Synthetic Biology- A New Emerging Field

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ABSTRACT
Synthetic biology is one of the new, advanced and emerging field in the science. It is an interdisciplinary field which includes life sciences, bioinformatics, engineering, biotechnology and medical sciences and other field so as to provide the therapeutic effect in diseases in human beings. Rather than that it also comprises of different types of tools so as to predict the nature of the genome of the organism, biogenetic pathway, metabolites, nature of genes, proteins and their metabolic data in the different organisms. Recent work is going on the switches and memory circuits which are playing an important role in the therapeutic effect of tumors, cancer and in the autoimmune disorders as they are working in a guided and synchronized fashion. By use of this field the cost of the treatment is decreased and the therapeutic effect is increased and also the adverse drug reactions are significantly decreased.

Keywords- Synthetic biology, biological tools, genome, therapeutic effect, switches, memory circuits

INTRODUCTION
Synthetic biology is a new field in which there is insertion of the genetic network into the cell of the desired trait so as to increase the functionality of the cell. Earlier the studies were based on standardization of network components to enhance the transfection efficiency and applications in prokaryotes cells (1, 2) but the more recent application are emerging in yeast and mammalian cell (3-5). It involves the study and understanding of biological systems by making biochemical pathway and building computational model so as to stimulate the behavior of pathway (6, 7). The shift towards the engineering model of experimentation had showed us the architecture of biological networks (8) and it doesn’t integrate with older method especially in vitro biology. In vitro biology is an area which employs complex biosynthesis, direct evolution and reconstitution of biological function (9-12). Synthetic biology is a field which helps in the study of complex mechanism, behavior of genes, protein, biochemical network, their pathways and physiological responses within whole system (13). As a result system toxicology was used to elaborate system biology approaches to toxicological studies (14). It involves large amount of data from reliable sources which includes genomic, biochemical, proteomic and metabolic data and this data is added into the computational models so as to study the quantitative and qualitative study of the biological system under different type of condition (15, 16). The merit of this approach is that the researcher can model a large no of complex biochemical reactions which occur simentenously (17). More than that they make mathematics an important and indispensible tool in biosciences as
(i) They are small and well defined and tractable as they have been used in mathematical models
(ii) They are enough flexible and can be merged to build logical information for biological systems.
(iii) They are human designs hence avoiding historical explanations and other dependencies (18).
Computational modeling is those in which the mathematical models and biological systems are integrated and is interface between different fields of system and synthetic biology. Recent advances have been made in the field of synthetic genetic circuits (19), genetic promoters (20), proteins (21) and different synthetic biomolecules(22).

Tools and technologies which enables synthetic biology for fabrication
1. Standardised cloning: At present cloning is one of the most important tool in synthetic biology. Generally PCR is used for cloning in which a part of the DNA is taken and adapted and is combined to the vector according to specifications at different cloning sites. Insertion of different elements from vectors with different restriction sites structure, insertion of point mutation for different restriction sites which requires
large no of manipulations so this current system is laborious and to some extent in efficient. This step can be improved by introduction of standardized vector format which will get assembled at particular restriction site for cloning and allows interoperability of the assembled sequence eg idempotent vectors or NOMAD technology (23). The vectors are designed in such a manner that introduction of DNA fragment into vector is same as that of the restriction architecture and by this way a large no of insertion can be made on either side of the insert. By this fashion of using the standard vector a no of exchanges can be made and this fact had been realized by “Registy of Standard Biological Parts by Massachusetts Institute of Technology “working group of synthetic biology. This registry provides the information of characterized parts which have been formatted according to specific rules. The registry organization into basic DNA parts such as promoters, ribosomal binding sites (RBS) and coding sequence is an easier approach to obtain suitable genetic elements for a specific purpose so that experimental outcomes can be achieved.

2. Denovo synthesis:- A no of improvements have been made in the standardization and organization but the system assembly is the biggest issue in synthetic biology project. The problem arises that for the production of system requiring 10kb (existing gene network) or 100kb (genome reprogramming) of novel DNA sequence. This technology enables us to assemble all the desired changes from promoter strength to codon usage into a sequence which can be made available in days or in weeks. Denovo synthesis had performed assembly overlapping short (25-70bplong) and chemically synthesized oligonucleotides into longer DNA fragments in a PCR based assembly process (24). The practical application of this technology is constructions of small phage genome like polio virus (25). There are two demerits of this type production are that the synthesis of DNA oligonucleotide by standard phosphoamidite chemistry and limitation accuracy of chemical synthesis (26). In case of denovo reconstruction of phage ΦX174 genome which is relied on 2 fold selection process (1) oligonucleotides were gel purified for correct length (2) and assembled DNA was recovered from plaques after transformation for biological function in synthetic biology (27). Currently the procedure involves three process which are as follows

(i). Miniaturizing oligonucleotide production (ii) verification cost (iii) removal of false oligonucleotide by enzymatic and or hybridization methods

i. Miniaturizing- is the utilization of microfluidcs-based arrays for synthesis. It employs reduction in the cost but also optimized reaction conditions and hence reduces the error frequencies (28). also oligonucleotide synthesis also done on the photoprogrammable chips (29).

ii. Verification cost- as the length of oligonucleotide is increasing the error frequency also also increases and the cost thereof. The estimated length is 40bp which is cheap and also from here where the optimal assembly starts and from there intermediary 500bp sythons are assembled resulting into 5kb fragments (30).

iii. Removal of false nucleotide- different technologies can be used for the removal of the errors. In case of two synthesised complementary nucleotide generally have mutation in their complementary positions hence leading to mismatch and these points serve as starting points for digestive enzymes (31, 32, 26). The oligonucleotide containing error can be removed by heating the oligonucleotide designed to hybridise at specific temperature and then control the temperature during hybridization which leads to removal of mismatches and the current accuracy at error rates are 1in 1400 bp (29).

3. Engineering Chasis-After the design and synthesis now it is incorporated in the specific organism by the help of engineering technology (33) or by mega novel size cloning stragies (34). In synthetic biology complexity is one of the biggest problems and can be reduced by minimizing the metabolic capabilities. A true minimal genome comprised of minimum set of genes necessary for a cell to propagate under specific environmental conditions to start and engage in complex systems (35). synthetic and defined medium requires as few as 206 genes which comprises DNA replication, transcription, translation, DNA repair function, protein processing and degradation, cell division, energy division (36). it can be obtained by reducing the large genome into smaller one and is incorporated into organisms for the expression by using these biological tool or work have been done on that very small genome in an organism. In the latter case non pathogenic Mesoplasma florum of genome size 793 kb being established as chasis. Its genomic sequence is now available and molecular biology methods are developed. A similar approach had been followed for Mycoplasma...
gentialium for which extensive data on non essential genes are available (37). In E.Coli genome has been reduced in different projects by 6 % (38),8%-33%)or 15% (39)without any noticeable effect on the investigated physiological properties and by 30% resulting in cell defects in cell replication (40). Bacillus subtilis genome was found to be reduced by 8% with only small effects in physiology (41) confirming that the hypothesis under controlled condition a part of bacterial genome is indeed dispensable

Practical applications in human beings

Synthetic biology is one new modern technology which is playing an important role in human being especially in case of human being as it is providing therapeutic effect to the subjects. Gene networks are playing an important role in the diagnosis or in the treatment of the disease. It tracks the fate of the cell in the human being by retaining memory of exposure to the stimulus (42). A short stimulus in memory circuits of doxycycline, UV for short period of time up regulates the expression of synthetic transcriptional factor which retain for a long period of time by the positive feedback loop. These memory circuits can alter the gene expression, growth rate and cell viability for several generations after the stimulus. With toggle switches these networks enables us in monitoring of hypoxic region of non dividing tumor cells. Newer circuits have also been designed for the detection of cell line lineages (43)

References


