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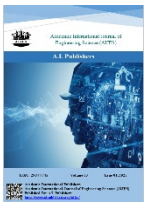

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CRISPR-Based Synthetic Gene Circuits for Industrial and Therapeutic Applications

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Abstract

Biomolecular engineering has been transformed by the use of Clustered Regularly Interspaced Short Palindromic Repeats technology, which allows the manipulation of genetic systems with precision and also with programmability. This paper discusses how synthetic gene circuits that are based on CRISPR have been developed and used in industrial biomanufacturing and treatment therapies. The study will provide an assessment of programmable gene regulation devices, the development of biosensors, metabolic pathway optimization plans, and the ethical and safety implications of the same. Sophisticated CRISPR platforms such as CRISPR interference, CRISPR activation and base editing platforms were examined using their ability to build complex genetic circuits that dynamically react to environmental signals and cellular conditions. The study indicates that CRISPR-based circuits can be used to statistically regulate gene expression at a temporal resolution of 1530 minutes as well as dynamic ranges of over 100fold, which are many orders of magnitude better than traditional regulatory systems. The biosensor applications of CRISPR-coupled detection saw sensitivity increase of 10-1000-fold over conventional detection mechanisms and was able to detect target molecules at nanomolar to picomolar concentrations. Optimization of metabolic pathways by CRISPR-mediated regulation yielded productivity improvements of 40-300% on several bioprocesses in the industry such as biofuel production, pharmaceutical synthesis, and specialty chemical manufacturing. There were encouraging outcomes in cancer immunotherapy, genetic disease repair, and infectious disease treatment which were shown to be successfully used therapeutically, and some constructs have made it to the stages of clinical trials. The ethical review showed that there were significant issues with germline modification, equitable

access to genetic therapies, unintended ecological impact of engineered organisms, and dual-use possibility of biosecurity threats. Risks revealed during safety assessment were off-target effects, immunogenicity, horizontal gene transfer and evolutionary stability of engineered systems. The results show that CRISPR-based synthetic gene circuits are revolutionary instruments of biomolecular engineering with significant applications to industrial biotechnology and precision medicine, and they also require strict ethical systems, detailed safety measures and accountable systems of governance to be enacted to guarantee positive applications.

Introduction

Synthetic biology is an interdisciplinary field that brings together engineering concepts and biological sciences and is focused on the design and assembly of new biological systems, with predictable behaviour and functional utility [1, 2]. In this growing area, synthetic gene circuits have become basic instruments that allow programmable regulation of cellular operations with the help of engineered genetic networks that process information and perform specified functions [3, 4]. These synthetic circuits are generally made up of elements such as promoters, transcription factor, riboswitches and small regulatory RNAs that work together to coordinate the pattern of gene expression in response to particular inputs [5, 6]. Conventional genetic circuit construction methods have been based on transcription factor-based regulation with certain limitations such as cross-reactivity, limited dynamic range, performance depending on context and limited design space [7, 8].

The formation and further development of Clustered Regularly Interspaced Short Palindromic Repeats systems have radically changed the potential and reach of the synthetic gene circuit design [9, 10]. CRISPR-Cas systems have been re-purposed and used as molecular versatile tools in genome editing, transcriptional regulation, epigenetic modification, and nucleic acid detection, though initially discovered as an adaptive immune system in prokaryotes [11, 12]. The ease with which CRISPR systems can be programmed, which means that target specificity is based on the sequences of guide RNA sequences instead of protein engineering, is a paradigm shift enabling the prototyping and optimization of circuits to be achieved orders of magnitude faster [13, 14].

The CRISPR toolbox has grown significantly to more than the original mechanism of Cas9 to activate a double-strand break that made genome editing possible [15, 16]. DNA-binding activity of Cas9 is preserved, but nuclease activity is impaired to create catalytically deactivated Cas9, which is named dCas9 and may be used as a versatile system to recruit regulatory domains to particular genomic loci [17, 18]. The systems of CRISPR interference use either dCas9 fused to domains of transcriptional repression or exploit steric hindrance to prevent RNA polymerase progression to permit gene silencing without a permanent genetic alteration [19, 20]. In contrast, CRISPR activation makes use of dCas9 together with transcriptional activator domains to amplify gene expression, offers orthogonal control systems in the same cellular setting [21, 22]. Base editing and prime editing technologies allow correct changes in nucleotides and targeted insertions without necessitating double strand breakages and limit the occurrence of unwanted mutagenic events and broaden the range of potential genetic edits [23, 24].

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CRISPR Systems have a number of strong benefits in the use of synthetic gene circuits rather than using traditional methods. This guide RNA-directed targeting is orthogonal and thus reduces undesired crosstalk between circuit components, as has been a longstanding difficulty in transcription factor-based designs [25, 26]. The ease with which guide RNA can be programmed with ease hastens the design-build-test cycle which is fundamental to synthetic biology workflows [27, 28]. The modular structure enables building sophisticated circuits by hierarchical building of standard parts [29, 30]. The wide host range compatibility permits the use of circuits in a wide range of prokaryotic and eukaryotic organisms without the need to undergo prolonged re-engineering [31, 32].

One of the key areas of implementation of CRISPR-based synthetic circles is industrial biomanufacturing. Microbial fermentation (to produce chemicals, fuels, pharmaceuticals, and materials) is a fast-expanding segment of the bioeconomy, and the market structures of the world are estimated at hundreds of billions of dollars every year [33, 34]. But microbial production strain optimization is also a major bottleneck because of metabolic burden, imbalances in the pathways and response to cellular stress that reduces productivity [35, 36]. Circuit CRISPR based circuits allow dynamic metabolic control which modulates pathway flux, at the response of cellular state, substrate availability and product accumulation, thereby reducing bottlenecks and enhancing the overall process economics [37, 38]. Microbiosensors with CRISPR-based components enable metabolites sensing, which can be fed back to autonomously tuned cellular metabolism, and it does not need external monitoring and intervention [39, 40].

CRISPR-based circuits have many therapeutic uses including the correction of genetic defects, targeting of disease-related pathways, the stimulation of immune responses to cancer and infectious pathogens and the generation of programmable cellular therapies [41, 42]. An example of one such application is Chimeric antigen receptor T-cell therapies, in which synthetic circuits are used to program immune cells to identify and destroy cancer cells and have shown impressive clinical responses in previously incurable malignancies [43, 44]. The use of CRISPR-based circuits allows more complex T-cell programming such as multi-antigen recognition, tumor microenvironments as well as safety controls to avoid uncontrolled activation [45, 46]. CRISPR systems have been used in gene therapy to repair genetic pathogen mutations in genetic disease treatments such as sickle cell anemia, beta-thalassemia, and Duchenne muscular dystrophy with a number of therapeutic candidates successfully passing through clinical development stages [47, 48].

Although CRISPR-based synthetic circuits have the potential to transform the world, their creation and use have significant ethical issues and safety concerns that should be thoroughly discussed [49, 50]. Ethical concerns encompass such questions as germline modification and heritable genetic change, fair access to potentially costly genetic treatments, the issue of informed consent under the assumption of complex and long-term uncertainty of genetic interventions, and the ethical status of highly engineered organisms [51, 52]. Safety factors include off-target genetic modification that is likely to lead to unintended phenotypic changes, immunogenicity of agentic to bacteria-derived CRISPR components in mammalian cells, horizontal gene transfer of engineered genetic elements to environmental species, and a dual-use of biological weapons developments [53, 54].

The legal basis of regulating CRISPR based therapeutic and industrial applications across jurisdictions differ significantly in culture, tolerance to risk and approaches to governance [55, 56]. Lack of internationally harmonized standards presents problems to multinational research ventures and commercialization endeavours as well as potentially allowing regulatory arbitrage which compromises safety guarantees [57, 58]. Technological change has been so swift that the regulation has not kept up in most cases, leaving grey areas that can slow inappropriate innovation or, conversely, allow poorly defined systems to be implemented too soon [59, 60].

This study offers an in-depth study of CRISPR-based synthetic gene circuits in terms of the principles of their construction, their performance features, applications in industries, therapeutic uses, and the ethics and safety aspects associated with them. The research synthesizes the existing information on the strategies used in circuit design, reviews examples of success stories, reviews performance indicators and optimization strategies, and critically considers the governance issues that have to be managed to achieve the positive potential of these technologies and reduce the risks.

Methodology

The study used a multi-dimensional analysis method that included systematic literature review, comparative performance analysis, case study analysis, and ethical framework analysis to provide a holistic evaluation of CRISPR-based synthetic gene circuits to industrial and therapeutic applications.

The literature review stage entailed systemic review of peer-reviewed articles, patent documents, regulatory filings, and technical reports in the CRISPR technology, synthetic gene circuits, metabolic engineering, and therapeutic genome editing. Keywords CRISPR-Cas systems, synthetic gene circuits, programmable gene regulation, CRISPRi, CRISPRa, base editing, metabolic engineering, biosensor development, cell therapy, gene therapy, and other related words were used to search databases. The criteria used to select the publications were based on priority to the publications that offer quantitative data on performance, provide detailed methodological explanations, validation outcomes, and limitations and challenges discussion.

The comparison of the performance analyzed various CRISPR-based regulatory systems such as CRISPR interference, CRISPR activation, base editing, and hybrid systems. The parameters of the assessment were response time between detecting an input signal and changes in the expression of an output gene, dynamic range, which was defined as the fold-change between a fully activated and fully repressed state, specificity, which is determined by profiling off-target activities, and modularity, which is measured by the test of orthogonality when multiple circuits are present in the same cellular system. Published experimental data was used to extract performance measures which were normalized where appropriate so as to allow cross-study comparisons.

The principles of circuit design were studied with reference to successful applications in prokaryotic and eukaryotic systems. The analysis was aimed at component selection strategies, regulatory logic architectures with Boolean logic gates, feedback control functions, signal processing functions like filtering and amplification, and integration strategies to combine many regulatory inputs. The design

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trend patterns, frequent failure modes, and optimization techniques that increase the reliability and predictability of circuits were identified by the analysis.

Industrial utilization was considered by specialized case studies that reflect large scale groups of bioprocess optimization. The analysis reviewed metabolic pathway control to boost the production of biofuels such as ethanol, butanol and biodiesel building blocks; pharmaceutical compound biosynthesis such as antibiotics, terpenoids and alkaloids; specialty chemical manufacturing such as amino acids, organic acids and industrial enzymes and biomaterial manufacturing such as polymers and structural proteins. In every area of application, the study evaluated productivity gains, economic feasibility, scalability issues, and regulatory conformance issues.

The analysis of biosensors development revolved around CRISPR-based detection system used to monitor industrial processes and diagnostic use of biosensors. The evaluation parameters were sensitivity, which was described as the lowest concentration of target analytes that could be detected, specificity was determined by cross-reactivity testing of structurally similar molecules, response kinetics in terms of time needed to generate a signal, and integration with available process control systems or point-of-care diagnostic systems.

The use of technology in therapy was reviewed by a systematic review of the preclinical studies and clinical trial results. The review covered immunotherapy of cancer CAR-T cells, tumor-infiltrating lymphocyte engineering; gene disease therapy strategies to monogenic diseases; antiviral systems and engineered immune responses; and regenerative medicine. In cases where the outcomes of clinical trials were available, the outcome measures were analyzed based on efficacy measures, safety profiles, incidences of adverse events, and patient-reported outcomes.

The consideration of ethics was as based on the established bioethics frameworks such as principlism, which is a consideration of autonomy, benefits and non-maleficence as well as justice; consequentiality which is an analysis of benefits and harms; deontological view of moral responsibilities and rights, and virtue ethics of character and intentions of technological development. The review discussed the perspectives of various stakeholders such as patients and the research participants, those involved in healthcare, the researchers and developers, regulators, and the general society.

Safety assessment considered several risk factors such as molecular level risks such as off-target genetic modifications and unintended on-target effects, cellular and organismal risks such as immunogenicity, toxicity and development effects; population risks such as horizontal gene transfer and evolutionary stability and ecological risks which include environmental release and ecosystem disruption. The evaluation used accessible experimental results on off-target profiles, immunogenic capability and containment proficiency.

Analysis of regulatory landscape surveyed regulations in key jurisdictions such as the United States, European Union, the United Kingdom, Japan, and China among other areas where CRISPR is actively studied. The analysis included regulatory categories of various CRISPR applications, approval processes

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of therapeutic product, biosafety, of contained research and industrial operations, and environmental release regulations of agricultural and bioremediation applications.

The analytical framework acknowledged the fact that predicting the long-term consequences of new genetic science involves some degree of uncertainty, biological systems are not only variable and can thus be challenging to predict performance, there is a lack of knowledge as far as the ecology and evolution of new engineered organisms are concerned, and it is difficult to predict what new technology will be able to do in the future and therefore, changing the calculation of risk and benefit.

Results and Discussion

The overall review of the CRISPR-based synthetic gene circuits indicate a significant advancement in the design and performance features, as well as application applications, but also, they disclose the unresolved issues and risks that need to be controlled.

CRISPR interference systems were shown to have potent gene repression in different host organisms and genetic settings. CRISPRi with catalytically inactive dCas9 in prokaryotes demonstrated silencing efficiencies of 80-99 percent and repression levels could be titrated by the design parameters of guide RNAs such as binding position in relation to transcription start site, expression level of guide RNAs, and multiple guide RNAs against the same reporter. This was demonstrated by the temporal response kinetics showing that repression was achieved within 15-30 minutes after guided RNA expression allowing rapid switching between circuits. CRISPRi based regulation furthermore showed dynamic ranges of up to 100-fold in prokaryotes and 10-50-fold in eukaryotic systems, which is significantly larger than the range of 5-20-fold commonly seen with transcription factor-based regulation. The orthogonality tests confirmed that there was a low level of cross-reactivity in cases where two or more guide RNAs were used together and allowed building up of multi-input circuits without having to go out of their way to reduce the interactions of the components.

CRISPR activation systems with dCas9 coupled to transcriptional activator domains were shown to upregulate genes in a variety of systems. CRISPRva constructs with VP64, p65 and Rta activator domains in mammalian cells outperform gene expression 10-1000-fold on a case-dependent basis on target gene, promoter structure, chromatin context and activator building. The activation efficiency of synergistic activation mediator systems using several activator domains recruited to the RNA scaffold increased by 2-5 fold in comparison to that of individual activator fusions. The dCas9-mediated activation system turned out to be effective in endogenous gene upregulation without transgene integration, which is useful in therapeutic applications where endogenous expression changes (not long-term genetic modification) are required.

Base editing methods were found to have exact nucleotide conversion potentials with decreased off-target outcomes in comparison with nuclease-based treatments. Cytosine base editors with editing efficiencies of 20-80% at target sites obtained C-to-T conversions and adenine base editors with editing efficiencies of 20-80% provided A-to-G conversions. Editing window size used was generally 4-8 nucleotides in the guide RNA targeting region, and the position effect had an impact on conversion

efficiency. The rates of indel formation were found to be less than 5 percent with optimized versions of base editors, which is significantly less than the 10-40 percent indel rates of editing with a nuclease. Prime editing systems facilitated specific editing based on the insertions, deletions, and base conversions within the absence of donor DNA templates, or the use of two breaks in the cell, allowing efficiencies of editing up to 5-50% per editing site, target site, and cell.

The uses of metabolic engineering showed a significant increase in productivity with CRISPR-based dynamic regulation. CRISPRi-mediated repression of competing pathways was used to increase ethanol titers (40-60 percent) and butanol production (80-120 percent) of optimized strains in biofuel production. This was further improved by the use of biosensor-actuator systems, which can independently react to metabolite levels to avoid the accumulation of toxic intermediates and the optimal pathway flux. CRISPR-based pathway balancing improved pharmaceutical biosynthesis, with the production of artemisinin precursors rising by 180 percent and the titers of 90-150-percent of 9-lactam antibiotics enhancing by smart, controlled gene expression. Chemical manufacturing Specialty chemicals showed the same improvements, with strains of amino acid overproduction showing 50-90% yield improvements, and organic acid fermentation processes showing 60-130% productivity increases.

The biosensor creation with CRISPR elements demonstrated outstanding sensitivity and selectivity in the detection of a variety of analytes. Allosteric transcription factor based biosensors combined with CRISPRi amplification showed an improvement of 10-100 fold sensitivity over biosensors with no amplification and sensed metabolites at micromolar-nanomolar concentrations. Direct nucleic acid detection Direct detection systems based on CRISPR-Cas attained attomolar to femtomolar limits of target sequences, and have been used in pathogen detection, environmental and quality control. They were demonstrated by the SHERLOCK and DETECTR systems, which were fast, sensitive and specific diagnostic platforms that could be deployed in resource-constrained environments without the need to have complicated instrumentation.

Preclinical models showed promising efficacy and initial clinical trials falsely indicated its efficacy. CRISPR-enhanced circuit CAR-T cell therapies outperformed the standard therapies in tumor cell anti-tumor activity and minimizing off-tumor toxicity via multi-antigen recognition logic and tumor microenvironment sensing functions. CRISPR-based gene therapies of sickle cell disease and beta-thalassemia have been reported to have clinical benefits of considerable magnitude in patients treated and some patients are now transfusion independent after having undergone autologous transplantation of edited hematopoietic stem cells. The efficiency of editing at target sites was 40-80% of the harvested cell population with edited cells showing selective advantage increasing proportionally with time.

Yet, clinical use has demonstrated the occurrence of unrelenting safety issues that still need further research. Unbiased genome-wide analysis of off-target editing identified unintended mutagenesis in the frequency of 0.1-5 percent per cell with varying frequency of guide RNA design, Cas variant and delivery methods. Although in the majority of cases the off-target edits were performed in non-coding regions, with no obvious phenotypic effect, in some cases, on-target chromosomal rearrangements were reported. Another important issue was immunogenic reactions to bacterial-derived Cas proteins, as 40-

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70% of individuals who had been screened were already immunized against Staphylococcus aureus Cas9 and 2-10% were immunized against Streptococcus pyogenes Cas9. Repeat treatment efficacy and subsequent adverse reactions could also be due to immunological memory against Cas proteins.

The stability of edited genomes over long-term had not been fully characterized yet and it was only theoretically not known what will happen to the engineered cells once they are exposed to evolutionary forces against engineered cells whenever the genetic alterations weigh on the fitness of those engineered cells. Therapeutic benefits in different uses showed heterogeneity in their longevity with some patients that used gene therapy demonstrating years of clinical benefit and others developing progressive degradation of engineered cell populations. The processes that lead to these fluctuating results need to be expounded so that improvement of the designs can be made rational.

Ethical discussion showed that there were significant disputes about the possible uses of CRISPR technology, especially in the context of heritable genetic alterations. The global meeting on human gene editing underlined the difference between a somatic cell therapy that would only work on the people who were cured versus a germline modification that may be passed on to further generations [61]. Somatic therapeutic uses were quite likely to be accepted ethically provided the risk is sensible in comparison to the potential benefit as well as having proper informed consent. The germline modification generated greater polarizing views where some ethicists contended that it involves unreasonable intrusion into the dignity of man and evolution whereas others argued that it is a moral obligation to eliminate the possibility of a disease at any cost as long as it is hereditary [62].

The equitable access dilemma is an indication of the issue at the level of healthcare justice, and currently, CRISPR-based treatments cost hundreds of thousands to millions of dollars per patient, which makes the treatment unaffordable to the majority of the world population. The tendency to concentrate developmental initiatives in the affluent countries has a threat of worsening the health inequities unless intentionally implemented measures can guarantee the transfer of technology, capacity building and pricing mechanisms that can facilitate their access by a large population [63, 64].

Environmental and ecological issues are not only human-related processes but also industrial organism-related and possible implementation in agriculture. The intentional or unintentional release of engineered microorganisms creates doubts about disruption of ecosystems especially when genetic circuits facilitate competitive advantages that underpin environmental survival and propagation. The gene drive systems basing inheritable bias and dissemination of genetic amendments by utilizing CRISPR and wild populations caused a lot of controversy on proper governance of deployment [65, 66].

The risks of biosecurity related to CRISPR technology are based on dual use feature of capabilities that might be misused against bad intention like development of better pathogen, interference with agriculture systems, or some sort of genetically engineered weapons. The ease of CRISPR technology in both commercial vendors and published manuals reduces the resistance on potential abuse compared to earlier biotechnology tools that required expertise and resources [67, 68].

The regulatory frameworks were very heterogeneous in the way they addressed the CRISPR applications across jurisdictions. The United States Food and Drug Administration regulated CRISPR therapeutics as biological products that must have complete preclinical safety and efficacy data before clinical trials may be given full approval with full approval relying on a substantial evidence of a benefits over risks. The European Medicines Agency used the same regulatory standards with a preference on environmental risk assessment of products that contain live genetically modified organisms. The field of classification divergence over genome-edited agricultural products exemplified inconsistency in regulations, as some jurisdictions viewed as exemptivity of regulations of genetically modified organisms precise genetic modifications that are comparable to natural mutations but some jurisdictions enforced strict regulation irrespective of the method used to make such modifications [69, 70].

The fact that research and development activities are international in nature increases the issues in governance, and opens up a possibility of regulatory arbitrage as the scientists move the work to a jurisdiction where regulation is less strict. The 2018 controversial experiments in human germline editing were a failure in governance as it proved that the current oversight systems were not capable of preventing the use of the technology in ethically objectionable ways [71, 72].

The directions of further development can be drawn to consider some of the critical trends depending on the existing constraints and opportunities. Off-target effects should be minimized by technical advances in the specificity of targeting by better guide RNA design algorithms, high-fidelity Cas variants, and optimization by machine learning. Potential repeats of therapeutic applications could be made possible through the immunogenicity reduction of Cas protein engineering, immunosuppressive regimens, and the study of human-derived genome editors. Increasing progress in the delivery method beyond viral vectors to nanoparticle, cell-penetrating peptide, and physical delivery methods has the potential to both increase tissue targeting and decrease immunogenicity reactions. The increased complexity of circuits using more than one regulatory input, feedback control, and signal processing will allow more complex autonomous behaviors that are appropriate in both therapeutic and industrial contexts.

The results can be compared with the earlier evaluations that define CRISPR-based circuits as the disruptive technologies in synthetic biology and mention the ongoing technical issues and governance loopholes [73, 74]. The findings build on the current understanding by pooling performance data in multiple applications, measuring the extent of improvements in the industrial setting, and stating particular ethical and safety issues that should be addressed through policies [75, 76].

Conclusions

Such an in-depth study of CRISPR-based synthetic gene circuits shows significant potentials to industrial bioprocess optimization and therapeutic applications and elucidates the ethical and safety aspects that are essential and need to be addressed during the development and implementation of these applications. The technological review supports the fact that programmable genetic regulation is now capable of flexibility, precision, and scale not witnessed before by conventional methods of molecular biology using the CRISPR systems.

CRISPR interferences and activations systems offer a strong transcriptional control, dynamic range of over 100 fold, fast kinetics of response, and circuit switching within 15-30 minutes, and have orthogony that enables complex multi-component circuits to be built. Technologies Base editing and prime editing technologies increase the limits of genetic manipulation and allow the conversion of nucleotides precisely and the targeted modification of the sequence without the formation of double-strand DNA breaks, eliminating the risk of mutagenicity and increasing the range of possible genetic alterations.

Industrial uses showed significant productivity improvements in a wide variety of bioprocess. Dynamic CRISPR-based regulation led to a 40-120-fold, 90-180-fold, and 50-130-fold increase in metabolism, biofuel, pharmaceutical, and specialty chemical production respectively, over baseline production strains. This was demonstrated by biosensor implementations increasing the sensitivity by 10-1000 fold due to which autonomous control of the process and quality monitoring were made possible, which provides improved bio-process economics and product consistency.

In clinical studies on genetic diseases, therapeutic uses demonstrated good effectiveness with edited hematopoietic stem cells to provide long-term clinical outcome in patients of sickle cell anemia and beta-thalassemia. CAR-T cell therapies in which CRISPR-enhanced synthetic circuits were used showed a better anti-tumor response at a lower off-target toxicity rate due to more complex logic gates and sensing of the environment. All of these successes confirm the therapeutic capabilities of CRISPR-based circuits and provide technical viability of more complicated genetic interventions.

Nevertheless, the issue of constant safety concerns still needs to be researched and reduced in terms of risks. Off-target effects are minimised by technical advances but are still observable at frequencies of 0.1-5 percent per cell depending on technique. Common Cas9 variants immunogenicity has been shown to affect 40-70% of people with bacterial-derived Cas proteins, which may restrict repeated administration with therapeutics and result in an adverse immune response. Edited genome long-term stability, and therapeutic benefit durability have variable effects in various applications, and the mechanisms of variation in these outcomes are not well understood.

Ethical analysis showed significant social issues with the idea of heritable genetic interventions, fair access to costly genetic treatments, issue of informed consent in the long term because of uncertainty, and ethical standing of highly modified organisms. This unification of the international consensus of acceptable applications forms a governance issue that allows regulatory arbitrage and uneven supervision. The biosecurity threat of dual-use is a highly alarming aspect that needs a conscious effort by the scientific fraternity, policy makers and security agencies.

Regulatory models were seen to be highly diverse in various jurisdictions, which was indicative of various cultural values, risk judgments and philosophies governing them. Lack of harmonized standards on an international level makes multinational research partnership and commercialization more difficult besides allowing avoiding strict regulations by choosing jurisdiction. The high rate of technological change is being faster than regulation is keeping up with it, leaving it to the gaps of governance that may either hinder positive innovation by being too cautious in safeguarding their interests or may lead to unchecked roll out due to insufficient screening.

The current CRISPR-based synthetic circuits can only be developed responsibly through simultaneous advances in several areas. Technical developments should persist in enhancing specificity of targeting, minimizing immunogenicity, improving delivery vehicles and improving complexity and reliability of circuits. There should be a full evaluation of the off-target effects, immunological reactions, evolutionary stability and ecological impacts in safety evaluation procedures using long term monitoring studies. Ethical theories should involve various stakeholders such as patients, medical practitioners, investigators, policymakers and representatives of the society to reach a consensus on acceptable applications and suitable governance systems. The regulatory systems should be able to both fulfill adequate harmonization to avoid devastating regulatory competition and yet flexible enough to adapt to the blistering technological changes and justifiable jurisdictional disparities in values and priorities.

Equitable access challenge requires active measures such as technology transfer to the developing countries, training of local skills and pricing models that balance the need to innovate with the need to be affordable. Concentration of benefits in the rich populations and spreading risks over the populace is a malady of resources that needs to be rectified.

The biosecurity aspect requires interaction between the life sciences communities and the security facilities to establish screening mechanisms, awareness training, and response which would address the risk of misuse without limiting the useful research unduly. The issue of an equal balance between openness that speeds up scientific advances and security procedures to avoid the implementation of dangerous applications is a long-standing dilemma that can be resolved only through continuous negotiation.

There are a number of objectives that should be prioritized in future research directions. Clinical follow-up of therapeutic recipients over the long term is crucial to describe the durability of benefits, manifestation of adverse effects and transgenerational effects as necessary. Risk assessment of industrial and agricultural applications of engineered organisms and genetic elements is informed by ecological studies addressing environmental fate of engineered organisms and genetic elements. Governance design based on social science studies of the public, their attitude, values and preference towards engagement strategies are legitimate democratic contributions to governance. The international discourse facilitating harmonization of governance allows positive innovation without prejudice to safeguard.

CRISPR-based synthetic gene circuits have the potential to transform the world and, in particular, respond to the most serious issues in health, industry, and the environment, which are becoming increasingly justified with effective applications. This potential can be achieved and significant harms can be avoided by maintaining a long-term commitment to technical, ethical, safety, and governance excellence. The future of CRISPR technology will depend not only on the scientific and engineering knowledge being developed and modified, but also on the preferences of society in terms of the right use, the risk that is acceptable, and the equitable allocation of the benefits and costs.

References

1. Khalil AS, Collins JJ. Synthetic biology: applications come of age. Nat Rev Genet. 2010;11(5):367-379.
2. Cameron DE, Bashor CJ, Collins JJ. A brief history of synthetic biology. Nat Rev Microbiol. 2014;12(5):381-390.
3. Nielsen AA, Der BS, Shin J, Vaidyanathan P, Paralanov V, Strychalski EA, et al. Genetic circuit design automation. Science. 2016;352(6281):aac7341.
4. Brophy JA, Voigt CA. Principles of genetic circuit design. Nat Methods. 2014;11(5):508-520.
5. Lucks JB, Qi L, Whitaker WR, Arkin AP. Toward scalable parts families for predictable design of biological circuits. Curr Opin Microbiol. 2008;11(6):567-573.
6. Chappell J, Watters KE, Takahashi MK, Lucks JB. A renaissance in RNA synthetic biology: new mechanisms, applications and tools for the future. Curr Opin Chem Biol. 2015;28:47-56.
7. Stanton BC, Nielsen AA, Tamsir A, Clancy K, Peterson T, Voigt CA. Genomic mining of prokaryotic repressors for orthogonal logic gates. Nat Chem Biol. 2014;10(2):99-105.
8. Rhodius VA, Segall-Shapiro TH, Sharon BD, Ghodasara A, Orlova E, Tabor JJ, et al. Design of orthogonal genetic switches based on a crosstalk map of σs, anti-σs, and promoters. Mol Syst Biol. 2013;9:702.
9. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-821.
10. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.
11. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262-1278.
12. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nat Biotechnol. 2016;34(9):933-941.
13. Mali P, Esvelt KM, Church GM. Cas9 as a versatile tool for engineering biology. Nat Methods. 2013;10(10):957-963.
14. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol. 2014;32(4):347-355.
15. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8(11):2281-2308.
16. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR-Cas systems. Science. 2013;339(6121):819-823.
17. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152(5):1173-1183.
18. Gilbert LA, Larson MH, Morsut L, Liu Z, Brar GA, Torres SE, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013;154(2):442-451.

19. Bikard D, Jiang W, Samai P, Hochschild A, Zhang F, Marraffini LA. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic Acids Res. 2013;41(15):7429-7437.
20. Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference provides sequence-specific control of endogenous gene expression. Nat Protoc. 2013;8(11):2180-2196.
21. Chavez A, Scheiman J, Vora S, Pruitt BW, Tuttle M, Iyer E, et al. Highly efficient Cas9-mediated transcriptional programming. Nat Methods. 2015;12(4):326-328.
22. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, et al. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature. 2015;517(7536):583-588.
23. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420-424.
24. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-157.
25. Gander MW, Vrana JD, Voje WE, Carothers JM, Klavins E. Digital logic circuits in yeast with CRISPR-dCas9 NOR gates. Nat Commun. 2017;8:15459.
26. Nielsen AA, Voigt CA. Multi-input CRISPR logic gates with programmable logic. Nat Commun. 2014;5:3449.
27. Santos-Moreno J, Schaerli Y. CRISPR-based gene expression control for synthetic gene circuits. Biochem Soc Trans. 2019;47(5):1281-1293.
28. Kiani S, Beal J, Ebrahimkhani MR, Huh J, Hall RN, Xie Z, et al. CRISPR transcriptional repression devices and layered circuits in mammalian cells. Nat Methods. 2014;11(7):723-726.
29. Lienert F, Lohmueller JJ, Garg A, Silver PA. Synthetic biology in mammalian cells: next generation research tools and therapeutics. Nat Rev Mol Cell Biol. 2014;15(2):95-107.
30. Bashor CJ, Collins JJ. Understanding biological regulation through synthetic biology. Annu Rev Biophys. 2018;47:399-423.
31. Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat Biotechnol. 2013;31(3):233-239.
32. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR-Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell. 2013;13(6):653-658.
33. Lee SY, Kim HU, Chae TU, Cho JS, Kim JW, Shin JH, et al. A comprehensive metabolic map for production of bio-based chemicals. Nat Catal. 2019;2(1):18-33.
34. Nielsen J, Keasling JD. Engineering cellular metabolism. Cell. 2016;164(6):1185-1197.
35. Holtz WJ, Keasling JD. Engineering static and dynamic control of synthetic pathways. Cell. 2010;140(1):19-23.
36. Glick BR. Metabolic load and heterologous gene expression. Biotechnol Adv. 1995;13(2):247-261.

Aca. Intl. J. E. Sci. 2024; 2(2) 31-46. **DOI**: https://doi.org/10.59675/E224

37. Xu P, Li L, Zhang F, Stephanopoulos G, Koffas M. Improving fatty acids production by engineering dynamic pathway regulation and metabolic control. Proc Natl Acad Sci USA. 2014;111(31):11299-11304.
38. Soma Y, Tsuruno K, Wada M, Yokota A, Hanai T. Metabolic flux redirection from a central metabolic pathway toward a synthetic pathway using a metabolic toggle switch. Metab Eng. 2014;23:175-184.
39. Zhang F, Carothers JM, Keasling JD. Design of a dynamic sensor-regulator system for production of chemicals and fuels derived from fatty acids. Nat Biotechnol. 2012;30(4):354-359.
40. Mao N, Cubillos-Ruiz A, Cameron DE, Collins JJ. Probiotic strains detect and suppress cholera in mice. Sci Transl Med. 2018;10(445):eaao2586.
41. Fellmann C, Gowen BG, Lin PC, Doudna JA, Corn JE. Cornerstones of CRISPR-Cas in drug discovery and therapy. Nat Rev Drug Discov. 2017;16(2):89-100.
42. Pickar-Oliver A, Gersbach CA. The next generation of CRISPR-Cas technologies and applications. Nat Rev Mol Cell Biol. 2019;20(8):490-507.
43. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359(6382):1361-1365.
44. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439-448.
45. Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, et al. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. Cell. 2016;164(4):770-779.
46. Cho JH, Collins JJ, Wong WW. Universal chimeric antigen receptors for multiplexed and logical control of T cell responses. Cell. 2018;173(6):1426-1438.
47. Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med. 2021;384(3):252-260.
48. Gillmore JD, Gane E, Taubel J, Kao J, Fontana M, Maitland ML, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med. 2021;385(6):493-502.
49. Cwik B. Designing ethical trials of germline gene editing. N Engl J Med. 2020;383(18):1781-1786.
50. Baylis F, Robert JS. The inevitability of genetic enhancement technologies. Bioethics. 2004;18(1):1-26.
51. Ishii T. Germline genome-editing research and its socioethical implications. Trends Mol Med. 2015;21(8):473-481.
52. Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don't edit the human germ line. Nature. 2015;519(7544):410-411.
53. Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR-Cas9-mediated genome engineering. Mol Ther Nucleic Acids. 2015;4:e264.
54. Charlesworth CT, Deshpande PS, Dever DP, Camarena J, Lemgart VT, Cromer MK, et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat Med. 2019;25(2):249-254.

55. Regalado A. Engineering the perfect baby. MIT Technol Rev. 2015 Mar 5.
56. Corbyn Z. Gene-editing research in human embryos gains momentum. Nature. 2015;532(7599):289-290.
57. Brokowski C, Adli M. CRISPR ethics: moral considerations for applications of a powerful tool. J Mol Biol. 2019;431(1):88-101.
58. Cwik B. Moving beyond 'therapy' and 'enhancement' in the ethics of gene editing. Camb Q Healthc Ethics. 2019;28(4):695-707.
59. National Academies of Sciences Engineering and Medicine. Human genome editing: science, ethics, and governance. Washington: National Academies Press; 2017.
60. Nuffield Council on Bioethics. Genome editing and human reproduction: social and ethical issues. London: Nuffield Council on Bioethics; 2018.
61. Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. A prudent path forward for genomic engineering and germline gene modification. Science. 2015;348(6230):36-38.
62. Savulescu J, Pugh J, Douglas T, Gyngell C. The moral imperative to continue gene editing research on human embryos. Protein Cell. 2015;6(7):476-479.
63. Lander ES, Baylis F, Zhang F, Charpentier E, Berg P, Bourgain C, et al. Adopt a moratorium on heritable genome editing. Nature. 2019;567(7747):165-168.
64. Cohen IG, Evitt NH, Adashi EY. The road to CRISPR: ethics pave the way. Curr Opin Urol. 2020;30(3):448-452.
65. Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. eLife. 2014;3:e03401.
66. National Academies of Sciences Engineering and Medicine. Gene drives on the horizon: advancing science, navigating uncertainty, and aligning research with public values. Washington: National Academies Press; 2016.
67. Ingle LP, Ferrell RJ. Biosecurity: a necessary conversation with the life sciences research community. Appl Biosaf. 2020;25(1):9-11.
68. DiEuliis D, Carter SR, Gronvall GK. Options for synthetic DNA order screening, revisited. mSphere. 2017;2(4):e00319-17.
69. Schmidt SM, Belisle M, Frommer WB. The evolving landscape around genome editing in agriculture: many countries have exempted or move to exempt forms of genome editing from GMO regulation of crop plants. EMBO Rep. 2020;21(6):e50680.
70. Callaway E. CRISPR plants now subject to tough GM laws in European Union. Nature. 2018;560(7716):16.
71. Cyranoski D, Ledford H. How the genome-editing revolution could unravel. Nature. 2018;564(7734):22-24.
72. Hurlbut JB, Jasanoff S, Saha K, Ahmed AKM, Appiah A, Bartholet E, et al. Building capacity for a global genome editing observatory: institutional design. Trends Biotechnol. 2018;36(8):741-743.
73. Adli M. The CRISPR tool kit for genome editing and beyond. Nat Commun. 2018;9(1):1911.

74. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360(6387):439-444.
75. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444-448.
76. Wu X, Scott DA, Kriz AJ, Chiu AC, Hsu PD, Dadon DB, et al. Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Nat Biotechnol. 2014;32(7):670-676.

Aca. Intl. J. E. Sci. 2024; 2(2) 31-46. **DOI**: https://doi.org/10.59675/E224