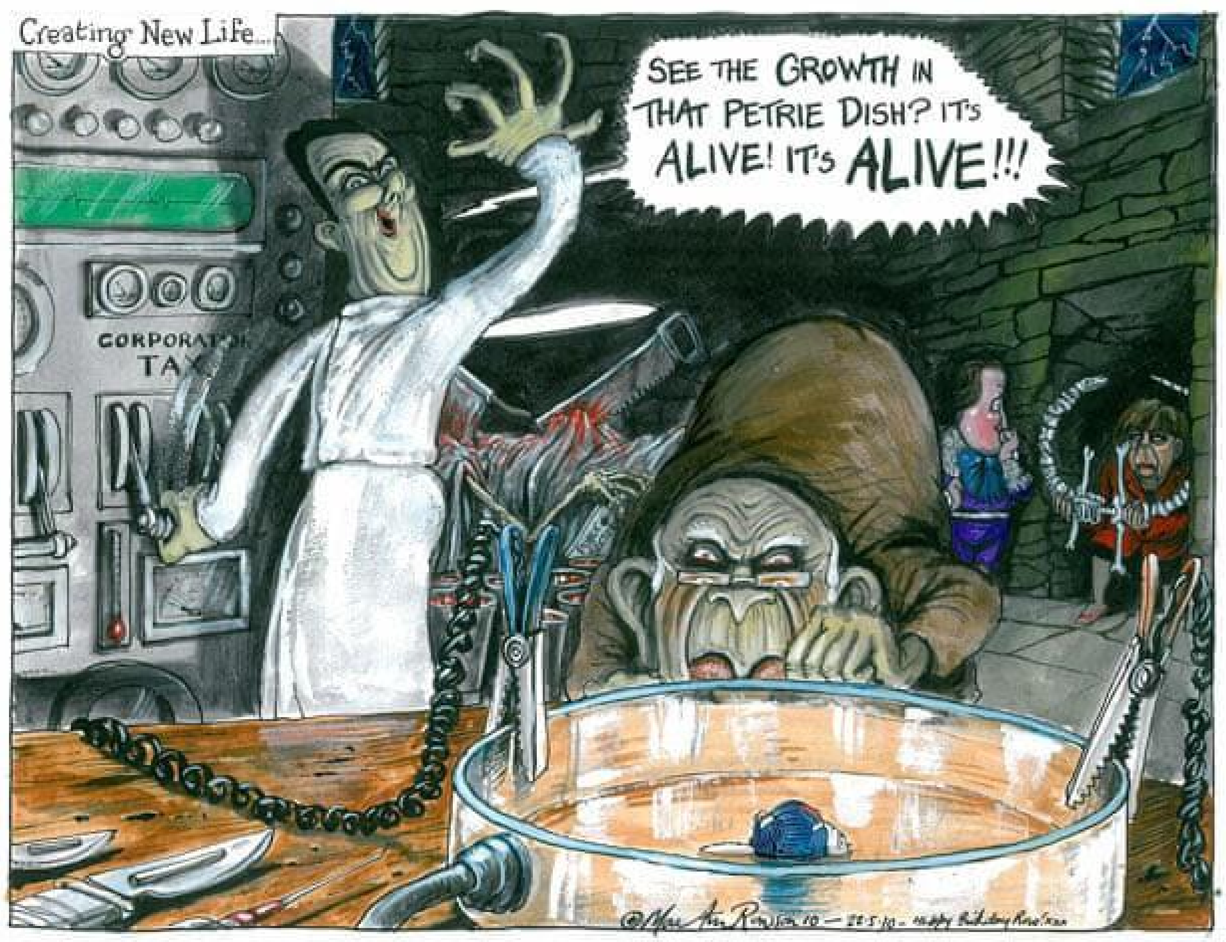


Image credits: The Guardian

The possible applications of synthetic life range from therapeutics to yielding environment-friendly solutions. It can help us understand the progression of various lethal diseases like COVID-19 and find quicker and more effective cures. It can be yielded to help degrade toxic wastes and thus help reduce our environmental footprint.

The creation of synthetic organisms with proper precautions undertaken might thus prove to be one of our greatest achievements of all time. Thus to conclude- even though a lot has been accomplished in this field, we mustn't forget that the baton still carries a lot of weight. And we still have a long way to go.

Biofuels- The Syn-Bio Way

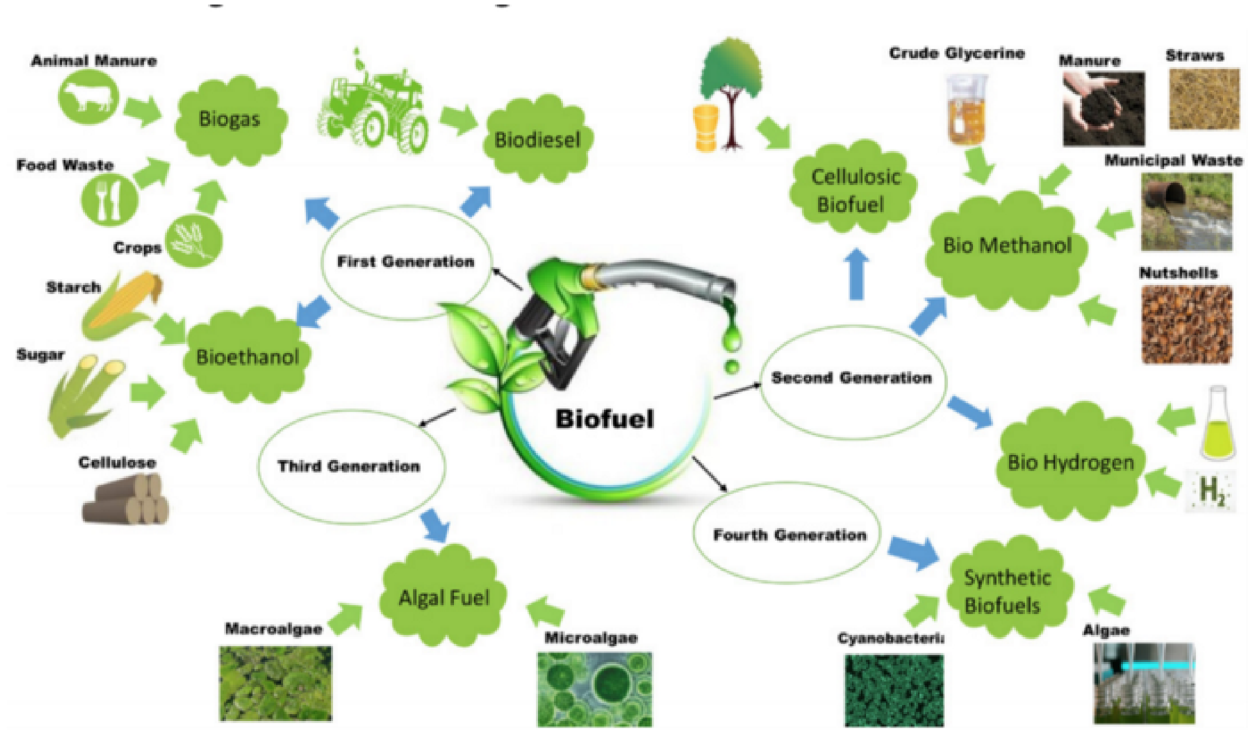
Introduction

Human life revolves around efficiently using various forms of energy. From morning till the day end, we use multiple energy sources to keep our everyday life going. This energy is provided by a source of fuel. We are aware of various fossil fuels such as petroleum, coal, and natural gas, which are currently in use for varied purposes. Apart from some of the detrimental effects on the environment, we cannot deny that the reserves of these fossil fuels are also rapidly depleting. So, why not shift our major source of fuels to biofuels which have already started contributing as fuels in certain specific sectors?

What is Biofuel?

Biofuel is a fuel derived from biomass .i.e. it is derived from any plant or animal source. Generally, it refers to the liquid and gaseous form of fuel used majorly in transportation. Unlike fossil fuel sources, biofuels are environment friendly (a bit questionable though), renewable and cost-effective, given the rising prices of petroleum. Biofuel is generally produced from energetic crops (low-cost and low-maintenance crops grown solely for energy production by combustion and not for food) or agricultural and industrial wastes of biological origin.

Biofuel Overview



Biofuel generations and types.

The use of biofuel dates back to 1937, when Rudolf Diesel envisioned vegetable oil as a source of fuel for his newly invented engine. The two common types of biofuels known are bioethanol and biodiesel.

Bioethanol is mainly produced by fermentation from carbohydrates produced in starch crops such as corn and sugarcane. It can also be derived from cellulosic biomass derived from grasses and trees. Bioethanol, in its purest form, can be used as a fuel source for vehicles. The underlying mechanism behind biodiesel production is a process called **transesterification**.

In 2019, biofuels contributed 3% to the global fuel for roads. According to the International Energy Agency target, to increase its contribution to more than 25% as the global fuel for roads by 2050, the production of biofuels needs to be hiked to an exponential level without posing any threat to the environment. This is because it'll require large scale use of plant and animal biomass, and thus, we need to find an alternative source to derive biofuels.

Synthetic biology seems to be a plausible solution for hiking production in the bioenergy sector.

Synthetic Biofuel Production

Synthetic biology is the science of programming organisms in such a way as to include devices and systems which will help the organism to perform a novel function.

Saccharomyces cerevisiae, or brewer's yeast, has been in use in the brewery industry for a long time, which provided scientists with the idea of using it for biofuel production. Budding yeast is known to produce ethanol and fatty acids in quite a large amount. But there's a limitation with its use in bioethanol manufacturing. It cannot be used for biofuel production as the process requires a high temperature. Biofuel production requires strains that can withstand stressful conditions such as extreme pH, high temperature, shearing forces, organic acids etc. Non-conventional yeast species like *Yarrowia lipolytica, Hanensula polymorpha, Pichia pastoris*, and *Kluyveromyces lactis* are a way out for this issue as these yeast species can withstand such harsh conditions. Moreover, these can use a variety of carbon sources as a substrate for the fermentation and production of biofuel (ethanol).

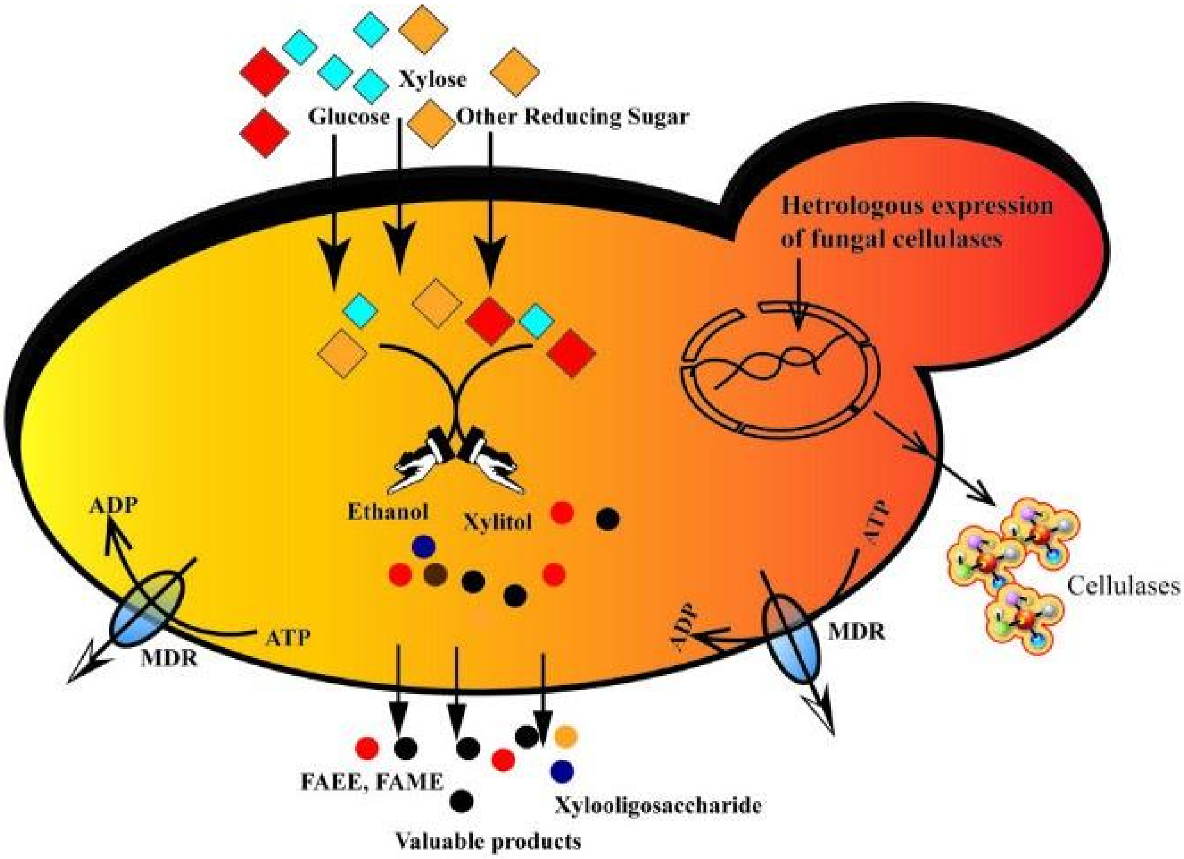
Synthetic biology uses the concept of producing microbial cell factories for biofuel production. For the cell factory to function efficiently, the promoter element (indirectly controls the protein expression level) must be chosen wisely to ensure heterologous protein (a protein that is not typically produced by the particular chassis organism) expression.

The yeast *Kluyveromyces lactis* uses the lactose inducible (activated only in the presence of lactose) promoter PLAC4 to produce the desired protein. The protein is secreted in a culture medium (any liquid or solid preparation made specifically for the growth, storage, or transport of microorganisms or other types of cells), making its purification easy. Significant biomass production in the culture makes *K. lactis* a great cell factory.

Pichia pastoris ferments xylose to ethanol by producing an almost negligible by-product, making it a very efficient and high yielding organism.

Yarrowia lipolytica is another non-conventional yeast strain used for biofuel production, especially fatty acid derived biofuels. Different target genes were made to overexpress and thus accumulate fatty acids.

Another non-conventional species of yeast, *Dekkera bruxellensis,* also yields a substantial amount of ethanol. Practical production of a high amount of biofuel was reduced due to the toxicity of the produced products for the yeast strain. But genetic manipulations came as a good resort to address this issue, and *D. bruxellensis* has been reported as one of the yeast strains in terms of product tolerance and production.



Yeast uses various carbon sources to produce ethanol (biofuel) after fermentation.

Conclusion

The production of biofuels by manipulating microbial cellular metabolism can be achieved through synthetic biology approaches combined with computational tools, as has been done in the case of yeast.

Production of biofuels using a synthetic biology approach might also help ensure that the environment is not being exploited for its production. All in all, the carbon footprint is also reduced compared to conventional fuels in use. Cost-saving methods of biofuel production can also do away with the high cost of fuels which is the current scenario. Overall, synthetic biology seems like the true friend who addresses all your issues and has a solution for many everyday problems faced by human beings.

The CRISPR Twins

A Story Of The World's First Genetically Modified Humans

Introduction

Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR is a technology that enables gene editing and is very likely to change the fate of the world.

To sum it up rather crudely, it's a way of specifically targeting a sequence of DNA that can then be altered for various applications. CRISPR is already widely used for scientific research and has the potential to radically transform medicine, allowing us to not only treat but also prevent innumerable diseases.

The Contentious Incident

A Chinese biophysicist, He Jiankuin, attempted to use this technique to make the world's very first CRISPR modified babies.

On 25th November 2018, He stunned the world by announcing that his team successfully created the world's first genome-edited babies, Lulu and Nana (pseudonyms).

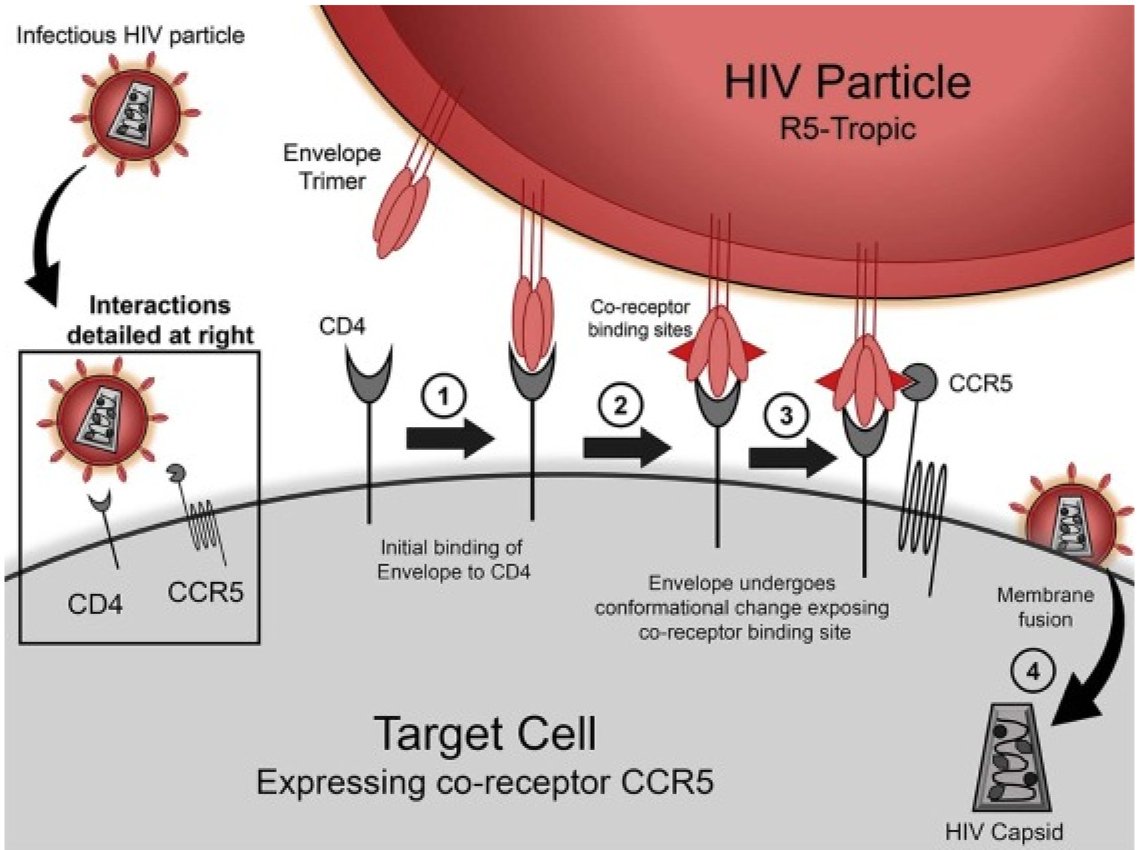
The twins were made from genetically modified embryos that were made HIV (Human Immunodeficiency Virus) resistant. The team recruited eight couples, each consisting of an HIV-positive father and HIV negative mother. During the in-vitro fertilization (fertilization of sperm and egg in laboratory settings, i.e. in a test tube rather than in the organism itself), the sperm was eradicated of HIV before insemination. Hence, the father-to-child transmission was no longer a concern.

The genomes of the embryos were edited using CRISPR/Cas9 by specifically targetting a gene, CCR5. CCR5 gene codes for a receptor that functions as an entry gate for HIV in cells. He was trying to create a specific mutation in the gene (*CCR5 Δ32*) that few people naturally have, which possibly confers innate resistance to HIV-1, as seen in the case of the Berlin Patient.

(Timothy Ray Brown was an American considered to be the first person cured of HIV/AIDS. Brown was called “The Berlin Patient” at the 2008 Conference on Retroviruses and Opportunistic Infections, where his cure was first announced, in order to preserve his anonymity.
)

He said that the girls still carried functional copies of *CCR5* along with disabled *CCR5,* given mosaicism inherent in the present state of the art of germ-line editing.

(Mosaicism is a condition in which cells within the same person have a different genetic makeup. This condition can affect any type of cell, including blood cells, egg, and sperm cells)



The work that He did could not protect the resulting children from the forms of HIV that use a different receptor instead of CCR5. A preimplantation genetic diagnosis process was used on the edited embryos, where three to five single cells were removed, and the editing was checked. He said that parents were offered the choice of using edited or unedited embryos. The couples' children could also pass the protective mutation to future generations. **The prospect of this irreversible genetic change is why the editing of human embryos, eggs, and sperms has been highly controversial.** The core concern is whether such germline editing would cross an ethical red line because it could ultimately alter our species.

He sought couples that had endured the HIV-associated stigma and discrimination and wanted to spare their children that same faith by making them less susceptible to contracting the virus. Although the intent behind this experiment appears to be noble at first glance, this experimentation with the human genome instigated immense controversy in the scientific community.

Scientists and ethicists excoriated He’s medical rationale for the experiment and expressed concerns about the well-being of the girls in the long run. He was accused of flouting government laws and conducting research in pursuance of personal fame and gain.

Even if He’s work did make the twin girls less likely to get HIV, it is possible that he inadvertently increased their susceptibility to other infectious diseases. There is also a possibility that his experimentation made unintended modifications in their genomes, as often happens in gene editing experiments in the laboratory, leaving the twins vulnerable to genetic diseases. Hence, under these regulations, such gene editing experiments on humans were deemed illegal.

Did a permissive scientific culture encourage the 'CRISPR babies' experiment?

To the Editor — The claim last November by He Jiankui to have engineered the first CRISPR-edited babies has ignited voluble condemnation from scientists worldwide. What, then, made this rogue researcher think that his 'achievement' would be welcomed? One possible factor is that He claimed that he was influenced by the words and actions of key US and UK scientists. He attended and was an invited speaker at several meetings about gene editing, including several focused on the ethical acceptability of human germline modification[1]. And he explicitly cited the 2017 US National Academies of Science report as support for what he did[2].

The Nuffield Council on Bioethics in the UK also issued a report in July 2018[3]. Notably, it concludes that germline genome editing could be permissible under certain circumstances. More strongly, it moves beyond the merely permissible to the ethically obligatory, saying in its final section (paragraph 5.2) that "there are moral reasons to continue with the present lines of research and to secure the conditions under which heritable genome editing would be permissible."

He Jiankui, who claimed to have carried out experiments creating CRISPR'd twins who were delivered in China in November. Credit: Anthony Wallace / Contributor / AFP / Getty

Here, we review the Nuffield Council report and show how its shortcomings are part of an increasingly permissive climate among elite scientists that may well have emboldened He. Without a robust and meaningful airing of the perils of human germline modification, these views are likely to encourage additional, more mainstream moves in the same dangerous direction.

The Nuffield findings

Though scantily noted by media in the United States, the Nuffield report was widely covered in the UK, where most accounts noted its permissive posture. Typical were headlines chosen by *The Guardian* ("Genetically modified babies given go ahead by UK ethics body")[4] and *The Telegraph* ("Designer babies on horizon as ethics council gives green light to genetically edited embryos")[5]. At the Nuffield Council's 23 July launch conference for the report last year, several members of the working party expressed surprise and displeasure about these characterizations.

The report's authors are correct in noting that they set out several technical and societal conditions for reproductive genome editing to be considered acceptable. However, the interpretation that dominated the media coverage is fair and accurate. Despite acknowledging the safety questions and social risks inherent in germline editing, the report downplays them. Instead, it emphasizes expansive potential benefits, including future use for, as the Council's Short Guide to the report puts it, "enhancing senses or abilities"[6]. In short, the report implicitly endorses inheritable genome editing for whatever purposes biotechnology companies could choose to commercialize or fertility clinics to market.

The report reasonably rejects assessing heritable genome editing within a medical framework because there is no existing person being treated, and recommends instead evaluating it as a reproductive option for parents. Together with the cheapness and accessibility of the new gene editing techniques, this approach would invite extensive commercialization. The report does insist, as one of two overarching criteria, that the welfare of the future child must be taken into account. But it is vague about what level of risk would be considered acceptable and how such determinations would be made in jurisdictions with no regulatory or oversight body.

Science and ethics

In terms of research methods that might suggest answers to these questions, the report recommends that the UK rely on follow-up studies (paragraph 4.95) rather than clinical trials. Even this more minimal standard, however, could not be met if an adult created as a result of germline editing or the parent of a child so created refused to be part of such a study. At the report launch, in response to a question from the audience, the panel admitted that this was indeed the case, as there is no way to enforce compliance with follow-up studies. Nor, of course, could these subjects or patients, however one construes them, withdraw from the experiment, as their genomes would have already been altered, probably irreversibly.

Neither does the report even once consider the welfare of the mother. Gene editing of course requires in vitro fertilization (IVF), with its attendant burdens, comparatively low success rates, and possible risks. The 'normalization' of

Conclusion

The technique has been earlier used on mice, monkeys, and 300 human embryos by He. The primary risk associated with CRISPR is that it can introduce accidental or off-target mutations. But He claimed that he found few to no unwanted changes in the embryo.

The attempt to create children protected from HIV falls into an ethical grey zone between treatment and enhancement. This procedure does not appear to cure any disease or disorder in the embryo but instead just claims to create a health advantage.

The birth of the first genetically modified humans could have been an incredible achievement for He, but where some see a new form of medicine, others see a slick slope to enhancements, designer babies, and a new form of eugenics.

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CELLULAR REPROGRAMMING

INTRODUCTION

Have you ever wondered how amazing it would be if Jennifer Aniston looked as young as Rachel Green at her "current" age? Or if your mom looked younger as she was when you were born? Or that cancer can be cured? It sounds like fiction but believe it or not, it could become a reality one day — Through cellular reprogramming…..

After conception, during development, a blastocyst forms, and the cells inside the blastocysts are called the inner cell mass. These cells, known as pluripotent cells/embryonic stem cells, divide and shape up into a more familiar form of a living being — absolute poetry in motion.

But can this process go in reverse? Let's find out…

What is cellular reprogramming?

Cellular reprogramming is **the process of reverting mature, specialized cells into induced pluripotent stem cells**, reversal of what happens during embryonic development. This ever-growing field aims to understand how cell fate is acquired, maintained, and inherited in health? What happens when cell identity is hijacked in disease? Harness cell fate engineering for therapeutic applications.

This restoration of the pliability of cells gives us humans control over its fate, which makes it potentially very powerful in terms of answering certain unsolved mysteries of how the body works in an unprecedented way.

It is a diverse and expanding discipline that studies cellular identity reversal or modification.

Timeline of developments:

Much research has gone into the field, which excites many scientists due to the prospective consequences being very far-reaching. So it would be exciting to know about the beginnings of the area and how it grew from there.

THE BEGINNING-

It was widely believed that the DNA in an adult cell was different from that of an embryonic cell, which was even a hypothesis proposed in the 1950s by Briggs and King. However, pioneering work by John Gurdon proved otherwise. He transferred the nuclei of adult frog cells into enucleated eggs. Now, these cells had all the characteristics of an embryonic stem cell, indicating that the adult nucleus had been reprogrammed so that it functioned just like an

embryonic nucleus. Conclusively showing that the genetic material in an embryo was the same as that in an adult cell. This set the ball rolling in the field of cell programming…..

EARLY DEVELOPMENTS-

In 1981: Martin Evans, Matt Kaufman, and Gail Martin discovered that cells from early mouse embryos exposed to the same culture environment can suspend developmental progression and continue to multiply while remaining pluripotent. These were mouse embryonic stem cells.

In 1997: For the first time, Ian Wilmut was the first to use the nuclear transfer of differentiated adult cells to generate a mammalian clone, a Finn Dorset sheep named Dolly, born in 1996 — a milestone in the history of cell reprogramming research.

Contrary to popular belief, Dolly isn't the first-ever cloned mammal. According to the University of Edinburgh's Centre for Regenerative Medicine, that distinction lies with another sheep, cloned from an embryo cell and born in Cambridge, UK, in 1984. Furthermore, Megan and Morag — two other sheep — had earlier been cloned from embryonic cells grown in a lab at Roslin in 1995.

But, what makes Dolly's creation so significant is that she was the first mammal to be cloned from an 'adult cell,' something considered impossible at the time.

LATER DEVELOPMENTS-

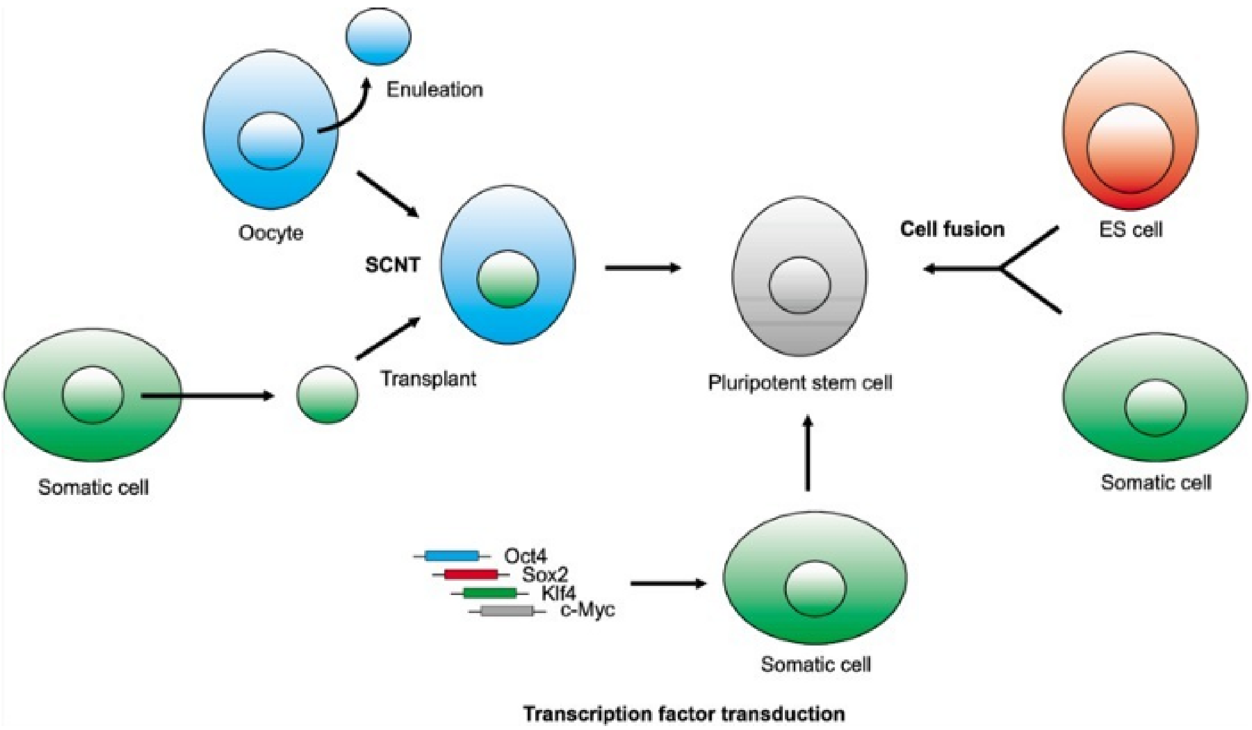
Another question that scientists had was, "we know that we can do this conversion if we take a nucleus and put it into an oocyte but is there a way to start with the adult cell and directly convert it back into a stem cell?"

Dr. Yamanaka showed this in **2006**. He and his team generated induced pluripotent stem cells (iPS cells) from adult mouse fibroblasts. When activated, skin cells from mice could be reprogrammed to immature stem cells, which, in turn, could grow into different types of cells within the body.

In 2012, John B. Gurdon and Shinya Yamanaka discovered that mature, specialized cells could be reprogrammed to become pluripotent.

There are currently three methods for achieving reprogramming-

1. **Somatic cell nuclear transfer** (**SCNT**)- It's a laboratory strategy for generating a viable embryo from a body cell and an egg cell. It consists of taking an enucleated oocyte (egg cell) and implanting a donor nucleus from a somatic (body) cell. When the donor nucleus of a somatic cell is transferred to an enucleated egg, some somatic proteins are also transferred; however, a large volume of egg cytoplasm dilutes somatic factors, allowing the embryonic transcriptional program to dominate and reprogram somatic chromatin such that egg proteins are produced. It is employed in both therapeutic and reproductive cloning. SCNT can develop a normal blastocyst from which embryonic stem cells can be derived for transplantation therapies (therapeutic cloning). In January 2018, a team of scientists in Shanghai declared the successful cloning of two female crab-eating macaques (Zhong Zhong and Hua Hua) from fetal nuclei.

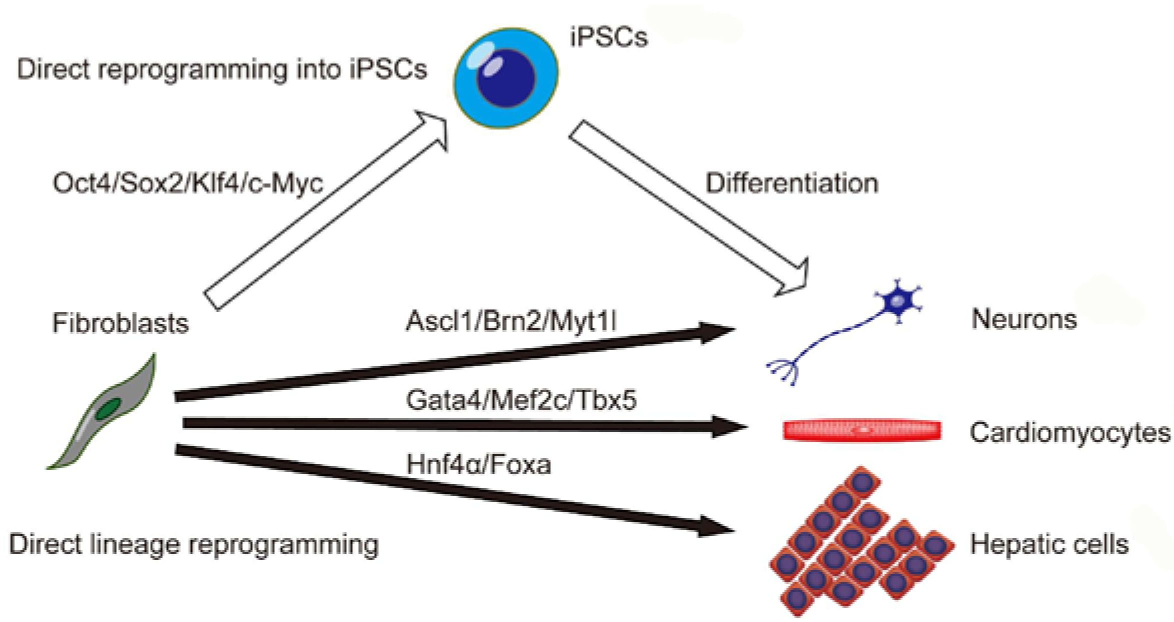


SCNT and Cell fusion — an illustration

1. **Cell fusion-** Fusion of unrelated cell types has been used to investigate cell plasticity; when two cells are fused, a heterokaryon with two separate nuclei is formed. In some cases, nuclei from fused partner cells merge, resulting in a stable hybrid cell. Hybrids generated from the fusion of embryonic stem cells and fibroblasts can behave like embryonic stem cells. In a small percentage of hybrid cells, stem cell-specific genes previously silenced in the somatic cell are reactivated from somatic chromosomes. Reprogrammed cells that display embryonic stem cell-like external appearance, growth, and gene expression have been isolated employing a genetic marker (indicating the reactivation of stem cell-specific genes). Heterokaryons and hybrids have also been generated by combining two somatic cells when hepatocytes are fused with myotubes (a developmental stage of a muscle fiber composed of multinucleate cells formed by fusion

of myoblasts), non-muscle nuclei in the resulting heterokaryons express muscle genes indicating that the muscle phenotype is dominant. Currently, low reprogramming efficiencies and high DNA ploidy of hybrid cells limit the clinical applications of cell fusion.

2. **Direct reprogramming**- It involves the ectopic expression of specific genes that confer a new transcriptional program on a cell. Recently the expression of four factors in fibroblasts led to the generation of stem cell-like cells or induced pluripotent stem cells. After viral integration, endogenous stem cell-specific genes are reactivated; these genes remain active even after viruses are silenced. Follow-up work has shown that iPS cells can be generated using non-integrating viruses that transiently expressed these four factors. In addition, direct reprogramming has also been used to create insulin-producing beta cells from pancreatic exocrine cells; this process is termed lineage reprogramming as a differentiated cell type is generated from another differentiated cell type.



Direct lineage programming — a simple overview

Utilizing this technique for various purposes:

Researchers now have the chance to study disease-specific cell types that earlier were inaccessible by reprogramming the cells from patients carrying disease-associated mutations and subsequent differentiation into the cell type of interest.

Cellular reprogramming: Recent advances in modeling neurological diseases-

The outstanding advancements in cellular reprogramming have made it feasible to examine the etiology of neurological diseases at the cellular level using neuronal populations derived from patients, which harbor the same genetic mutations thought to be connected to the risk for pathology.

Therapeutic implications include-

1. Ability to establish new humanized disease models for understanding mechanisms.
2. Conduct high-throughput screening for novel biogenic compounds to reverse or prevent the disease phenotype.
3. Identify and engineer genetic rescue of causal mutations.
4. Develop patient-specific cellular replacement strategies.

Two examples — involving Alzheimer's and Parkinson's disease are explained below -

Modeling Alzheimer's Disease by Cellular Reprogramming Technologies-

As we've seen earlier, researchers can now take a person's skin cells and reprogram them back into stem cells. Then they use those to grow whatever cells they are interested in researching. In Alzheimer's disease, living human neurons can now be sourced from patients safely and efficiently and then used to model the disease in the lab.

Cellular reprogramming: An innovative approach to modeling Parkinson's disease-

In the case of PD (Parkinson's disease), reprogramming is advancing rapidly. Cell lines have been produced from patients carrying mutations in various disease-associated genes, including SNCA (α-synuclein), PARK2 (parkin), PINK1 (phosphatase and tensin homolog deleted on chromosome 10-induced putative kinase 1), PARK7 (DJ-1), and LRRK2 (leucine-rich repeat kinase 2), also idiopathic cases.

Overall, these cells offer a unique opportunity to study dopaminergic neurons carrying a ‘Parkinsonian genome.’

Beyond modeling diseases, reprogramming can also be used to treat diseases.

Stem cells for retinal diseases- Induced Pluripotent Stem Cell (iPSC)-Derived Retinal Pigment Epithelium(RPE).

The ability to create RPE cells from iPSCs theoretically lessens the risk of immune rejection. Yet, in reality, this and other critical safety and efficacy parameters may depend on the reprogramming technique and the cell type produced. Regardless, iPSC-derived RPE transplantation holds enormous promise and several research efforts with pathways to human use are underway.

Theoretically, this therapy will allow new retinal cells to form from stem cells to replace the damaged cells in the diseased retina.

Also, stem cells can perform multiple functions, like immunoregulation, anti-apoptosis of neurons, and neurotrophin secretion. With recent progress in experimental stem cell applications, phase I/II clinical trials have been approved. These latest stem cell transplantation studies unveiled that this therapy is a promising approach to restoring visual function in eyes with degenerative retinal diseases. The reported improvements in visual function are encouraging and promising.

Nonetheless, it shouldn’t be forgotten that sight-threatening vitreoretinal complications may develop after intravitreal and subretinal applications. More extensive studies with more extended follow-up periods are required to determine the place that this treatment will hold in the future. There are currently many studies in progress regarding the use of stem cells in different retinal diseases; the results are highly anticipated.

Cellular reprogramming and its application in regenerative medicine-

Mice with humanized sickle cell anemia can be cured by transplantation with hematopoietic progenitors generated in vitro from autologous iPS cells following correction of the defective gene by homologous recombination.

Although the concept of technologies mentioned above sounds simple and would make us think that we have found our answers to questions in medicine mankind has been trying to answer for decades, it would be important to note that such technologies have several imperfections and are at very nascent stages of their being. More research and optimization are required to use them in mainstream medicine.

Future prospects

While this technology has been developed and refined, there have also been significant ongoing developments in other complementary technologies like gene editing, progenitor cell production, and tissue engineering.

These are converging to the point that will enable us to treat almost any disease. Personalized medicine may someday become a reality. With cellular harvest and reprogramming, genetic and tissue engineering might become routine procedures. These technologies are the foundations of what is becoming a fully functional field of regenerative medicine.

Cell reprogramming techniques may be used for cancer treatment: A promising therapy converting malignancy to benignity.

Organ regeneration: This can be done from induced pluripotent stem cells.

Reprogramming could also be used as a “**clinical trial in a dish**” to evaluate newly developed drugs’ general efficacy, safety and facilitate precision medicine by testing on human patient cells before they would be tested in animal models or people.

Can we just take a moment here to think how excellent this technique is? Imagine what would happen if we can cure diseases like Parkinson’s and cancers (which have no cure till date) and how great it would be to get personalized treatments and medicines; the possibilities are enormous. Still, it’s just the beginning of a long road.

Cancer Treatment Using Synthetic Biology

Introduction

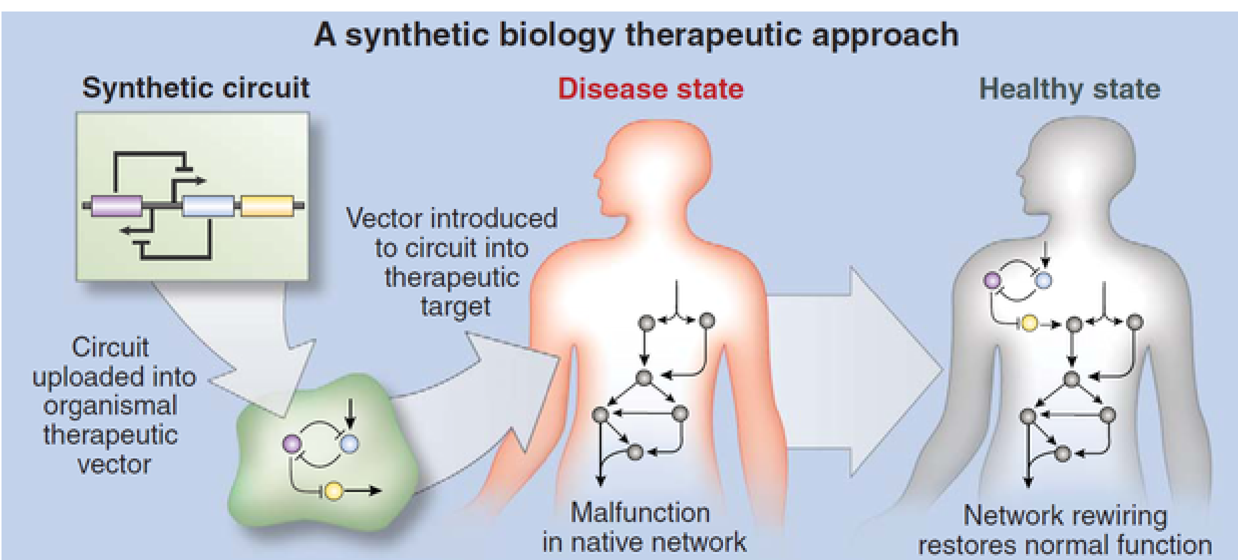
Black death, Spanish flu, Influenza, plague, H1N1, Cholera are a few examples of deadly pandemics we overcame. But fighting the war against cancer has become a Herculean task despite major advancements in treating cancer.

Cancer is the 2nd leading cause of death globally, accounting for about 9.6 million deaths or approximately 1 in 6 deaths, in 2018 only. In 2020 alone, about 10 million people died due to cancer, and about 9 million new cancer patients were diagnosed globally. In India, over a million cancer patients are being diagnosed in a year, and about 7.84 cancer-related deaths occurred in 2018. It is also estimated that about 2.25 million people are living with cancer in India. Chemotherapy, Hormone therapy, Radiotherapy, Immunotherapy, Surgery are some main cancer treatment techniques.

It's no surprise that millions of dollars and a huge amount of manpower is being put into discovering novel (new) ways to cure cancer. This has resulted in the birth of many inter-disciplinary fields of research.

One such inter-disciplinary field is synthetic biology.

Synthetic Biology

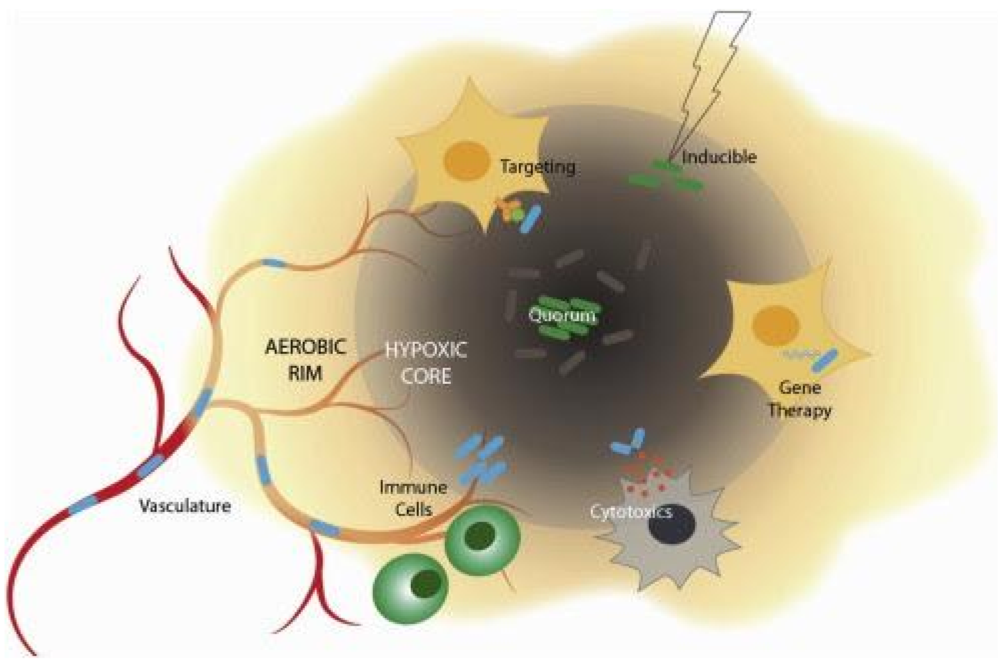


Therapeutic Approach Using Synthetic Biology

Synthetic biology aims to apply engineering principles to biology by modulating the behaviour of living organisms. It mainly involves harnessing the power of nature to solve problems in medicine, agriculture and manufacturing. It is an emerging application of the engineering of bacteria as a cancer therapy by the programming of therapeutic, safety, and specificity features through genetic modification.

Engineering normal cells to kill cancer cells or engineering cancerous cells to kill themselves are new ways to treat cancer.

Role Of Synthetic Biology In Cancer Treatment



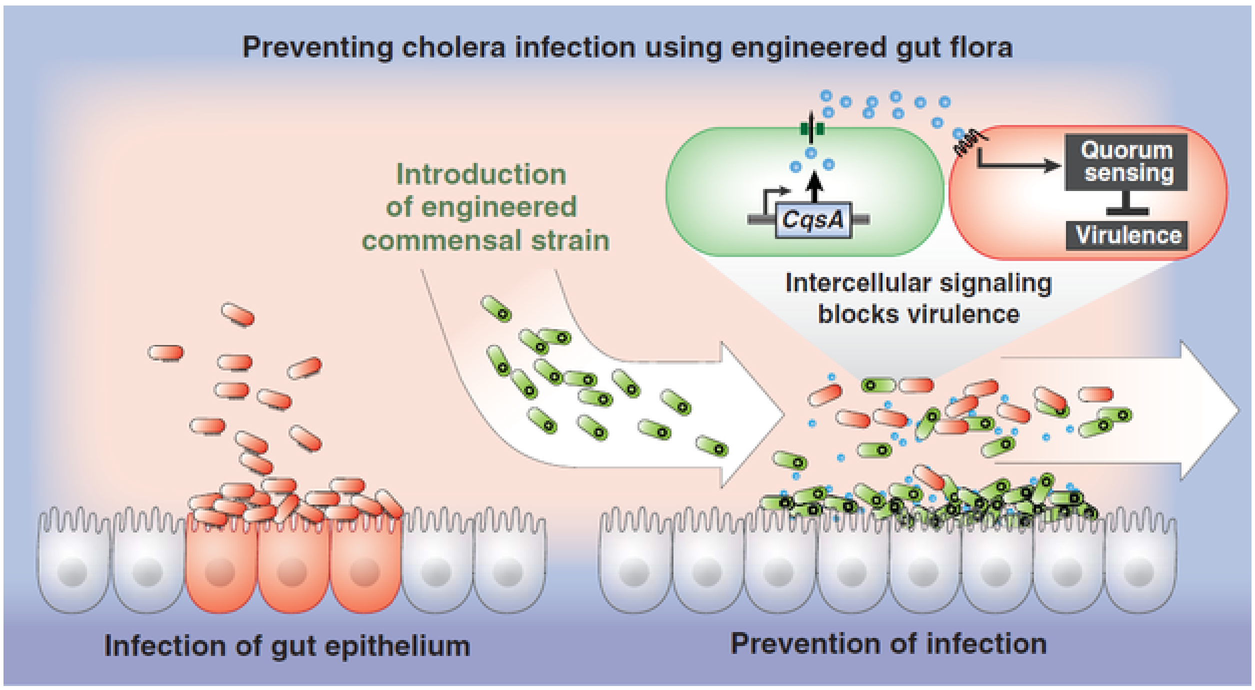
Advances In Bacterial Cancer Therapies

Although the experiments were confined to cells grown in the laboratory, the researchers believe the results could lead to a new type of cancer therapy. Synthetic proteins deliver highly targeted and customizable treatments to sidestep the sometimes devastating side effects of current options.

According to a study by researchers at the Stanford University School of Medicine, synthetic proteins are engineered to recognize overly active biological pathways. Thus sparing the healthy cells and killing only the cancer cells.

A key feature of this approach is that it is intended to be readily adopted by other bioengineering groups. To date, it remains difficult and time-consuming to develop genetic programmes when relying upon trial and error. It is also challenging to implement biological functions beyond relatively simple ones, the team said.

The research team used a "toolkit" of genetic parts invented in the lab and paired these parts with computational tools for simulating many potential genetic programmes before conducting experiments. They found that a wide variety of genetic programmes, each of which carries out a desired and useful function in a human cell, can be constructed such that each programme works as predicted. Not only that, but the designs worked the first time, the researchers noted.



Making the engineered bacteria code for the CsqA gene, cholera infection can be prevented

The signalling of the cells — The signalling pathways are useful to get a response from external factors for a healthy cell as the cancerous cell relies on the receptors that span the cell membrane.

Conclusion

We can conclude that we have found some methods using synthetic biology that mainly works on signalling and receptors for cancer treatment. For any drug to be recognizable, we must first look into the receptor it is going for. By using synthetic biology, we tend to make a synthetic protein that can be used to capture by receptors and hence to help in the treatment.

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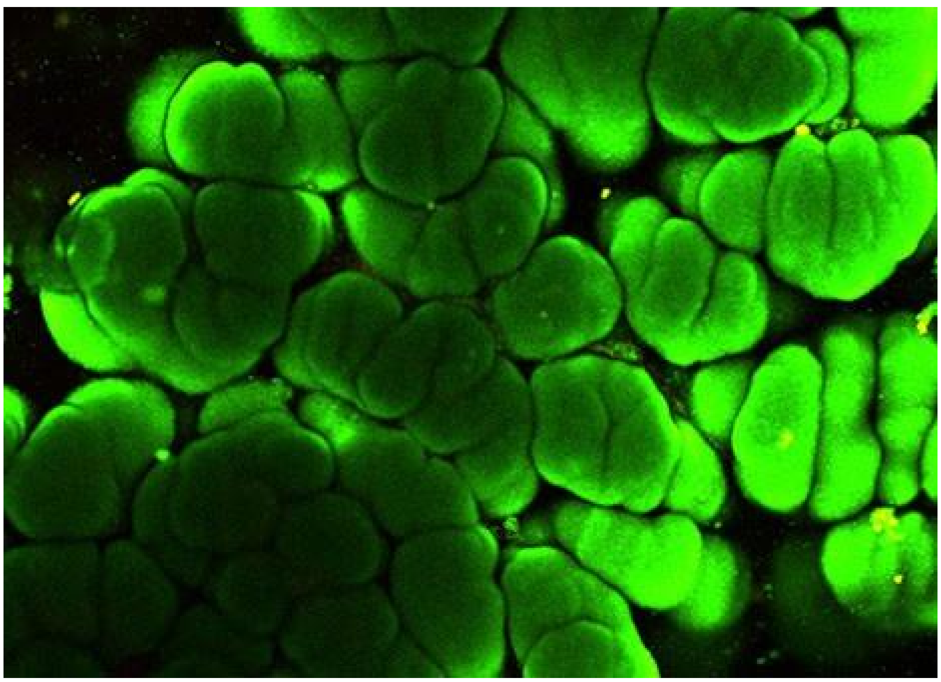
Synthetic biology used to target cancer cells while sparing healthy tissue

Synthetic proteins engineered to recognize overly active biological pathways can kill cancer cells while sparing their…

www.sciencedaily.com

Biofilms: The Microbial Megacities

Introduction



Microbial Biofilms

Biofilms are the assemblage of associated microbial cells enclosed in an extracellular polymeric substance matrix composed of polysaccharides,

proteins, lipids, and nucleic acids that enhance surface adherence and microbial aggregation.

Biofilms form on both biotic and abiotic surfaces. Micro-organisms that form biofilms are bacteria, fungi, and protists. Generally, they are created by the single cell or small groups of cells, and then they divide and differentiate.

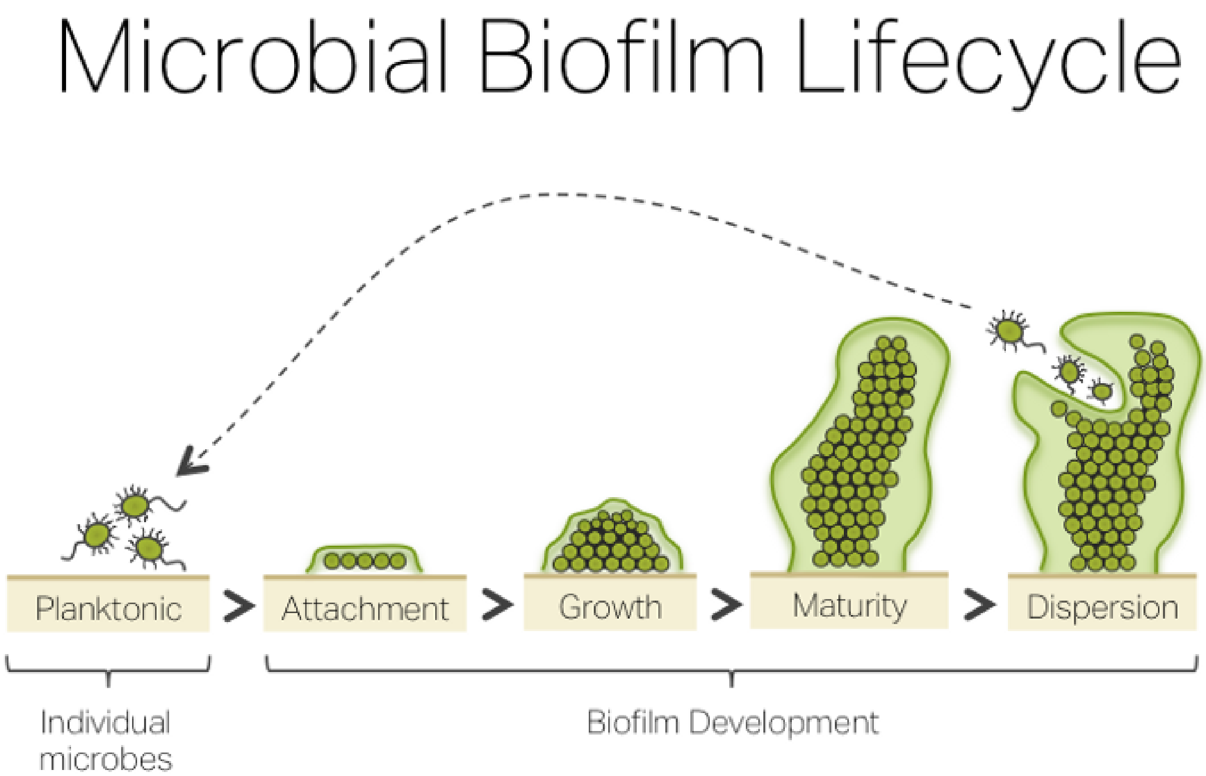
Biofilms account for nearly 80% of all bacteria on the planet, occupying environments spanning from miles underneath the ocean floor to inside the human gastrointestinal tract.

Bacteria within biofilms can undergo significant shifts in gene expression and participate in emergent social behaviours, including division of labour and coordinated growth.

Due to their prevalence in nature and natural emergent properties, biofilms present synthetic biology with the attractive opportunity to deliver and operate engineered gene circuits in a range of desired target environments, such as soil and the microbiome.

The collective organization has been a goal for the field of synthetic biology, and tapping into the native capabilities found in biofilms may enable the next generation of spatiotemporally controlled gene circuit designs.

While domesticated bacterial strains can be used to prototype new synthetic designs in the lab, installing these cells into nature remains a challenge as they experience diverse environmental conditions that can impact cellular fitness. To address this, recent efforts have focused on expanding synthetic biology toward new bacterial species beyond Escherichia coli. The field of synthetic biology has begun to utilize non-model and undomesticated bacterial species by creating new genetic parts and a broad range of genetic transformation methods.



The typical lifecycle of a microbial biofilm

Challenges

Future challenges will include maintaining and containment of these engineered functions in their native contexts, such as soil and the microbiome. Challenges that must be considered for engineering biofilms include extracting microscopic and macroscopic measurements among millions of biofilm cells and contending with bacterial cell fate changes during biofilm community development.

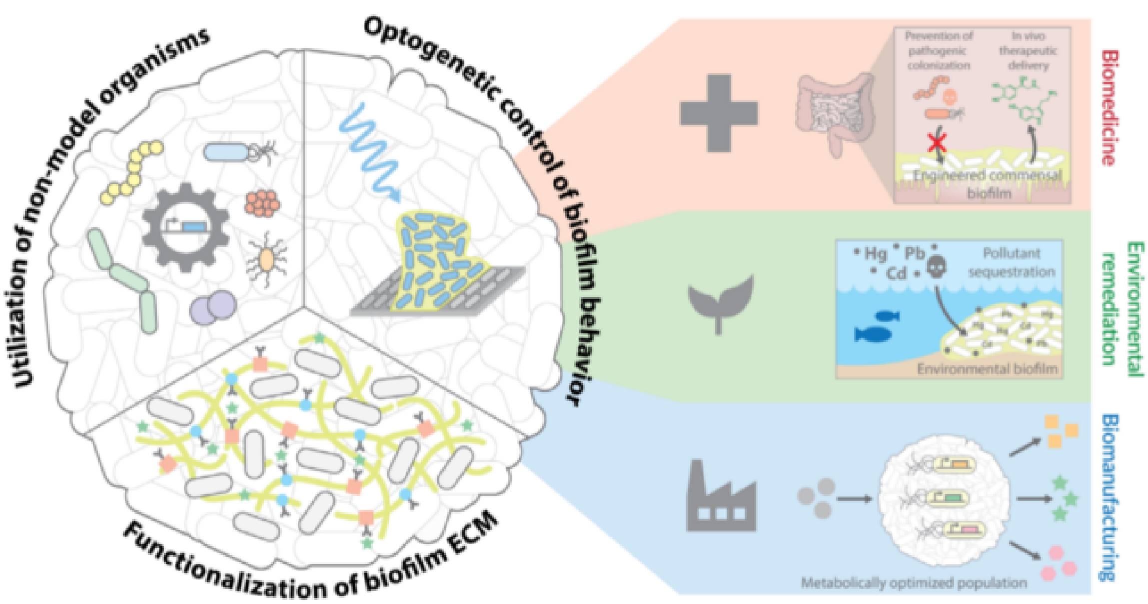
Opportunities

Bacterial biofilms currently provide benefits for wastewater treatment and microbial fuel cells due to their ability to adhere, densely pack and persist in the environment.

With an improved understanding of biofilm biology and creating new synthetic biology tools, biofilms are poised to advance artificial biology efforts in medicine.

Potential applications for synthetic biology in biofilms are:

- **Environmental remediation:** Removal of heavy metals and hazardous compounds by storing them safely in the biofilm matrix.
- **Biofouling prevention:** Seeding surfaces with engineered biofilms to prevent attachment of microbial species.
- **Novel biomaterials:** Biofilm ECM (Extracellular Matrix) with engineered biochemical properties to enable novel biomaterials.
- **Biomanufacturing:** Metabolic burden split across multiple cell populations within a biofilm for increased efficiency.
- **Microbiome diagnostics:** Biofilms as sentinel organisms in the mammalian gut to sense pathogens.
- **Microbiome therapeutics:** Engineered biofilms regulate host-microbiome through therapeutic production and environmental remediation.



Tools and potential applications for synthetic biology in bacterial biofilms

Conclusion and Future Perspectives

Developing biofilms as next-generation synthetic biology holds great promise, yet to realize this vision, several important challenges remain.

Current tools have only begun to address non-model biofilm-forming bacterial species and their complex social behaviours. The heterogeneity and temporal dynamics associated with cell state and species composition in biofilms remain poorly understood.

Furthermore, the environmental persistence of biofilms raises some concern about biocontainment, as biofilms have been associated with chronic infections and biofouling.

Despite these challenges, the opportunity remains to adapt the complex social behaviours of biofilms (e.g., cell-to-cell signalling, division of labour, and matrix production) for medicine, biomanufacturing, and environmental remediation. The physical robustness and environmental persistence of biofilms could enable new living materials and proper deployment of engineered bacteria into target environments. These advances may also prove valuable way beyond synthetic biology, ecology, and medicine.

References:

What Are Biofilms?

(Image credit: Lighthunter | Shutterstock) Biofilms are a collective of one or more types of microorganisms that can…

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https://aiche.onlinelibrary.wiley.com/doi/abs/10.1002/btpr.3123

Biofilm architecture: An emerging synthetic biology target

Synthetic biologists are exploiting biofilms as an effective mechanism for producing various outputs. Metabolic…

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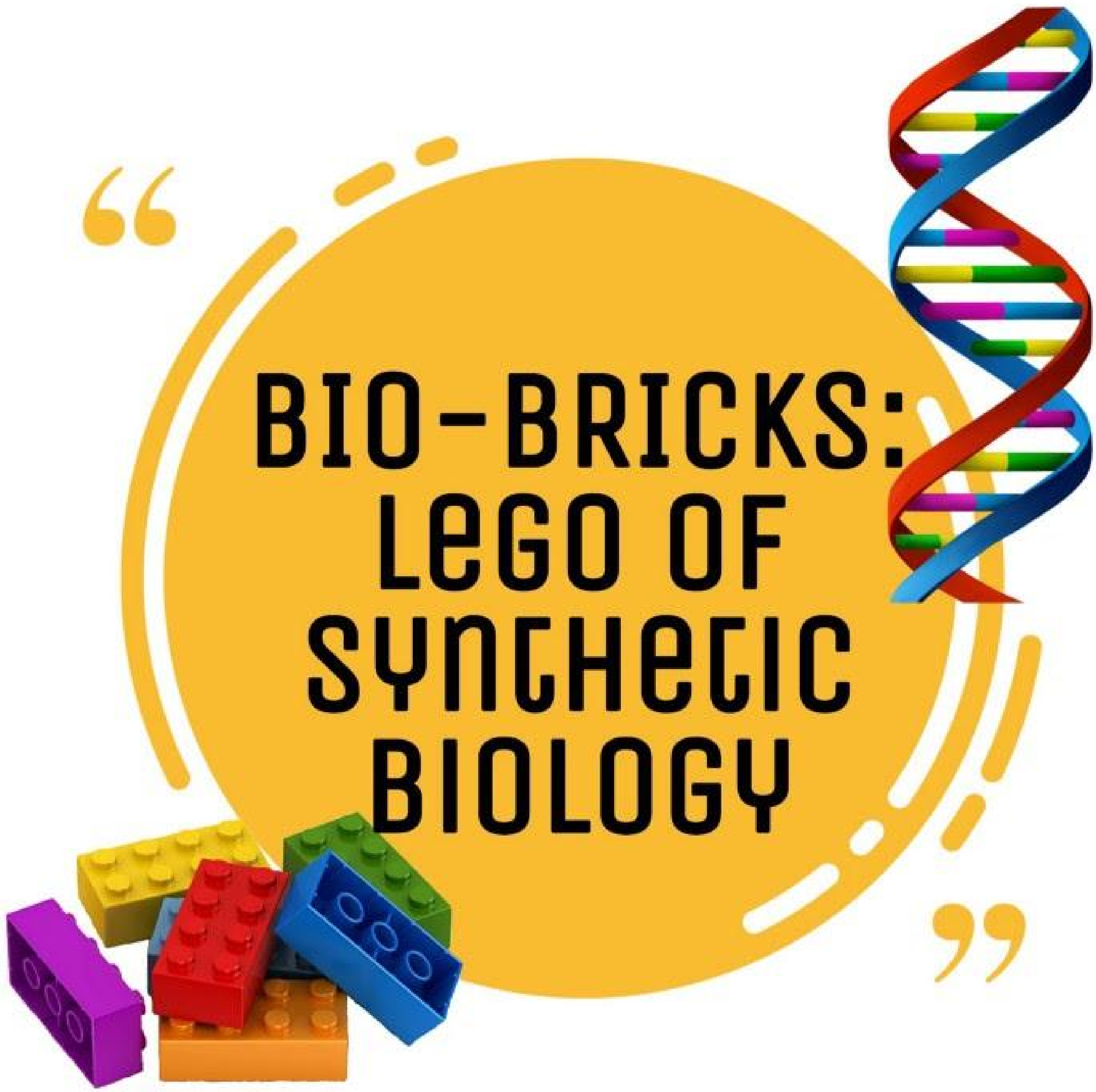
Bio- Bricks: The Lego Of Synthetic Biology

Introduction



Bet every scientist once played lego and built whatever they imagined, be it robots, bridges, buildings or minifigures of their favourite Star Wars characters. But who would have thought that synthetic biology has some similarities with this! Did lego bricks instil something in your hidden child because it did for so many researchers working with synthetic biology.

Now, the lab is a creative workplace with customizable biobricks ready to produce synthetic biology products such as therapeutic drugs, enzymes, pharmaceuticals, food processing systems, biofuels and many more.

LONG LONG AGO….OKAY, NOT VERY LONG THOUGH

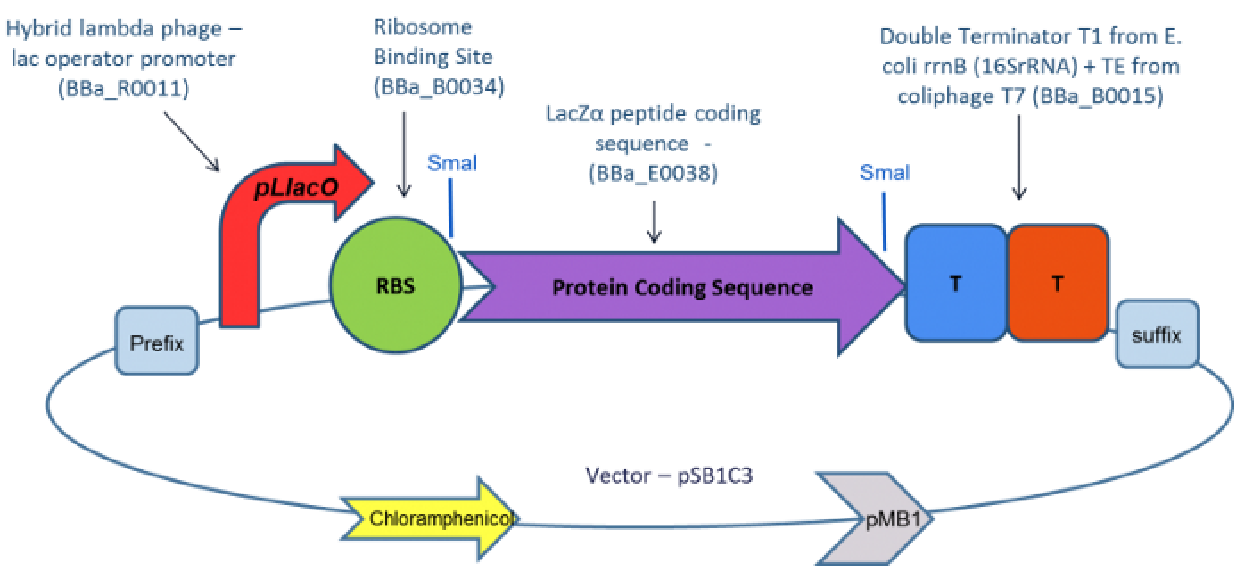
[BioBricks](#) parts were introduced and described by [Tom Knight](#) at [MIT](#) in 2003.

Since then, various research groups have utilized the BioBrick standard parts to engineer novel biological devices and systems from drug delivery systems to synthetic babies (yes, just like the RPG games' character customisation! But, this lies in the grey zone of bioethics.)

What Exactly Are Bio-Bricks?

> Like lego being the building blocks to create different working and static models, the bio-bricks are DNA sequences that can perform various desired biological functions and mechanisms with feedback mechanisms.

A BioBrick, in short, is a man-made elementary DNA sequence that codes for RNA or Protein and can be readily assembled to produce more complex biological systems.



Example of a circuit consisting of bio-bricks

These parts share a common interface and are designed to be composed and incorporated into living cells to construct new biological systems. Now researchers are working towards making new genes or combinations of genes, using the four letters — or nucleotides A's, T's, G's, and C's — that make up DNA and a trial and error method to determine the best working combination for the desired process to be engineered.

BIO BRICKS GOT SOME PRINCIPLES TO FOLLOW

The BioBrick parts are used by applying the engineering principles of **abstraction** and **modularization.**

I know, some fancy words right there, right? Let's make it simpler.

ABSTRACTION is an engineering principle that allows us to ignore unnecessary details and focus only on a particular level of organization. (YES YES, Just like abstract photography!)

In synthetic biology, we use an abstraction hierarchy to help us organize our projects.

First and foremost, since synthetic biology programs encompass living systems using DNA, we must begin by ensuring we have all of the physical DNA required to build our sensor.

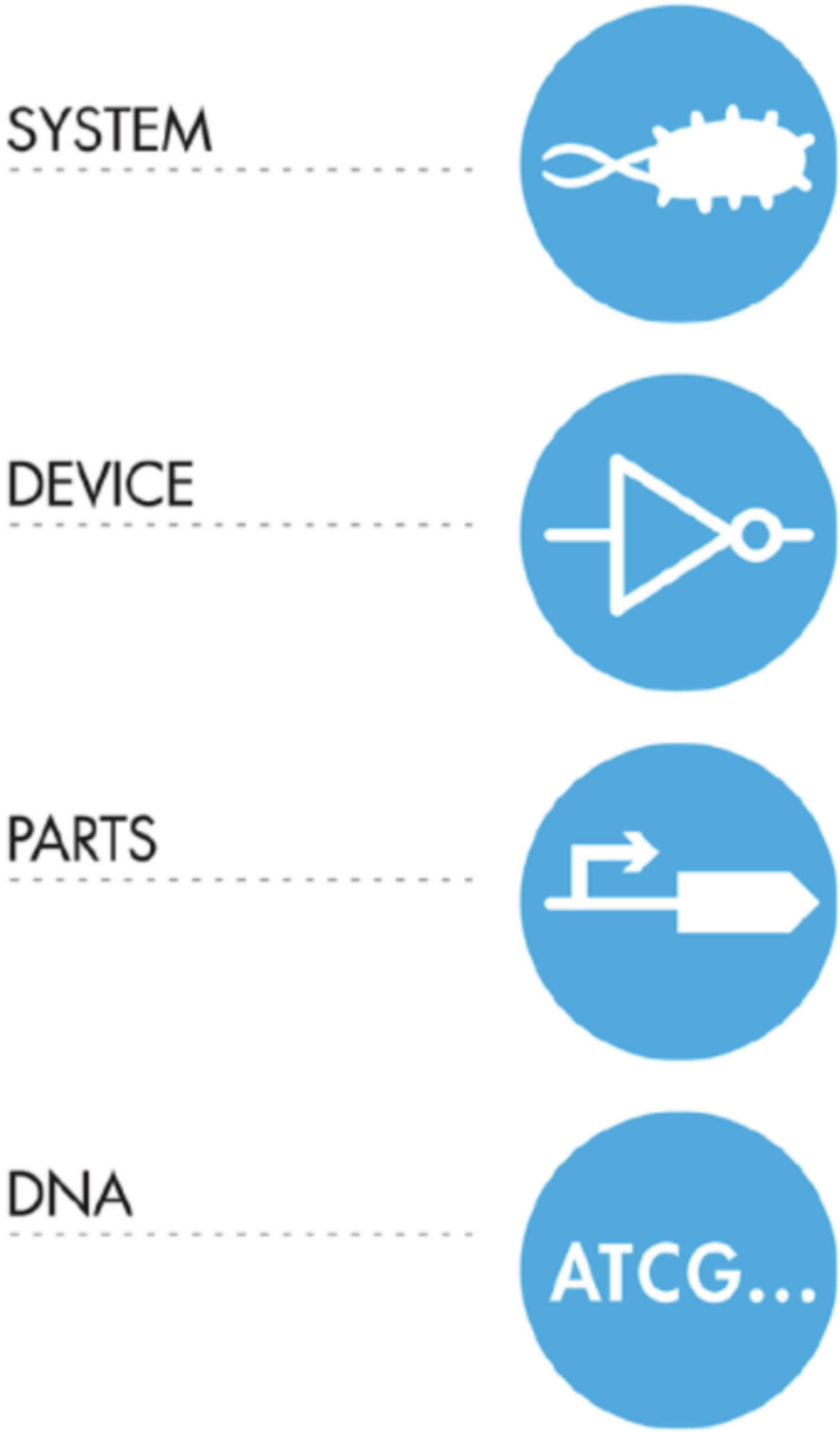
This takes us to the first level of abstraction: ensuring the availability of DNA sequences.

Once we have all of the DNA, we no longer have to deal with individual DNA sequences.

We can give each functional unit of DNA a part name and arrange it in the hierarchy of importance and order, which is the second level of abstraction.

There are three levels to the hierarchy:

1. **Parts:** Pieces of DNA that form a functional unit (example: promoter, Ribosomal binding site.)
2. **Device:** Collection set of parts with a defined function. In simple terms, a set of BioBrick parts put together forms a device.
3. **System:** Combination of a set of devices that performs the desired function.



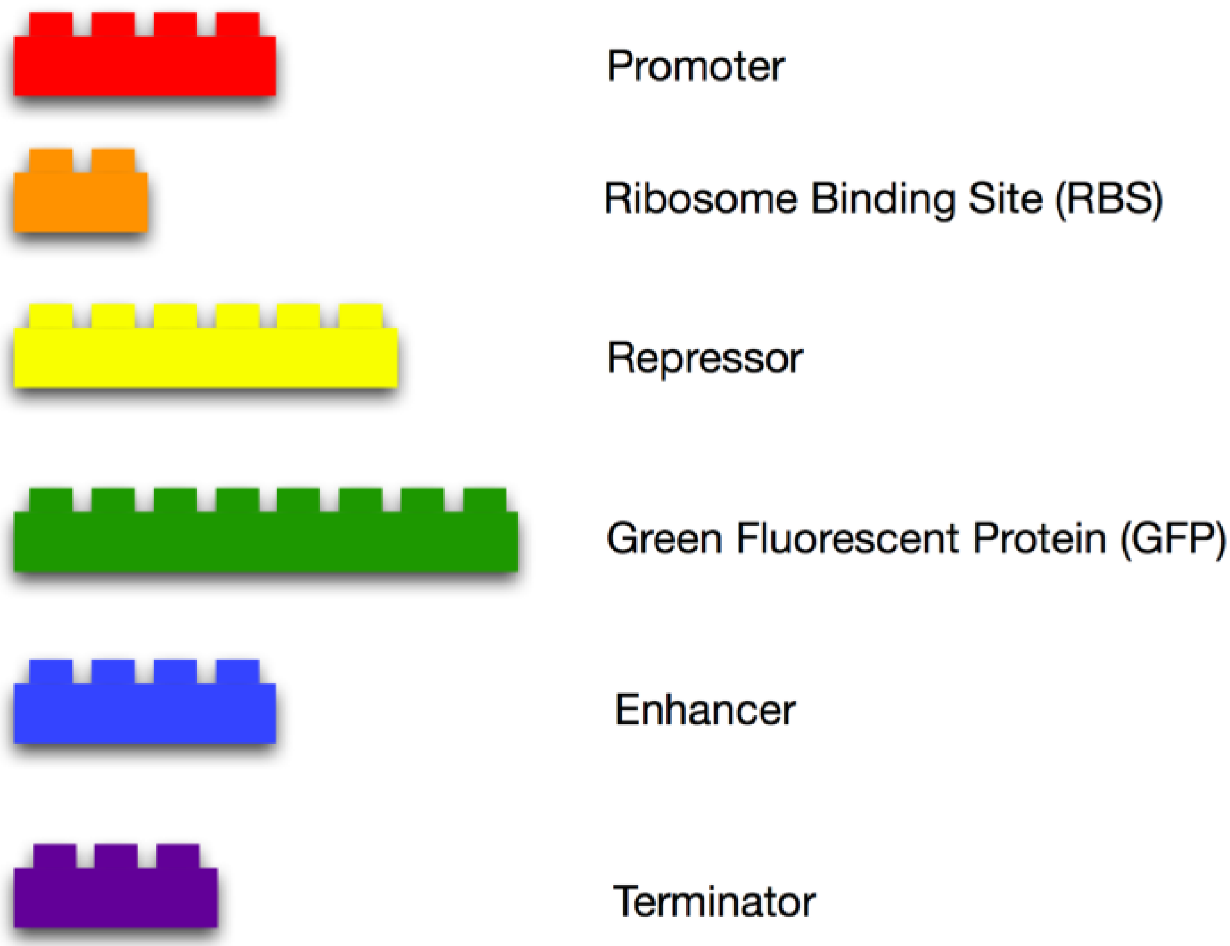
The third level of abstraction by combining parts into devices.

A device is composed of several parts connected in a meaningful way. When dealing with devices, we just have to know their inputs and outputs to know their overall behaviour.

The fourth level is the final compilation for the formation of the system.

MODULARISATION is the degree to which components of a system can be separated and recombined. In industrial design, modularity refers to the technique that allows building larger systems by combining smaller sub-systems. In biological sciences, the term is often used to design “functional blocks” in organisms simply.

Examples Of Bio-Bricks



Commonly used (and some essential) components in a genetic circuit

- **Promoter:** DNA sequence that initiates the transcriptional machinery (binding site of RNA polymerase, which is the enzyme that is responsible for carrying out transcription) and leads to the transcription of the downstream DNA sequence.

- **Ribosome Binding Site (RBS):** RNA sequence found in mRNA(messenger RNA) to which ribosomes can bind and initiate translation.
- **Repressor:** Protein that turns off the expression of one or more genes in a feedback loop. The repressor protein works by binding to the gene's promoter region, thereby preventing messenger RNA production (mRNA).
- **Green Fluorescent Protein (GFP):** Protein that exhibits green fluorescence when exposed to the blue to ultraviolet range. Typically used as a reporter of expression.
- **Enhancer:** Regulatory DNA sequences, when attached with specific proteins called transcription factors, enhancing the transcription of genes.
- **Terminator:** DNA sequence that marks the end of transcription and stops RNA polymerase from going any further.

BIO-BRICKS ASSEMBLY

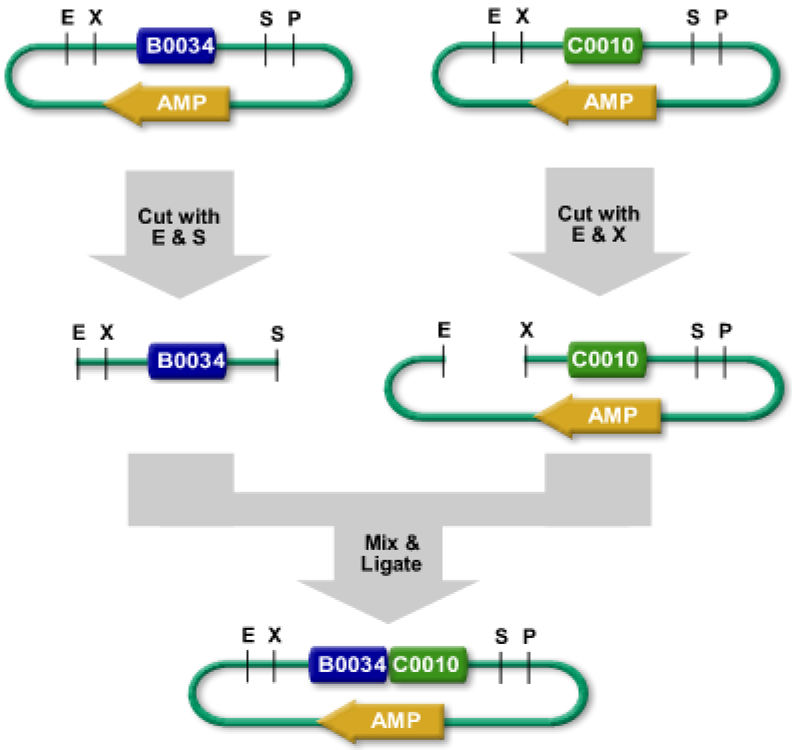
BioBrick parts can be incorporated into a circular plasmid (extrachromosomal self-replicating DNA loop) in which the functional part is flanked by universal and precisely defined upstream and downstream sequences, called prefix and suffix, respectively. It is important to note that the prefixes, suffixes and the plasmid are not BioBricks.

Let's again imagine bio-bricks as legos. Each lego block has standard-sized protrusions on its surface and grooves below, which allows any two blocks to be put together with relative ease. Similarly, each BioBrick is constructed such that two compatible parts (with the same protrusions and grooves) can be readily assembled. This compatibility is governed by the ***assembly standards**.*

The most common type of assembly is -

Standard Assembly

This uses normal cloning techniques based on restriction enzymes (scissors cutting the DNA strand at specific locations), ligation (joining the gene of interest in the sites where restriction enzymes have cut the strand), transformation (genetic material transferred to the desired host) and purification.



E(EcoRI), X(XbaI), S(SpeI) and P(PstI) are restriction enzymes that cut at specific sites. Ligating the so-called 'digested' DNA fragments yields a new genetic circuit.

The Parts Registry

The parts registry is where the exchange of data and material takes place throughout the synthetic biology community.

The registry is open access, whereby anyone can submit a BioBrick part. Most of the BioBrick submission is from students participating in the annual iGEM competition hosted every summer. The Registry allows the exchange of data and materials online, allowing rapid re-use and modifications of parts by the participating community.

Conclusion

We now know the functioning and the mechanism of the basic units in synthetic biology and have a deeper understanding of how synthetic biology can be yielded for producing genetically engineered organisms, devising cancer treatments, enhancing biofuel production, and producing essential and life-saving compounds like human-compatible insulin from microbes.

Bio-bricks opens a very different perspective of synthetic biology- the one of controlling all the components at the very basic level and working out the ideal combination for overcoming various challenges in fields of medicine, pharmacy, agriculture and biological resource management.

References:

Catalog

Catalog List We're in the process of developing new support for the specification of devices in the Registry. For the…

parts.igem.org

Biobricks

In this Timeline article, Collins and colleagues chart the history of synthetic biology since its inception just over a…

www.nature.com

Biobricks

Two main strategies developed on the principle of restriction digestion and ligation includes BioBrick [38] and Golden…

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Weapons Against Diabetes

Understanding methods devised with the help of synthetic biology to fight diabetes

Introduction

Diabetes, a disease that affects 422 million people worldwide, is reported as one of the leading causes of death in the world.

Diabetes is associated with high glucose levels in the blood, leading to severe damage to body tissues and organs over time.

There are two types of diabetes- Type I diabetes (T1D) and Type II diabetes (T2D). T1D features loss of pancreatic insulin-producing beta-cells. In T2D, either the body becomes resistant to insulin or does not produce any.

Synthetic biology helps produce novel genetic circuits (network of genes) that show promising results in controlling diabetes when implanted into mice. Scientists mostly engineer Human Embryonic Kidney 293 (HEK 293) cells to test for the effect of a new genetic circuit. The reason being better expression profiles of the circuit in these cells.

This article will focus on some recent methods developed using synthetic biology to treat diabetes.

Correcting Insulin resistance

Insulin resistance is considered a prediabetic symptom. This disease, primarily caused due to obesity, makes the body insulin resistant.

Insulin resistance for a greater time can lead to type II diabetes.

An insulin sensor device is designed by engineering mammalian cells to attenuate insulin resistance.

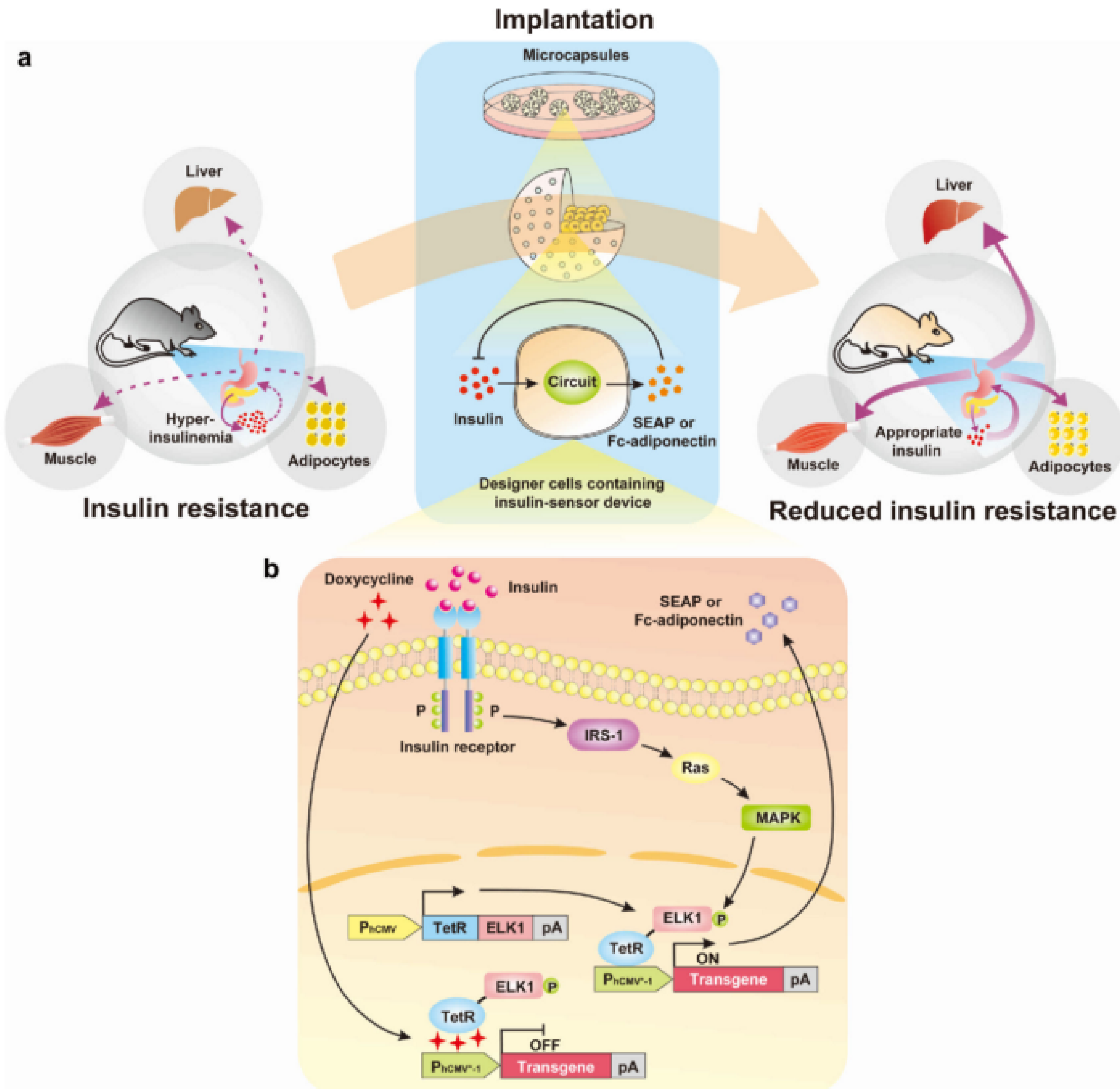
These designer cells express an insulin receptor that can sense the excess insulin in the extracellular (outside of the cell) environment. Sensing insulin leads to the initiation of a signalling pathway that activates a synthetically made transcription factor. This transcription factor binds to a synthetically produced promoter of a transgene (promotes the expression of an incorporated gene). This leads to the expression of a compound named ***adiponectin***.

Adiponectin can reverse obesity-induced insulin resistance. In the experiments, adiponectin also showed to attenuate the high blood glucose levels. However, the long-term high-dose administration of adiponectin can lead to some severe side effects. The amount of adiponectin is controlled by administering an antibiotic-Doxycycline, which stops transgene expression by binding to its promoter.

This synthetically produced device is also modulated by the extracellular insulin levels, and thus it is self-adjusting to produce adiponectin with the varying amount of insulin levels.

This self-adjusting nature also helps to prevent overdosing adiponectin.

These studies saw the engineering of HEK-293 cells, but future therapeutics might see the engineering of other cells to be effective in a broad range of organisms.



Synthetic insulin-sensor device

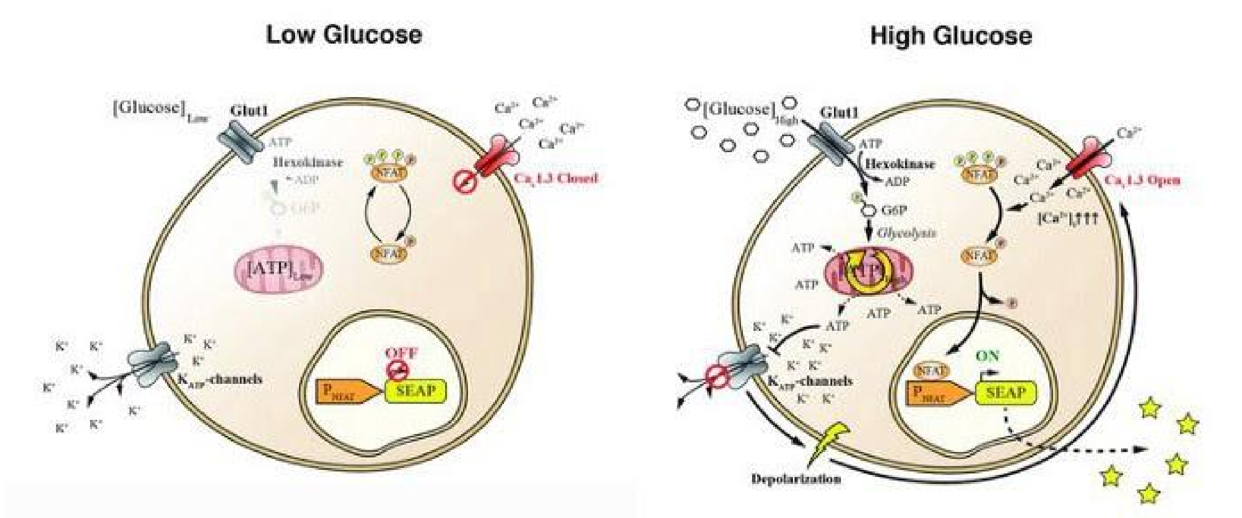
Insulin sensor device- High amounts of insulin outside the cells is sensed by the insulin receptor, which further leads to the expression of transgene producing Adiponectin.

Beta-cell-mimetic Designer Cells

These designer cells are produced by cotransfection of a voltage-gated calcium ion channel and a gene producing insulin or GLP-1 protein in HEK-293 cells.

High extra-cellular glucose levels lead to the activation of calcium(Ca) ion channels on the membrane, resulting in high amounts of Ca ions inside the cells. The high level of Ca ions excites the transcription system leading to the production of insulin or GLP-1 proteins. Ca ions dephosphorylate NFAT(a transcription factor), triggering a series of events that activates the transgene promoter leading to the production of desired proteins. The mechanism of entry of Ca ions on sensing high glucose levels is similar to that used by pancreatic beta cells. The specific ion channel used shows higher sensitivity towards the glucose levels compared to others used in experimentation. Coupling of this specific channel to insulin production by HEK-293 cells engineering results in beta-cell-mimetic HEK-beta cells. The transgene that produces insulin is used for treating T1D, and T2D is cured using the GLP-1 producing transgene.

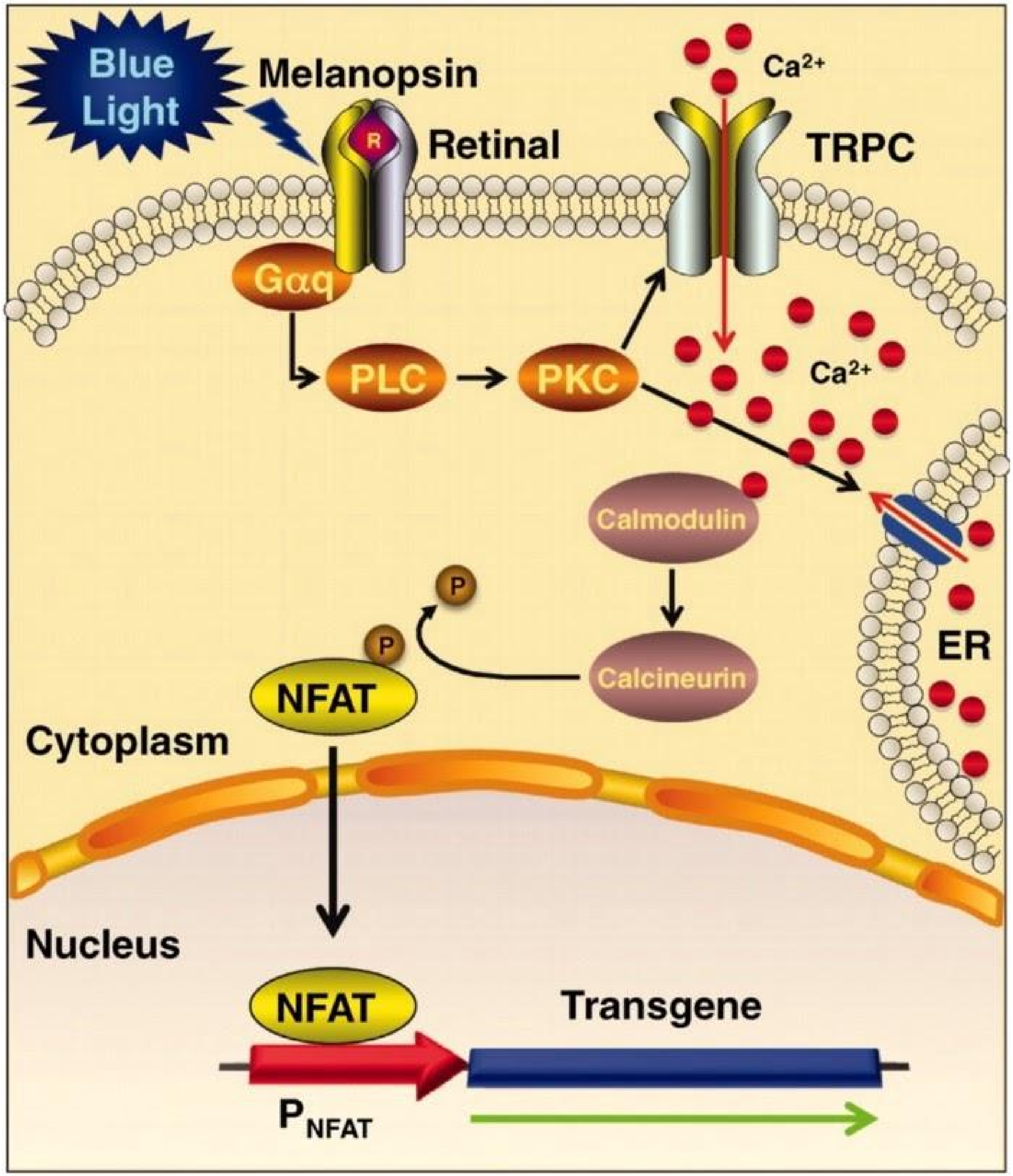
This genetic circuit is a reversible synthetic excitation-transcription coupling system. After the glucose returns to normal, Ca ion mediated activation of transgene stops keeps the body protected from hypoglycemic side effects. The existing therapeutics do not provide this advantage.



The image shows the two conditions- one of low extracellular glucose and the Ca voltage-gated channels close. The 2nd image shows High extracellular glucose which activates the Ca ion channels and subsequently helps to the expression of the transgene.

Optogenetic Transcription Device

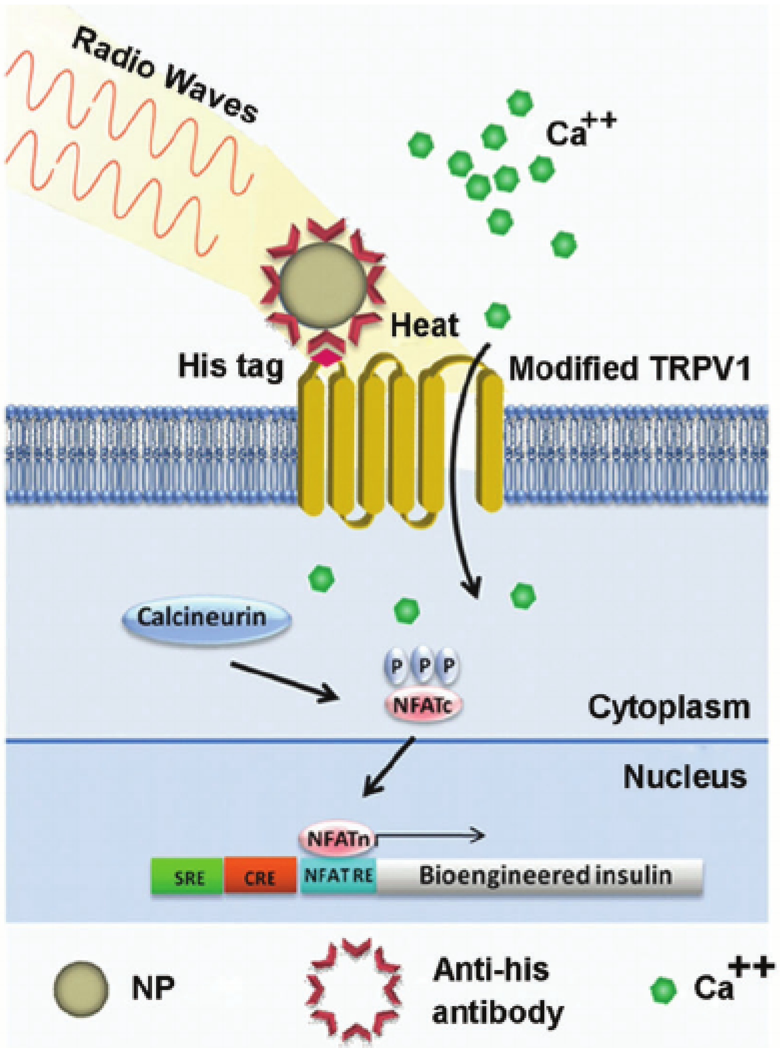
This device uses the light pulse to maintain glucose homeostasis in the blood. The transcription device sees the engineering of HEK-293 cells. Scientists transfect HEK-293 cells by melanopsin-producing and a variant of GLP-1 producing genes to fight T2D. Melanopsin is a photopigment that senses light and leads to the increment of calcium ions in the cell. An increment in Ca leads to a downstream process, resulting from which NFAT moves into the nucleus. NFAT binds to the promoter of the transgene and leads to the production of the GLP-1 variant. This GLP-1 variant is responsible for maintaining blood glucose levels in T2D. As the glucose levels come to normal, the GLP-1 variant shuts down the insulinotropic actions and thus prevents hypoglycemia. Varying the exposure time of cells to light pulse helps us control transgene expression. Scientists irradiate the cells with blue light because it activates the melanopsin to allow the Ca ions entry. Varying the intensity and period of exposure can help preprogram the engineered cells in different ways.



The Blue light pulse activates the melanopsin which further leads to the Ca ion channels which allow more Ca ions to come inside the cells and lead to signalling processes and expression of transgene producing GLP-1 variant.

Radio-Wave heating of Iron Oxide Nanoparticles

This includes engineering HEK-293 cells to express modified temperature-sensitive channel TRPV1 and modified human proinsulin genes. Iron oxide nanoparticles are coated with specific antibodies that will get attached to the modified TRPV1 channel (antibody could not be attached to normal TRPV1 channel). Heating the nanoparticles by radio frequency helps to heat modified TRPV1 and allow the entry of Ca ions inside the cell as a result. These Ca ions lead to the expression of specific transcription factors that activate the transgene and lead to the production of modified proinsulin protein, which can be converted to insulin. Thus, this device activated by heating from Radio Frequency can serve as a potential therapeutic for diabetes. The use of nanoparticles is to provide target specificity and proper heating of the modified channels. Iron oxide nanoparticles heat at a relatively low frequency of 465kHz and thus also helps to minimize surrounding tissue heating.



The nano-particle binds to the channel via the antibody. The nano-particles gets heated by Radiofrequency which in turn increases the channel's temperature and allows entry of Ca ions in the cells. Increment of Ca ions in cells leads to the expression of bio-engineered insulin.

Conclusion

All methods discussed in the article were tested on mice and on engineered HEK-293 cells. Most of them use Ca ions to regulate the activation of the useful transgenes. The efficacy of these methods needs to be tested on the human system, which will help reduce the risk of diabetes in the human body.

All in all, these synthetic circuits show a great potential to produce effective therapeutics not just against diabetes but also against many other diseases.

For further reading, check out team IISER Bhopal's 2020 iGEM project on creating modified beta cells producing insulin in the gut.

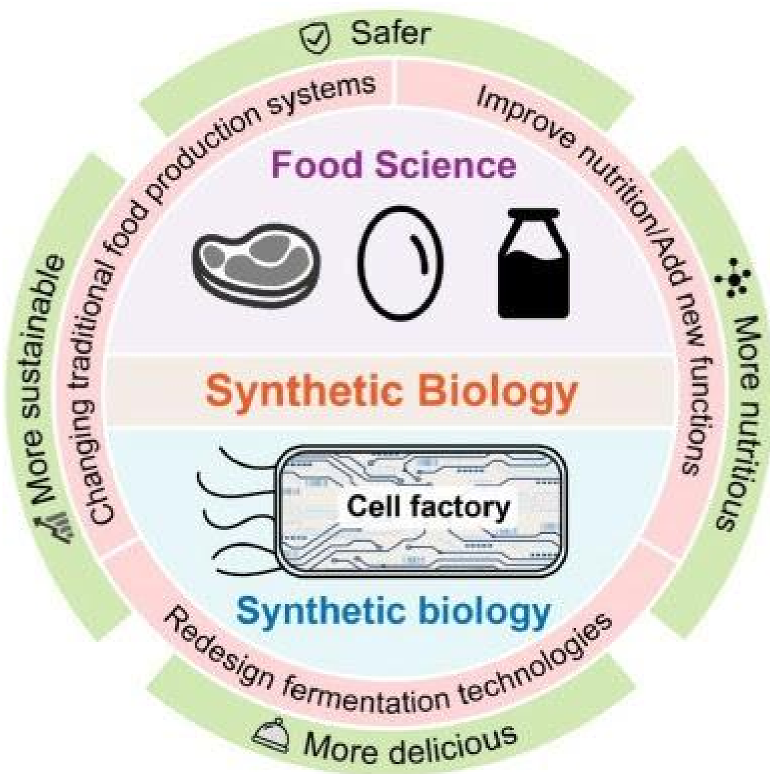
Making Food Out of Thin Air

Exploring an innovative method to get rid of excess atmospheric carbon dioxide

Introduction

With the global population about to explode to 10 billion people by 2050, the reality is that our current food system will not last and will not be able to meet the needs of our future global population.

The world's food systems are under greater strain. Population growth coupled with escalating demand for meat and dairy, which accounts for more than 50% of food-related greenhouse gases, and 80% of all agricultural land use, means that there is an eminent need to find more sustainable sources of food. Also, due to the excess carbon emissions, climate change has greatly influenced agriculture and dairy industries.



Food Science and Synthetic Biology

With the help of synthetic biology, we can now convert Carbon dioxide (CO_2) into delicious, life-sustaining nutrition.

The Technology

The technology behind it comes from an idea first explored by NASA in the 1960s when scientists while trying to figure out how to feed astronauts in space, discovered that it was possible to use microbes to convert CO_2 produced by astronauts — into food.

The idea is to feed atmospheric CO_2 to microbes in fermentation tanks to get a final product- a protein-rich flour that can be used just like soy or pea flour.

This protein flour can then be made into a plethora of delicious and nutritious, seemingly meat-like products.

This process is possible because of microbes called hydrogenotrophs that metabolize hydrogen and carbon dioxide for energy. These microbes convert carbon dioxide to methane using energy derived from hydrogen (H_2) molecules.

About The Microbes

These microbes are strictly anaerobic, meaning they can't survive in the presence of oxygen. They are cultivated in fermentation tanks.

Fermentation tanks used for the process

Electricity from renewable sources is used to perform electrolysis, the process of using electricity to break apart water molecules into oxygen and hydrogen gas.

H_2 is then supplied to the microbes, which the microbes use to generate energy for growth while consuming CO_2 captured from the atmosphere.

As a result, once they've proliferated, they can be dehydrated and processed into a flour-like product that is rich in protein and carbohydrates.

The Plus Points

- Unlike soy or other plant protein, it's a "complete protein," with the same amino acid profile as protein in beef or chicken.
- It also has vitamins such as B12 that aren't typically found in vegan food.
- Unlike some animal protein, it doesn't have any antibiotics and hormones.
- The process runs on renewable energy.
- It is made without the need for traditional land, water and weather requirements, making it a neat solution to the environmental crisis.
- It can be made in days instead of months, thus reducing the strain on the food supply chain.
- It can be used as a useful alternative to meat products, thus increasing its usefulness manifold times.

Meat production isn't environmentally sustainable as producing a single quarter-pound (around 113 grams) grain-fed beef burger consumes around 1,700 litres of water and results in about 3 kilograms of greenhouse gas emissions. The environmental cost of meat production is disproportionate to its output, and livestock farming occupies roughly 80% of agricultural land, which contributes to significant deforestation, but accounts for only 18% of calories consumed worldwide.

Conclusion

Environmental friendly alternatives are the need of the hour. The benefit is the ability to withstand worsening climate conditions and remain resource-efficient. Additionally, hydrogenotrophic organisms are easily found in nature, and therefore they can be easily acquired. Since the whole process takes place in an enclosed tank unaffected by environmental conditions like weather or soil quality, the protein-yielding microbes can be cultivated practically anywhere at any time. This provides a potential food source in deserts and other areas where agriculture is not well supported. Now that making food out of air no longer sounds like crazy sorcery, you may be thinking, wow, that's neat, but will I really be spooning powdered carbon dioxide-eating microbes into my mouth. It's most likely that the protein flour would be incorporated into protein-enriched foods and beverages like pasta, bread, and shakes or into more sustainable feed for livestock and other animals. Moreover, we can also combine it with existing alternative-protein products, like plant-based burgers and yoghurts that depend on plant protein isolates, to further cut down their environmental costs of production as they expand to meet growing consumer demand.

Air-based Protein

At last, the biggest advantage about these is that you're killing two birds with one stone — capturing carbon waste while generating food products and removing the strain from agricultural markets. These will play a big role in improving our environment.

References

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Synthetic Phyto-antimicrobials

Curbing antibiotic resistance using synthetic biology

Introduction

Antibiotic resistance is one of the most challenging global health threats in our society. Phyto-antimicrobials are prospective alternatives/adjuvants to antibiotics for combating Anti-Microbial Resistance (AMR). Synthetic biology can be used effectively for the production of the desired phyto-antimicrobials. The AMR crisis versus slower discovery of new antibiotics has highlighted a daunting task to control these drug-resistant superbugs. Several phyto-antimicrobials have been identified and tested in recent years with direct-killing and/or drug-resistance reversal potencies. Synthetically produced phyto-antimicrobials may hold the key to combat AMR due to their ability to target major microbial drug-resistance mechanisms, some of which include cell membrane, drug-efflux pumps, cell communication, and biofilms.

Plant tissue culture used to create synthetic phyto-antimicrobials.

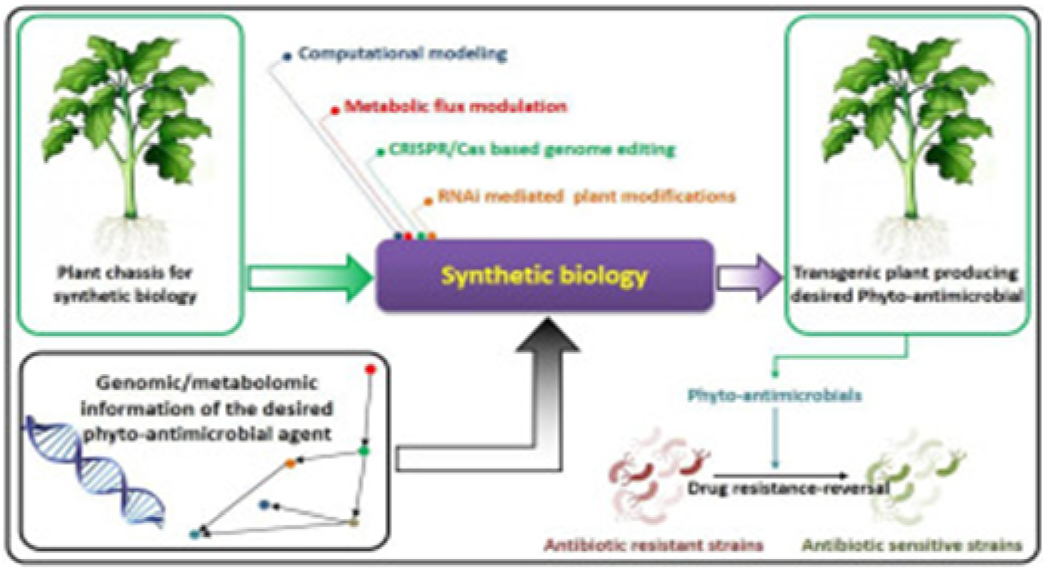
What are Phyto-antimicrobials?

Antimicrobials sourced from plants are known as phyto-antimicrobials. They can also be produced synthetically. Phyto-antimicrobials can be regarded as an alternative source of antibiotics.

Production By Using Synthetic Biology

Synthetic biology strategies integrate several biological methods such as metabolic engineering, RNA-interference, genome editing/engineering and/or systems by using a particular chassis, here a plant chassis, which is used as an engineerable platform.

With the help of advancing SynBio toolkit, successful attempts have been made towards introducing an entire gene cluster, reconstituting the metabolic pathway, or transferring a synthetic pathway into heterologous plant systems, thus highlighting the sheer potential of this field.



[Using Synthetic Biology To Fight AMR]

Importance Of Plant-derived Anti-microbials

Although [synthetic antimicrobial] agents have been already approved in many countries, the usage of natural compounds derived from microbes, animals, or plants attracts the attention of many researchers and since they have the potential to be modified according to purposes with the help of Syn-bio techniques. These compounds have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens. Among all available options, synthetically modified plant-derived antimicrobials have displayed the most potential for combating bacterial infections. They can restore the clinical application of older antimicrobials by increasing their potency and thus avoiding resistance development.

Conclusion

There is ample evidence suggesting that medicinal plants can be effectively used for treating infectious diseases. Plants hold great promise as sources of novel antimicrobial agents that can be reframed using Syn-bio techniques. They are readily available, cheap and also have minimal side effects. Plant-derived compounds like phyto-antimicrobials, especially synthetically modified ones, have been found to counter several infectious diseases and various human pathogens. Some of these show both intrinsic antibacterial activity and antibiotic resistance modifying activities. While not effective as antibiotics, some of them can overcome AMR in bacteria when combined with antibiotics.

Future Perspective

The spread of antibiotic-resistant microorganisms has been a big threat to devising a successful therapy for microbial infections. So far, there is an urgent need to develop a new strategy to combat antibiotic resistance. Synthetically produced phyto-antimicrobials are characterized by diverse chemical structures and mechanisms of action. These provide attractive therapeutic tools for discovering bioactive products in the coming years. However, continued research should be conducted to better understand the exact mechanisms and the pharmacodynamic and pharmacokinetic properties of phyto- antimicrobials, thus forming the base foundation for developing novel phyto-antimicrobials using synthetic biology techniques.

DNA- the solution to our data storage problem?

Introduction

DNA as data storage

We are voracious consumers of digital data. Our generation has generated and consumed more data in the last few years than the whole of human history put together. But as the adage goes, with great data comes a greater storage problem. IBM's 1956 hard drive was the size of a goods truck, and all that it could store was equivalent to the size of this article! Today's handheld hard drives of the order of TBs are a far leap from those times, but these won't suffice for the soon to come future.

As engineers always do, those confronted with this problem looked for inspiration in mother nature. Nature has an elegant solution to data storage that is hitherto unsurpassed- DNA. For the uninitiated, DNA is a molecule that serves as an instruction manual to build organisms, like ourselves, from scratch. It is the hereditary material in all living organisms and is passed on from generation to generation.

Similar to how computers use the binary system of 0s and 1s to store data, DNA encodes data in the quaternary system of four alphabets A, T, G, and C. Human DNA has 3.1467 billion of these letters running into GBs of information, which is stored in a tiny microscopic piece of real estate in our cells called the nucleus. DNA is extremely low maintenance. It being a robust molecule, is not easily prone to environmental degradation.

It is estimated that all the data we have today if stored as DNA, can comfortably fit in the dicky of your car! In addition, it requires much less energy and infrastructure to preserve DNA, making it environmentally sustainable.

If you are like me, you must be thinking at this point — all these facts are great, but how do you actually use DNA for data storage!?

Getting To The Brass Tacks Of DNA Data Encoding

Encoding digital (binary) data into the quaternary language of DNA is posed with its unique problems. For starters, de-novo synthesis of long fragments of DNA is difficult and time-consuming, not to mention exorbitantly expensive. Companies like CodexDNA are at the forefront of developing this technology.

Sequencing technologies in vogue are rattled with homopolymeric stretches, i.e., the same base repeated multiple times. For example, in the sequence TAGAAAAAAT, the multitude of As in the middle will be difficult to accurately sequence. In addition, any form of repeats like the TTAC unit in the sequence GTTACGTTACGTTACGTTAC will pose a challenge to the assembly software. An assembly software is one that takes in raw data from the sequencer and gives out a contiguous sequence as its output. Sequencing and assembly together let us retrieve the data stored in DNA.

While there have been continuous and substantial efforts to optimise this process since the late eighties, let us look at two important recent developments.

George Church of Harvard, in his famous 2012 experiment to encode his book into DNA, used a rather simplistic method. A ‘0’ was randomly assigned to either an A or a T, and a ‘1’ was similarly assigned to either a G or a C. Homopolymers that we talked about before were limited to a maximum size of three. These little homopolymers were still enough to cause some menace while retrieving data.

European Bioinformatics Institute researcher Nicholas (Nick) Goldman came up with a novel idea to solve the problem of repeats. Binary data {0,1} was first converted into a base-3 system {0, 1, 2} where bits become tirts. The first number was assigned a C if ‘0’, a G if ‘1’ and a T if ‘2’. The next number was encoded according to the table shown. (Goldman N., 2013)

Previous nucleotide written	Next trit to encode		
	0	1	2
A	C	G	T
C	G	T	A
G	T	A	C
T	A	C	G

Goldman’s scheme for data encoding

Perhaps an example will help elucidate the process. Let us take the same one-letter word ‘a’. Its binary representation is ‘01100001’

Binary — 01100001

Ternary — 010121

DNA base encoding -

First digit: The digit is a 0, so C

Second digit: The previous is C, and the next is a 1, so T

Third digit: The previous is T, and the next is a 0, so A

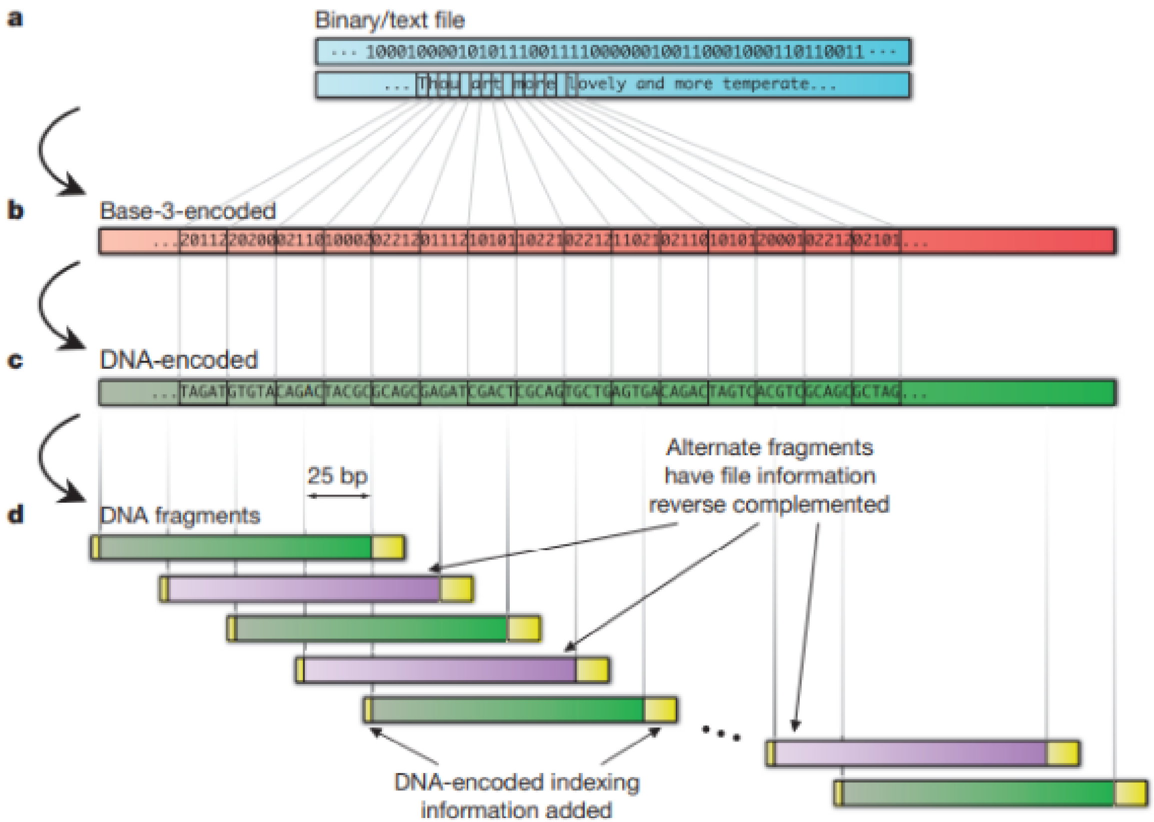
Fourth digit: The previous is A, and the next is a 1, so G

Fifth digit: The previous is G, and the next is a 2, so C

Sixth digit: The previous is C, and the next is a 1, so T

The sequence is thus encoded as CTAGCT

As there are 3 numbers and 4 bases to represent them, homopolymers are out of the question. In addition to this ingenious technique, the data was distributed into DNA fragments of 117 nucleotide length each, with the beginning and end parts designed for indexing. The fragments overlapped each other for about ¾ th of their lengths to prevent assembly errors to the greatest extent possible. A look at this illustrative figure from the paper will instantly help you visualise the idea.



(Goldman N., 2013)

Goldman’s group decided to encode Martin Luther King’s famous speech, a sonnet of Shakespeare’s, a photograph of their institute, and the classic Watson-Crick paper into their DNA hard drive. As irony would have it, all but one of their files could be recovered flawlessly- the Watson-Crick DNA structure paper sticking out like a sore thumb! It turned out that a self-complementary region in the responsible DNA fragment was the culprit.

Goldman's paper came out in 2013. Since then, we have seen this technology growing by leaps and bounds. A relatively recent work by Dina Zielinski in 2017 shows the progress of DNA storage work over the years and builds on it. Those interested can read about her method published in Science, termed DNA fountain, by using this link.

In a presentation about his work, Dr Goldman handed a microfuge tube containing DNA to attendees. A bitcoin was encoded into the sequence and the first one to retrieve the data from this would have the bitcoin for him/her to keep! If you are gasping with your mouth wide open, this was way back in 2015 when a bitcoin was valued at about Rs 20,000 comparable to the sequencing costs of the time. Not quite the prize-worthy price it is today!

Conclusion

Scientists are yet to explore the possibility of encoding data in living organisms which would if successful, greatly simplify the process of maintaining the integrity of the data. Other than the ethical considerations, one hurdle is that organisms, like bacteria, will only keep the encoded DNA in their genomic DNA if it gives them an evolutionary advantage. Scientists of the future will have to look for ways to fool these bacteria into thinking that keeping DNA-encoded versions of our family photographs is the one-stop solution to all their life problems! Maybe we can take business tips from those selling ‘astro-suraksha-kavach’ on Tele-ads!

DNA is the data storage technology of the future. Future hard drives might look like pen drives with integrated cell cultures and a mini DNA sequencer-synthesiser built-in! All the digital data you can ever produce or consume in your life can be stored in this DNA drive. The day is not far when we upload our files to the ‘cell’ instead of the ‘cloud’.

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Synbio Meets Conservation

Synthetic biology, or synbio, employs the latest and most advanced gene-editing tools, such as the “cut-and-paste” technology known as CRISPR-Cas9. Combined with new techniques to digitise and automate the design and modelling of various genetic elements, scientists can now engineer organisms to produce novel food ingredients or to rewire the switches that express genes that control certain functions. In terms of conservation, synbio could potentially address several areas of concern, such as curbing invasive species, reducing pressures from wildlife trade, improving resistance to disease, and even bringing a species back from the brink of extinction

https://cdn.britannica.com/q:60/71/174271-050-B90CC219/Siberian-tiger-Longleat-Safari-Adventure-Park-England.jpg

Introduction

Synthetic biology refers to technologies that allow humans to make precise alterations to the genes of organisms. Synthetic biology applications have important positive and negative implications for biodiversity conservation depending on how they are designed and targeted.

Potential benefits range from protecting threatened species to providing synthetic alternatives to wildlife products. Potential detrimental effects include changes to ecological roles played by target organisms and negative impacts on the livelihoods of indigenous and local communities that largely depend on biodiversity.

The use of synthetic biology needs to be informed by case-by-case assessments guided by empirical evidence and incorporating traditional knowledge and ethical values in decision-making.

What Are Endangered And Threatened Species?

Endangered And Threatened Species

Under the Endangered Species Act, a species is considered “endangered” if it is in danger of extinction throughout all or a significant portion of its range. A species is considered “threatened” if it is likely to become endangered in the foreseeable future.

Impact Of Synbio On Conservation

Depending on how they are designed and targeted, certain synthetic biology applications have the potential to enhance or disrupt biodiversity conservation, acting through both direct and indirect pathways.

Synthetic Biology At CSIRO

Synthetic biology is an emerging field of research that combines genetics, chemistry and engineering. Scientists working in synthetic biology design, build, and test DNA to enable plants, animals and other organisms (e.g. bacteria, fungi, algae) to function in desired ways. These organisms could then be used to help in the management of environmental and societal problems such as pollution, waste, land degradation and biodiversity loss.

The CSIRO Synthetic Biology Future Science Platform has developed a range of synthetic biology techniques, such as genetic engineering, gene editing and gene marking.

Conclusion

Synthetic biology is an emerging discipline that can be viewed as the endpoint or the prescriptive, intentional phase of biology.

As such, it raises many of the same ethical questions as traditional genetic engineering. Because synthetic biology is so new, it isn't easy to accurately assess its ramifications. If it lives up to at least some of the applications scientists currently believe are possible, synthetic biology could be of considerable benefit and provide solutions to some of humanity’s most pressing problems. It is worth thinking about the ethical questions that synthetic biology raises at this early stage in its development and consider strategies that may prevent misuse.

Synbio Meets Wildlife Conservation

Imagine a place where endangered species like the Asiatic lions and Bengal tigers run freely in the wilderness. Imagine a world where wild animals are not massacred for luxury products anymore. Imagine a scenario where the spread of an invasive alien species such as the lantana is no longer a threat to the native biodiversity.

Earth's biodiversity is declining rapidly. While nature conservationists are trying hard to conserve the remaining species, the ecological interactions are way past disrupted. However, the newly emerging field of Synthetic biology can provide a helping hand in nature conservation by using some of the greatest gene-editing tools available such as CRISPR-Cas 9, to perform tweaky lil' manipulations in the genetic material of organisms.

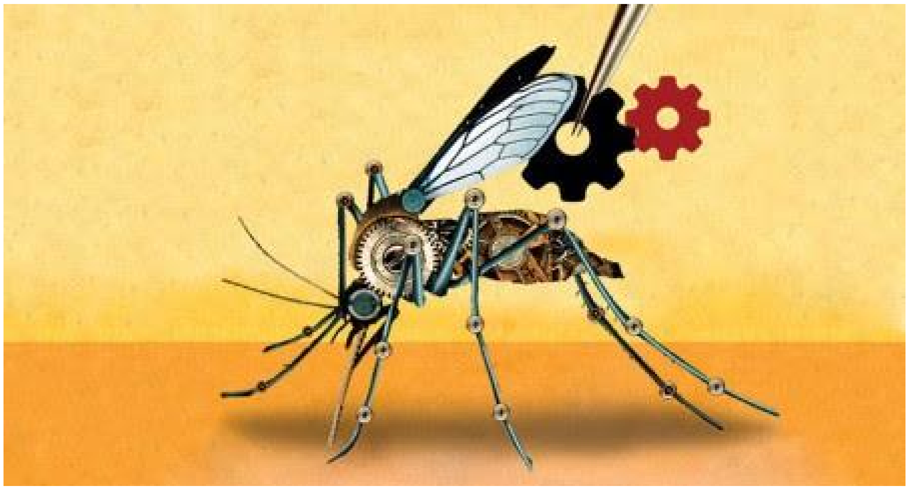
Synthetic biology can act as a tremendous tool to prevent biodiversity loss, restore biological interactions and conserve natural ecosystems. Research into how synthetic biology can help restore the natural interactions could help us clean polluted waters, control invasive species, reduce pressures from wildlife trade, improve disease resistance, and even bring a species back from the brink of extinction.

Invasive Species

One of the major threats to biodiversity is the spread of invasive species that disrupt natural habitats and push native species to extinction.

What if we genetically tweak these invasive species to stop them from spreading?

For instance, on the Hawaiian Islands, mosquitos transmitting avian malaria has lead to a rapid decline in the native bird species and disrupted the island ecosystem.

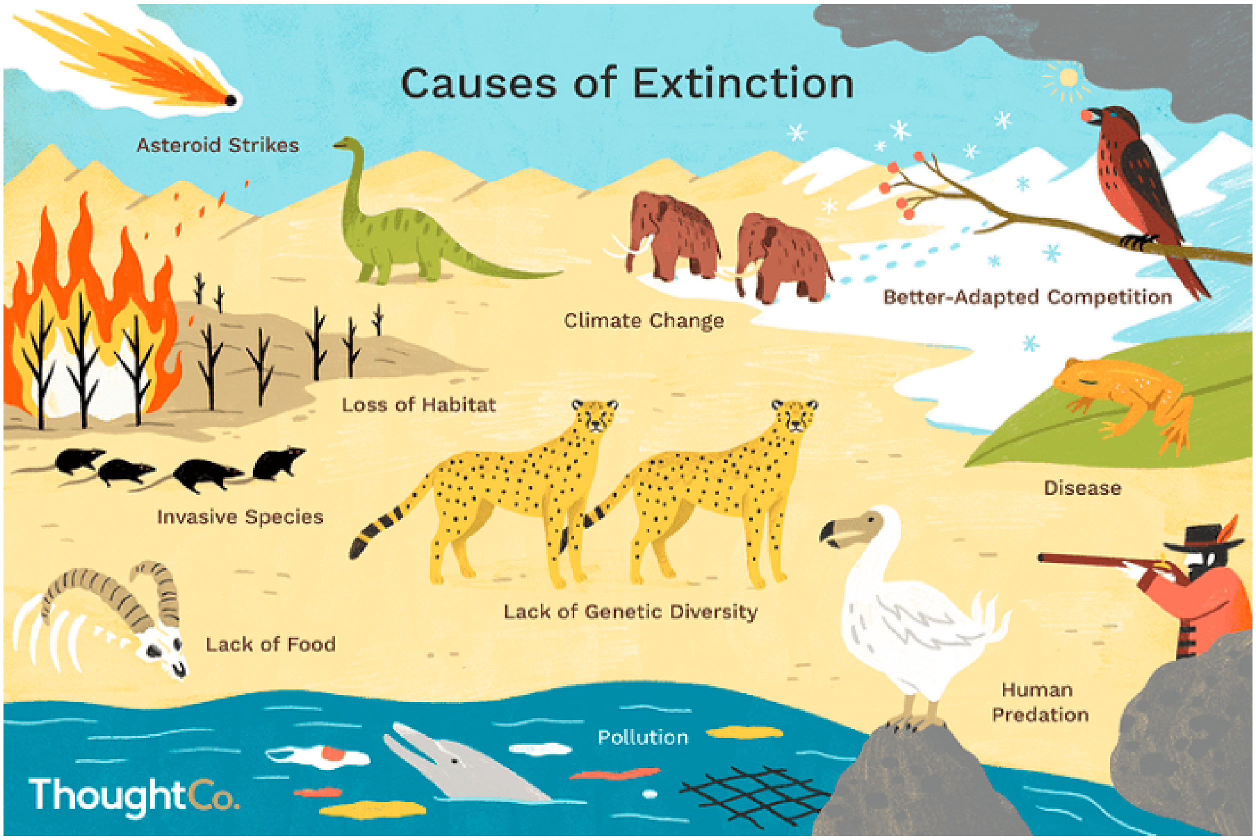


Gene drive in mosquitos

What if we could wipe out avian malaria without spraying any toxic pesticides by releasing male mosquitoes that have been engineered to be reproductively sterile?

Such genetic manipulations can be made to spread across the population by a method known as ‘gene drive’ to reduce the invasive species and protect the native biodiversity. Eradicating such invasive species could restore ecosystems and let the evolutionary processes resume unfettered.

Bringing Species From The Brink Of Extinction



While seeing dodos and woolly mammoths running in the wild might be fascinating, it is more important to focus on preserving the existing species by applying synbio techniques. Assisted reproductive techniques such as gamete cryopreservation, artificial insemination, embryo transfer, and in vitro fertilization could allow the propagation of wild endangered species and give a push to the critical species that are long gone from the wild.

Najin (left) and her daughter Fatu (right): the last two Northern white rhinos

For instance, the Northern white rhinos of Africa have been in decline for decades. By 2018, the population had dwindled to two remaining females, who are the last of their kind in the world. In a desperate attempt to conserve this critically endangered species, scientists are now using IVF procedures and genetic methods to create embryos from fresh eggs of the two remaining female rhinos and frozen sperm from dead males. Even though they have been successful in doing so, it would probably take a long time to see these rhinos being functional on the wild grasslands of Africa.

No More Wild Products

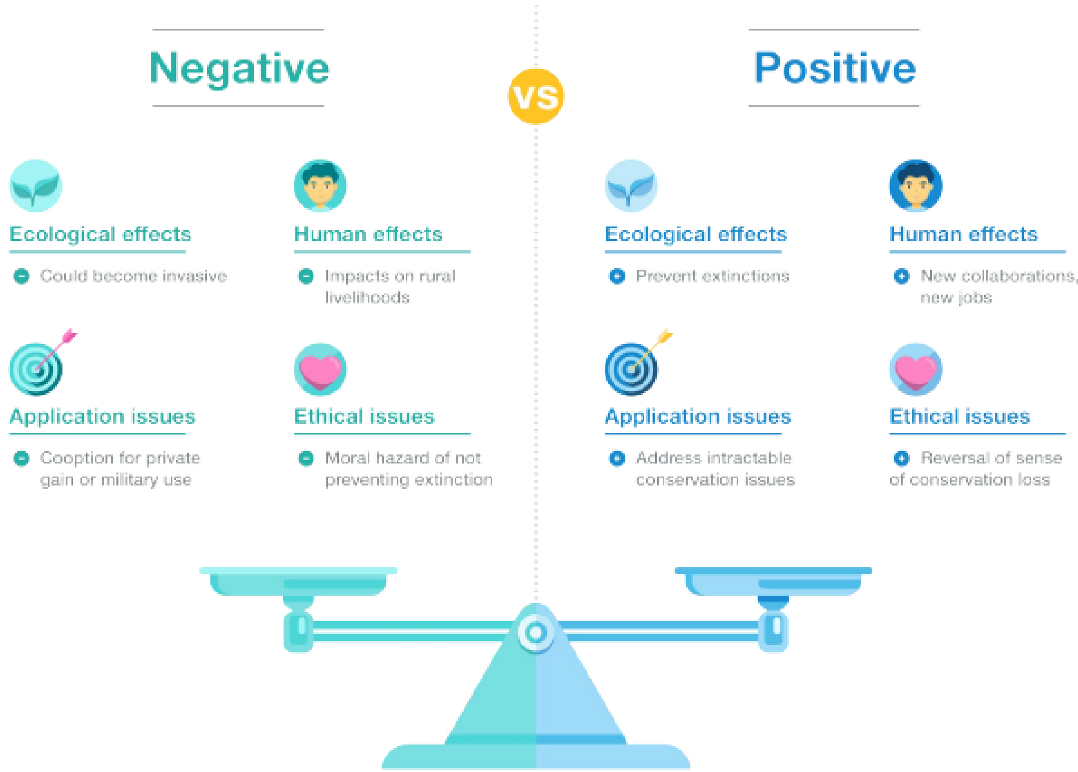
Biosynthesis isn't something new to us, one of the classic examples being insulin production by engineered bacteria. The synthetic production of human insulin revolutionized the field of synthetic biology. We are now able to produce abundant amounts of insulin without worrying about domesticating animals for it. This same process can reduce the commercial need to extract biological products from wild species.

For instance, Horseshoe crabs, aka. the living fossils have been exploited for their blood by pharmaceutical companies for decades. The horseshoe crabs are highly sensitive to bacterial toxins, so drug companies extensively use them to test for any possible product injected into the human body, causing a dangerous decline in their population.

However, the pharmaceutical companies have finally started committing to an alternative that doesn't harm animals. Scientists have engineered a synthetic replacement for horseshoe crab blood cells that have been commercially available for more than 15 years; it has yet to be broadly adopted.

Should SynBio Interfere?

With benefits comes the cost. While synbio can potentially impact the field of nature conservation, some might argue that playing with nature's DNA could be an ethically false way to save the natural world, and that too with unforeseen consequences.



Examples of the anticipated costs and benefits of conservation applications of synthetic biology

However, targeted manipulation of certain species might be one of our last hopes to help conserve biodiversity and save the declining species. Careful use of synthetic biology techniques in nature conservation might allow us to witness critical animals from just being a few cells in a test tube to be running freely in the wild.

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[Restoring ecosystems through synthetic biology - DNV]()

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Synthetic biology and its implications for biodiversity conservation

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[The Muggle Dittany: Plant Synthetic Biology-Based Therapeutics]

Introduction

The Harry Potter Favorite: Essence of Dittany

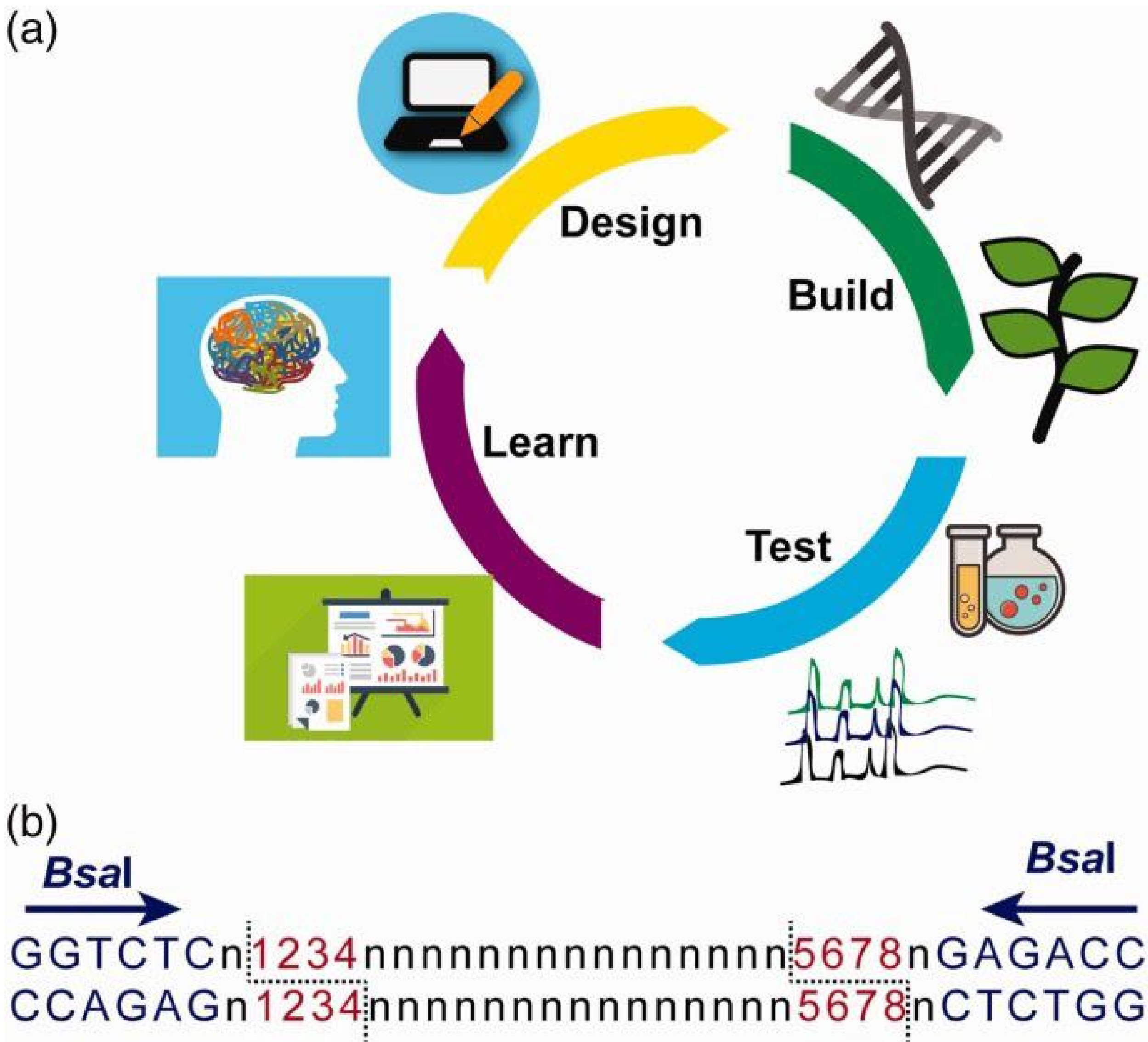
For a witch or a wizard reading the title, they must be now on their nostalgic tour to the woods where Hermione Granger poured the essence of dittany on Ron's wounds after he splinched. But for the confused Muggles (non-magical people), we owe a mini explanation- In the Harry Potter series, dittany was the all-heal medicine used on wounds.

Many years ago, the existence of medicine as powerful as the imaginary dittany would be laughed off in conversations witnessed by little pharmacies on the nooks and corners of London when the books were first released; but now, decades later, the narrative has undergone quite the change!

Owing to the fast improvement in Synthetic Biology, plant SynBio has ended up as a go-to for improving next-gen medicines. This article summarizes contemporary development towards robust and predictable engineering of plants to benefit the manufacturing of recent and new-gen medicines.

What is artificial biology in this context?

“Synthetic biology aims to make biology easier to engineer…. It can be thought of as a biology-based toolkit that uses abstraction, standardization, and automated construction to change how we build biological systems and expand the range of possible products.”



Plant synthetic biology concepts (a) The design–build–test–learn (DBTL) cycle (b) Type IIS restriction site-based DNA parts.

Plant Synthetic Biology Concepts and Techniques

The engineering concept of the design-build-test–learn (DBTL) cycle reinforces the application of synthetic biology to organismic engineering. Synthetic biology speeds up the rate at which we move around the DBTL cycle. Using this foundation, recently, a plant standard has been developed based on Type IIS restriction endonuclease assembly.

The eventual goal of Synthetic Biology is to possess biological blueprints to understand and modify an organism. For plants, these goals are still very distant. However, progress has surely been made with the help of techniques like —

(a) **Transgenic techniques** that have been used in early plant synthetic biology approaches

(b) The development of precise **gene-editing** technologies can precisely manipulate a DNA sequence and gene expression levels

(c) **Gene stacking and whole pathway engineering** are methods that allow a relatively easy and rapid way to rearrange genes in a metabolic pathway and to test out different combinations.

This can be essential to alleviate metabolic bottlenecks or identify potential co-factors during plant research. All of these techniques play an important role in making medicines using plant-based synthetic biology.

Application in Pharmaceutical Production

The concept of plant-based pharmaceutical production (pharming) has been worked on for 30 years and recently has led to innovative results. Originally, this was based on the concept of production of edible vaccines in crop plants. These would be cheap to produce as they avoid the expensive costs associated with manufacturing or supply chain issues such as refrigeration.

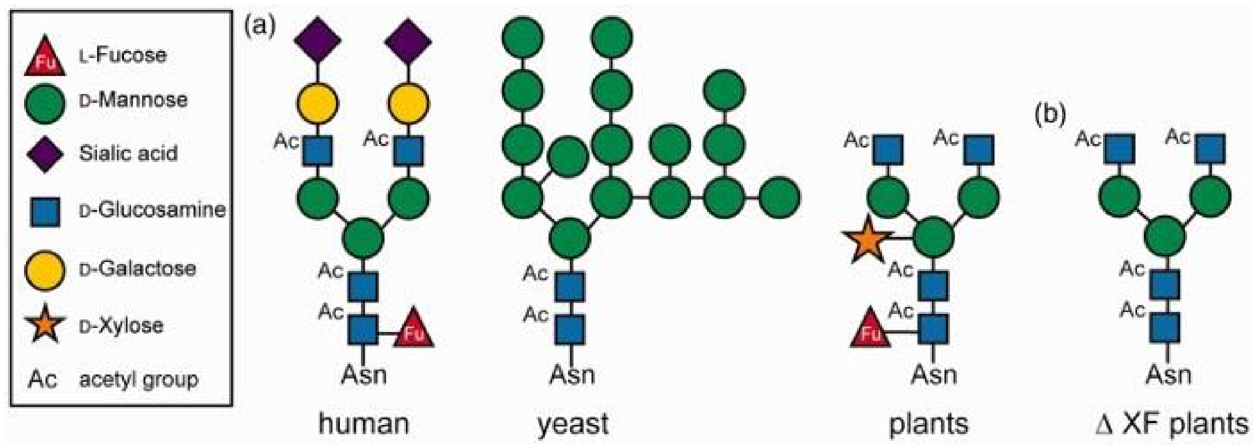
However, it rapidly became clear that this goal was not achievable. The challenges of regulating dose could not be overcome. As a result, with a modified approach, there is now acceptance that while plant-produced vaccines and pharmaceuticals have many benefits over other expression systems, some processing of the plant material will be required before the vaccination of the patient.

Biopharming has gained increased importance in the last few decades. Widespread research efforts towards making a plant-made vaccine are being conducted.

In the field of therapeutics, plants have been chosen for the production of monoclonal antibodies (mAbs), as opposed to other industrial production systems such as bacteria, yeast, insect, or mammalian cells due to a combination of reasons based on cost, safety, and the potential of plant systems to produce mAbs with the correct post-translational modifications.

For example, mammalian production systems, which dominate the market, are expensive with very less prospect of scaling up, whereas bacteria are unable to produce most of the correct post-translational glycosylation required for the mAb activity.

Yeast, insect cells, and plants, since they are eukaryotes, can perform complex post-translational glycosylations. However, in all these systems, the exact nature of the glycosylation differs depending on the species, and all show significant differences to mammalian systems. As a result, the concept of glycoengineering has been developed, in which the expression system is modified to produce mammalian-like glycosylated mAbs. Plants have proved to be particularly amenable to glycoengineering, and this has been accelerated by synthetic biology tools.



Glycoengineering of proteins in plants. (a) Simplified example structures of *N*-linked glycosylation in humans, plants, and yeast (b) *N*-linked glycosylation in ΔXF tobacco plants, which produce humanized glycans on proteins. They lack the immunogenic β1,2-xylose and core α1,3-fucose.

Examples of Plant Therapeutics

Perhaps the most well-known example of a plant-produced therapeutic mAb is ZMapp™, a promising treatment for the Ebola virus. This therapeutic consists of three mouse/human chimeric mAbs that target the virions' surface glycoprotein and are produced in a strain of *Nicotiana benthamiana* (tobacco) called ΔXF. ΔXF plants have reduced xylosyltransferase (XT) and fucosyltransferase (FT) activity and produce authentic human protein glycosylation.

Additional plant-produced therapeutics are currently in clinical trials or have been approved. For example, Elelyso™ (taliglucerase-α), a treatment for Gaucher's disease, is produced in carrot cell culture and was approved by the FDA in 2012.

Plants also hold promise for the commercial production of pharmaceuticals or pharmaceutical precursors.

One example is the anti-malarial drug artemisinin, the main ingredient in the ACT therapies (first-line malaria therapy in endemic countries) used globally. Artemisinin is found at relatively low abundance in the *Artemisia annua* plant, which means production can't meet the increasing demands. Scientists have now learnt that the precursor, artemisinic acid, can be converted chemically in a low-cost process to artemisinin, and therefore it has become an important biotechnological target. The initial strategy used synthetic biology to engineer artemisinic acid production in yeast by overexpressing 14 genes and conditionally repressing two more. While this approach was highly successful, producing yields over 25 g/L, the costs of large-scale yeast fermentation have made this challenging to produce commercially.

More recently, a similar strategy has been used to produce artemisinic acid in tobacco, making use of the COSTREL gene stacking strategy. This has resulted in yields of over 120 mg/kg of biomass. The scientists estimate that 200 km^2 of tobacco fields would be enough to meet the current global demand for artemisinin.

Generation of the potent anti-malarial drug artemisinin in tobacco has emerged as a stellar success in the world of Plant SynBio-Based Therapeutics.

Where on from here?

As the precision with which plants can be engineered increases, so will the range of applications. The discovery of novel plant compounds with immune system modulating activities has already become an increasingly important area of research, and SynBio aids to the progress in this field by fueling new possibilities. The identification, characterization and SynBio based mimicking of plant compounds that augment new or existing vaccines have been and will be of significant interest to immunologists all around the globe.

Finally, it remains to be seen how the public will view synthetic biology and gene-editing in light of previous concerns over GMOs and medicines as products of synthetic biology.