Review Article

Engineered Microbiome – The Successor of Medicines

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ABSTRACT

The human gut is home to a diverse collection of microorganisms that play a vital role in the body's metabolism and in enhancing the immune response in the body. The studies on the interactions of the gut microbiota in the body reveal the correlation of the microbes with various chronic diseases like diabetes, obesity, inflammatory bowel disease, etc. In fact, the microbiome has an influence on various viral infections as well. Therefore, it can be concluded that these microorganisms have the potential to be used as therapeutics. The engineered microbiome would have higher efficiency, cost-effective and most importantly, would be non-invasive, thereby being advantageous than current therapeutics. The current market is greatly dominated by biologics and hence, a greater capital is being invested in this sector. However, there are certain safety concerns related to the engineered microbiota and therefore, a thorough research must be done to combat with the limitations. The review gives detailed information on the potential use of engineered microbiome as therapeutics and also highlights a few safety concerns pertaining to the technology.

Keywords: Engineered Microbiome, Biologics, Therapeutics, Gut microbiota.

INTRODUCTION

A Wide varieties of microbes colonize the human body. It is evident that the interactions between the microbiota and the human system have different physiological as well as psychological effects and determine the health of the individual.[1]These microbes are of utmost importance as they help in regulating the metabolic functions and aid in maintaining immune homeostasis.[2] The human microbiota consists of a trillion of organisms belonging to more than 1000 species.[3] The microbial population that colonizes the human mucosal surfaces, the skin and most importantly the GIT (Gastrointestinal Tract), is of utmost importance as it helps in maintaining the health status of the body.[4-8]In fact, an alteration in the microbiome in the GI tract may cause chronic illnesses like diabetes, obesity, certain metabolic disorders, etc.[8-13] as shown in Figure 1.

With the advent of various sequencing techniques and bioinformatics tools, the sequences of gut microbiota are being explored in order to understand the interactions between them and the host.[3] It is found that around 33% of the gut microbiota is common to all the human beings and the remaining 67% differs from person to person due to various factors like diet, lifestyle and environment. It also changes as the person get older.[10, 14-18]Since it is evident that there is an influence of the microbiome on the host, the microbiota can be used as potential therapies for various diseases.[19]

Firmicutes and *Bacteroidetes* phyla dominate the adult gut ecosystem and this microbiota of the gut serves to be a target with immense promise for various clinical treatment procedures. The diagnosis and treatment of a variety of diseases that are linked to the GIT or that of the gut can be done by potent engineered microflora.[20]The bacterial strain must be precisely selected as it is crucial to develop a design and engineer effective therapy. The bacterial strain must always be a non-pathogenic one, must be vulnerable to the desired genetic manipulation and must ideally adapt to the environment where the therapeutic action is required.[2]The engineered strain then undergoes clinical trials before being launched in the market. For instance, Steidler et. al engineered a strain of *Lactococcus lactis* to deliver an anti-inflammatory cytokine called Interleukin, IL-10 in the GI tract.[21] It successfully cleared clinical phase 1[22] but failed in phase 2a.[23]

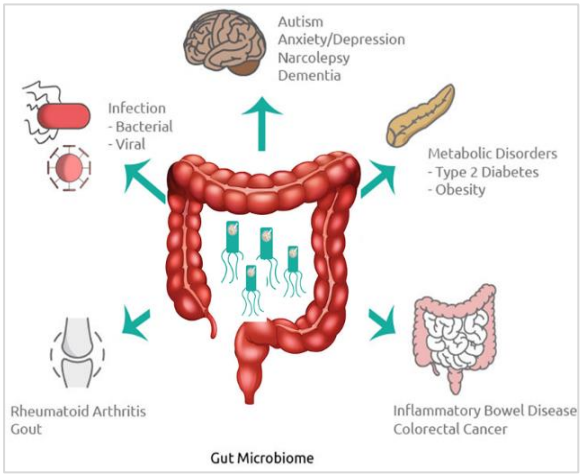

Figure 1: There is an influence of the gut microbiota on the various chronic ailments.[8-13, 49]

Gut Microbiota as Aa New Treatment Target

Dietary supplements have a therapeutic role on gut microbial population and manipulation of it can only be done after precisely identifying the role of each microbial family present in the gut. The manipulation initially focuses to reduce the number and different types of harmful populations and eventually increase the beneficial ones. A number of known therapeutics, namely the antibiotics, the prebiotics and probiotics can help to alternate the microbiota diversity.[20] Use of prebiotics and probiotics, collectively called synbiotics, are being frequently being used at present to confer health benefits to the individuals.[24]Probiotics are the live organisms that are administered to improve the health of the host. These enhance the host's intestinal barrier and the immune system as a whole.[25] They are also used in the treatment of certain GI diseases like enterocolitis[26] and Inflammatory Bowel Disorder (IBD).[27] Non-viable constituents of food are called prebiotics, which enhance the health status of the individual by modulating the microbiome. Examples would include gluco oligosaccharides and fructooligosaccharides.[28]Faecal transplantation refers to the process of transfer of certain bacterial populations present in the faeces from a healthy individual (donor) to an individual who has a disrupted microbiota (recipient). Presently, recurrent Clostridium difficile infection is being treated by faecal transplantation and it is done by bringing back the microbiota which is lashed out by the usage ofantibiotics. The results seem to be quite promising despite of the emergence of a few problems, [29] that are highlighted by Nagpal et. al.

There is a possible treatment for genetic diseases like phenyl ketonuria. Individuals who are suffering from phenyl ketonuria (PKU) by birthare unable to process and metabolize the amino acid or phenylalanine, which is commonly found in majority of the proteins. Despite of following a strict diet, which consists of extremely low protein content,[30]people with PKU suffer from cognitive and neurological impairments that are caused by an accumulation of phenylalanine.[31] Two enzymes help in the complete breakdown of phenylalanine and the genes coding for these enzymes were inserted into a strain of *Escherichia coli*, "Nissle". Nissle is a bacterium that is present in the feces but does not colonize the human body.[32, 33] The engineered strain has capability to degrade phenylalanine and is a potential treatment for the patients.[34]

The technical and economic impediments that are presently related with the use of these potent drugs and medicines globally are seen to be overcome with the precise and controlled delivery of the biologics*in situ*. This would hence, permit the production of the expensive and complex drugs in the body locally, in a sustainable manner. The pharmaceutical industry is now advancing towards the use of biologics, which are therapeutics that are solely based on macromolecules, like enzymes, antibodies, growth factors, and cytokines.[2]Smart probiotics make use of the bacteria that sense the levels of one or more gut biomarkers, compute whether the profile of those biomarkers is diagnostic of the disease, and respond to the condition by delivering an optimum dose of certain appropriate and specific therapeutics.[35-38] This is shown in Figure 2. A higher level of specificity is achieved due to the large size of these biologics, and the non-target side-effects is greatly reduced. Rational design and molecular evolution of the biologics must be taken into consideration while engineering them, in order to improve and enhance their functional properties. The engineered microbiome, when used as therapeutics, would be non-invasive, cost-effective and have higher efficacy than the current synthetic medications.[39]

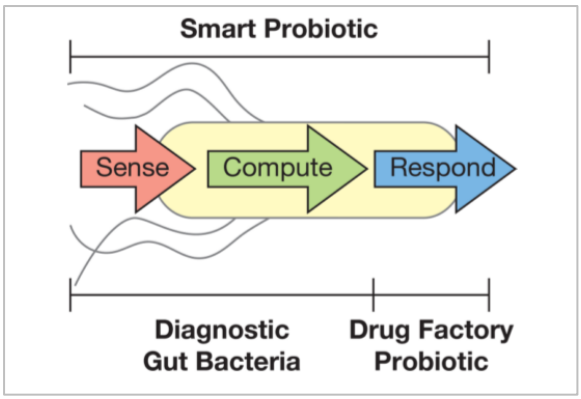

Figure 2: The diagrammatic representation of Smart Probiotics.[39]

Mechanism of Action

The mechanism of action of gut microbiome in the treatment of diseases depends on the technique chosen to treat the disease and its pathology. For instance, in the treatment of colon cancer, it was observed in murine models that, *Bacteroides fragilis*, secreted enterotoxin (ETBF) which changed the host's immune system,[40-42]*Enterococcus faecalis*, was found to secrete superoxides which polarized to M1 macrophages,[43,44]*Fusobacterium nucleatum*, secreted fap2 protein which engaged the T cell immunoreceptor with Ig and ITIM domains (TIGIT) and hence avoids natural killer (NK) cell toxicity.[45]Also, probiotics are found to have many health benefits such as, immune system modulation by strengthening non-specific and antigen-specific defense against infections and tumors, treat blood lipids and heart disease by altering the activity of Bile Salt Hydrolase (BSH) enzyme and treat inflammatory bowel diseases and type 1 diabetes by enhancing mucosal barrier function.[24] Genetic diseases can be treated by genetically engineering native gut microbes and inserting desired genes which would result in the production of the required protein or function to treat the disease.

The Current Market Scenario

The list that showed the rankings of the top drugs that provide the highest incomes to the pharmaceuticals is now dominated by Biologics. With over 50 molecules that

have already been permitted for the treatment of the most commonly prevalent diseases like autoimmune diseases, inflammation, and cancer, and whose turnover surpasses 74 billion euros (approx. INR 5,803 crores) per year, antibodies are the main biologics that are exhibiting the highest increase.[46]

Biologics now elucidate the rapid-growing segment of prescription drug spending. Biologics are projected to account for more than half of the sales generated by the top 100 products by 2022. This market is forecast to reach $399.5 billion (approx. INR 28,000 crores) by 2025, according to Grand View Research, and by 2022, biologics are expected to contribute 52% of the top 100 product sales, according to an Evaluate Pharma report. IQVIA Institute for Human Data Science in a report from April 2018 finds that the balance of medicine spending has shifted strongly to specialty medicines, which drove $9.8 billion (approx. INR 694 crores) of the $12 billion (approx. INR 833 crores) net growth of new brands and now accounts for 46.5% of per-person, per-year spending on medicines. The biologic market grew by 12.6% in 2017, accounting for $11.5 billion (approx. INR 800 crores) in spending. It is predicted that over the next five years, 20% of the 40 to 45 new active substances projected to be launched each year will be next-generation biotherapeutics, such as regenerative medicines, gene therapies, and cell-based therapies, with costs approaching $100,000 (approx. INR 70 lakhs).[46]

Most of the commercial successes in biologics are based on monoclonal antibodies. All of the top-selling biologics are monoclonal antibodies, including AbbVie's Humira, with 2017 sales of $18.4 billion (approx. INR 1,278 crores); Roche's Rituxan with 2017 sales of $9.2 billion (approx. INR 640 crores); and Pfizer/Amgen's Enbrel with 2017 sales of $7.9 billion (approx. INR 549 crores). According to a survey by Catalent Biologics, monoclonal antibodies (MAbs) (biosimilars in particular), conjugated therapeutics (including antibody drug conjugates), and gene and cell therapies (such as CAR-T cells) are expected to dominate in the next five to 10 years.[46]

Biologics can be made available to those who currently lack access by the production of biosimilars. July 2018 marked the most recent biosimilar approval and that was for Pfizer's Nivestym, which was also the second biosimilar approved to Amgen's Neupogen.The global biosimilars and follow-on biologics market was estimated to have been $7.7 billion (approx. INR 535 crores) in 2017 and it is expected to grow at a CAGR of 23.8%, according to Visiongain. The market is dominated by biosimilar monoclonal antibodies, a submarket that will be estimated to be a 24% share by 2022.[46]

In the United States, the demand for biologics is expected to grow 6.5 per cent per year to $102 billion (approx. INR 7,086 crores) in 2015, according to research by The Freedonia Group. Specialty drug spending has speeded up, accounting for 16.3 per cent of plan costs and a whopping 70.1 per cent of drug trend, according to Medco's "2011 Drug Trend Report".[47]

According to The Hindu, in the second quarter of FY19, Biocon has reported a brilliant performance by the Research Services, Small Molecules business and Biologics segment, which has resulted in a better-than-expected profit primarily due to the commercial launch of biosimilar MAbs or Monoclonal antibodies and also the launch of a drug (biosimilar Pegfilgrastim), that was useful for cancer treatment in the United States.[48]

Safety Concerns

Before these engineered bacteria enter the market, numerous obstacles and challenges still need to be resolved. Scientists require a better knowledge of the gut microbiome and its interaction with the host. The stability of the engineered strain is questionable as they are "less fit" than that of the wild strain,[49] and also their behavior might be unexpectedly altered.[50]

The most vulnerable fact is that the human genes that are incorporated into the engineered microbes could be transferred to other microflora that colonizes the host and thereby, the consequences are not known. In order to prevent this, several attempts have been made to alter the chromosomes of a bacterium, and not its plasmids. Biological 'kill switches'[51, 52] or DNA degradation devices[53] have also been built and these would prevent the survival of the microbe outside the body. The strategy however was not very successful. If not controlled in the right manner, the engineered microbiome might be potentially harmful as other bacteria might also produce the protein. Given these uncertainties, a school of thought retaliate the testing of these microbes in humans and consider them "highly risky".[54]

Potential Applications and Future Scope

Smart Probiotics

Probiotics are live organisms (microbes), which are isolated, grown and consumed to provide health benefits.[39, 55] Probiotics communicate and signal the human body directly which result in useful and desired positive effects. This could be by chemical or physical modes or by modifying the gut microbiota.[56] A more efficient and interesting form to produce health benefits using microbes is by using smart probiotics. These smart probiotics can diagnose and treat diseases.[57] These are genetically engineered and have the ability to sense the levels or concentrations of gut biomarker(s), analyse if the level(s) is/are normal or not and then deliver a dose of the appropriate therapeutics, if required.[35, 36, 38] The efficiency of drug delivery is high and lesser side effects are observed in the case of both, smart and natural probiotics, as they deliver drugs to the affected tissue, locally. Drugs produced by these bacteria are on-site which eliminates the need for various purification processes and thus brings down the expenses. A huge advantage of smart probiotics over traditional probiotics

is its specificity and reliability to activate therapeutic pathways. This is due to the adoption and usage of well-characterized gene regulatory networks. Also, the genetic pathways responsible for the production of therapeutic compounds are well-understood and defined in smart probiotics. The process of developing smart probiotics involves the usage of iterative cycles of design, construction, testing, and learning. These help in improving the performance of these smart probiotics.

The fields of synthetic biology and genetic engineering are widely credited for the concept of smart probiotics. Synthetic biology is a new, emerging field of engineering wherein living organisms are programmed, genetically, to carry out desired actions which would be helpful for the fields of medicine, agriculture, energy, manufacturing, and fundamental biology research.[58-61] In the context of smart probiotics, organisms are made to express genetically encoded sensors, such as signal transduction pathways, which sense certain specific chemical or physical inputs within or external to the cell, by the means of genetic engineering. These sensors convert the chemical or physical signals into biological outputs such as transcription of a gene transcription. These biological outputs, serve as inputs to a network of interacting regulatory molecules, such as transcription factors, called a genetic circuit.[57, 62, 63]The functioning and activity of actuator genes are controlled by these circuits. These actuator genes are responsible for functions such as metabolic pathways or secreted proteins, which instruct the cell to either change its state or change its environment.[57] Figure 3 depicts the outline of the types of sense, compute, and respond behaviour an engineered gut bacterium can exhibit.

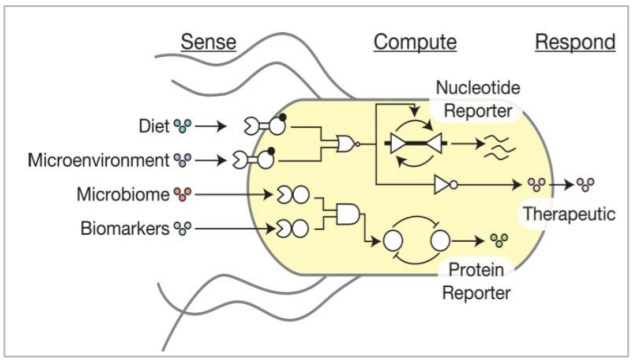

Figure 3: An outline of the types of sense, compute, and respond behaviour an engineered gut bacterium can exhibit.[57]

Genetically modified natural probiotic, *Lactococcus lactis*, made to secrete the human anti-inflammatory cytokine protein interleukin-10 (IL-10), when administered orally has been observed to reduced colon inflammation (colitis) by 50% in mice. Furthermore, concentrations of IL-10 are required to be 10,000-fold lesser when delivered in the gastrointestinal tract by L. lactis compared to intraperitoneal administration which thus, increases the efficacy and decreases the potential for unwanted side effects.[64] Figure 4 shows the IL-10 treatment of inflammation.

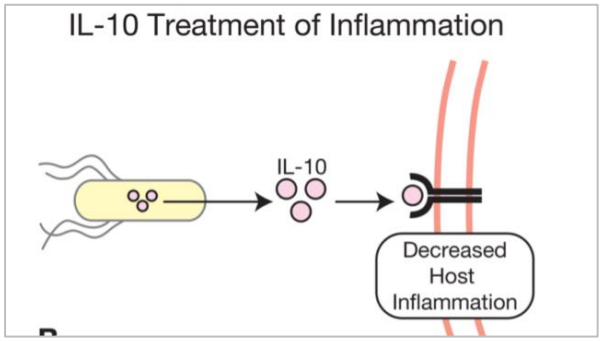

Figure 4: Interleukin-10 (IL-10) Treatment of Inflammation.[57]

Future advances in our understanding of the biology of the diseases linked to the gut will result in advances and improvements in smart probiotic designs and applications and also increase the number of diseases that can be treated using this technology.[57]

In-Situ Manipulation of Gut Microbiota

In-situ micro biome engineering allows us to study and manipulate the microbial communities in their native state eliminating the need for culturing them individually in laboratories by mimicking the native environment. Conventional methods to engineer micro biome *in-situ* include chemical, cellular, and phage-based methods. *In-situ* genome engineering is an emerging frontier in micro biome modulation.[65]

The composition and function of a micro biome can be affected by availability of biochemicals.[66, 67] Targeted biochemical modulation of microbiota may also involve the use of xenobiotics, which are foreign compounds designed to modulate the function or growth of the microbes. For example, β-glucuronidase inhibitors have shown to decrease the toxicity of a chemotherapeutic drug by inactivating the enzymes produced by the bacteria which can reactivate the drug.[68]

Live bacterial strains or communities also have the ability to manipulate the micro biome and are nowadays being used for the same. Cellular approaches can yield better interaction and function[65]For example, probiotic Lactobacilli are observed to have decreased pathogenic infections when used in livestock.[69]

Bacteriophages (phages) are the most abundant, diverse, and rapidly replicating life forms on Earth.[70]Phages replicate by infecting a host microbe and hijacking its replication machinery. Phages have been engineered genetically to deliver desired DNA strands or to alter the specificity of the host. For example, phages have been designed to deliver genes which increase the sensitivity of microbes towards antibiotics[71] or to deliver bio film dispersal enzymes.[72]

Faecal microbiota transplantation is one of the cellular based *in-situ* micro biome manipulation methods. In this

method, the native microbial community is replaced by a healthy microbial community obtained from the faeces of a healthy donor. The mechanism of manipulation is unknown.[65]

In genetically engineering the microbiota, rather than targeting specific strains or functions, the metagenomic content of a community of microbes can directly be altered. While there are a lot of differences in genomes between microbes, the metagenome of a community is more constant, [73] and controls its biochemical and cellular functionalities. For example, it is better to add a metabolic pathway directly into the genetic material of a native microbe than to introduce a completely foreign strain of microbe which contains the genes of the required pathway. This reduces off-target effects and helps in manipulating the microbiota with high-specificity.[65]

Gut Microbiota and Cancer Treatment

Tumorigenesis is one of the most studied pathologies linked with the gut microbiome.[74]It has been observed that both, local gastro-intestinal cancers and other tumors, have links with the gut microbiota.[75]Metabolomics and metagenomics studies have shown the dual role of the gut micro biome in the prevention of cancer, tumorigenesis and also in anti-cancer therapy.[76] Short chain fatty acids (SCFAs) derived from microbes may have an anti-cancer effect. For example, butyrate and propionate from the gut bacteria have been shown to be able to inhibit the deacetylases of the host's tumour cells which shows a general anti-cancer effect. Such a mechanism has been observed to show anti-tumoral effect in vitro and in vivo of butyrate in colorectal cancer (CRC) and lymphoma.[77, 78]

The main aim of anti-cancer therapies is of being effective in the targeted removal of the malignancy. Almost every anti-cancer treatment comes with side effects of its toxicity to normal cells. Some of these may even compromise the lives of the patients.[79]Gut Microbiota as a Tumour-Suppressor, Gut Microbiota as a Tumour-Promoter, Gut Microbiota and Anti-Cancer Therapy, Modulation of gut Microbiota to Enhance Chemotherapy and Immunotherapy Efficacy, Use of Probiotics in Oncology and Use of Faecal Microbiota Transplantation (FMT) in Oncology have been described in recent studies.[74] The outcomes of the anti-cancer therapies may be heavily influenced by the modulation of gut micro biome. In fact, anti-cancer treatments, including radiotherapy, chemotherapy and immunotherapy, have been observed to modify patients' micro biome and, the composition of the micro biome can deeply affect patients' response to such treatments.[80] It has been observed that, when affected by dysbiosis, the microbiota can influence cancer pathogenesis and also its therapeutic outcomes. The ability of gut microbiota to metabolize anti-tumoral compounds, and to modulate the immune response and inflammation pathways of its host, is tightly linked to regulation of the anti-cancer treatment. These two effects of the microbiota on the immune response of the host and the therapeutics may explain the involvement of the patient's microbiota and the efficacy of immunotherapy and chemotherapy.[81]

With regard to chemotherapy, mice lacking the presence of microbes are observed to not respond to oxaliplatin drug treatment. The explanation for this effect is that commensal micro biome members within the gut of the mice may be producing toll-like receptors (TLR) agonists, which promote the rise of an oxidative stress and tumour cell death. Consequently, without a healthy gut microbial community there would be depletion in microbiota-dependent ROS production, thus the response to the chemotherapy would be less effective.[82]

The transfer of gut microbiota between individuals has been used to cure many infections or in the treatment of diseases like dysbiosis and gut inflammatory disease. For example, recurrent *Clostridium difficile* duodenal infection has been cured using Faecal Microbiota Transplantation (FMT).[83,84] FMT has also been used to treat Graft Versus Host Disease (GVHD) post-allogeneic stem cell transplantation.[85] With respect to anti-tumour therapeutic applications, it has been demonstrated in mice to have good efficacy in reducing colon tumorigenesis, but the efficacy in clinical trials phase is still needed to be proven.[86] Several clinical trials are going on which are designed to analyse the use of FMT in cancer patients.

Gut Microbiota and Viral Infections

Viruses are found to have certain impacts on the microbiota of humans. Microorganisms in the body are known to enhance the actions of a few viruses like poliovirus, reovirus,[87] etc., while in some cases, they inhibit the viral actions like dengue virus,[88] rotaviruses,[89-92] influenza virus,[93-96] etc. however, the effects of the micro biome on certain viruses like the adenoviruses, HIV and noroviruses[97] are still unknown and are being extensively researched. The current scenario demands treatment procedures for the new COVID-19 or Novel Corona Virus (2019) disease, which has been declared as a global pandemic by the World Health Organisation.[98] Pathological studies have revealed the SARS corona virus has an impact on the gut micro biome as well.[99,100]As engineering micro biome has a lot of potential therapeutic properties, a study and research might be conducted to understand the impact of micro biome on the functions of the deadly Corona virus and eventually, these therapeutics might be used to treat the disease.

CONCLUSION

A healthy gut microbiota caters to a number of important functions by influencing the host metabolism. It also helps in the modulation of the host immunity and provides protection against disease-causing pathogens and other toxic substances. The ongoing research is mainly focusing in understanding the relationship between the disease and the microbial population and the research is

succeeding logarithmically. The engineered strains of bacteria would have the capability to combat and fight against distinct pathogens and their specific antigens, and also contribute to overcome the trouble caused due to antibiotic resistance. Using the mentioned techniques to treat diseases like diabetes, GVHD, cancers, etc. will most definitely be beneficial and efficient in the near future. The treatments and therapies would be economical as it only requires the growth of the desired microbe and does not require any complex purification steps. It is optimistically believed that people would witness an era, in the near future, where the individual's gut microflora will widely be used for the diagnosis and treatment of multiple health issues and problems. Interestingly, the microbial population of the gut at infancy might eventually be used in the prediction of a number of issues and ailments, and the diet of the individual would be exclusively planned according to the respective microbiome profile, for a healthy living. The structure, density and function of the gut microbiota must be understood properly. Only after this can new therapeutic targets be identified and utilized for a healthier gut as well as improved overall well-being.

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