Chapter

# Introductory Chapter: The Role of Genetic Engineering Technology in the Manipulation of Genetics of Organisms and Synthetic Biology

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## 1. Introduction

Synthetic biology is a new interdisciplinary science that involves synthesis of biological components, systems, and organisms using genetic engineering technology. The manipulation of DNA or the introduction of DNA of one organism into another organism leads to synthetic biology.

The manipulation of the genomes of the organisms is revolutionizing the genetics of the organisms. The cloning of the genes is an important genetic engineering technology and is required for studying the biological properties of the genes, the DNA fragments, and the organization of the genomes. Other techniques that are part of the genetic engineering technology are isolation of DNA, restriction digestion of the DNA, ligation, sequence analyses, and expression.

What
Genetic engineering technology
Does
Turns
Biology to medicine
Genes off and on
Controls gene expressions
Produces transgenic organisms
Synthetic organisms
Organisms with new genes
Modified genes
Man-made genes and genomes
Man-made organisms
Imagine
Man-made men and women
With superintelligence
Superstrength
Super-synthetic biology
Engineered synthetic biology

The genes have specific chromosomal loci and are determined by linkage analysis and deletion mapping. The eukaryotic genes are too large and have introns and exons. The gene is transcribed into mRNA which is further translated into proteins, and the proteins perform various functions in the body.

Genes define the molecular biology of the normal as well as the abnormal states of an organism. Mutations in genes can cause serious changes in the organism. During cell division, DNA is copied, and sometimes, the copy differs in some of the deoxynucleotides, and this is called mutation. Most of the mutations occur naturally and they could lead to evolution. Mutations are also caused by many other ways, such as exposure to chemicals or radiations, and the cell of the organism ends up with DNA slightly different than the original DNA. The DNA damaging agents induce a set of responses, termed as "SOS" in *Escherichia coli*, which involve the transcriptional activation of various genes required for DNA repair. In the yeast *Saccharomyces cerevisiae*, a large number of DNA repair genes have been identified.

The yeast, *Saccharomyces cerevisiae*, is used as a model organism for studying fundamental aspects of eukaryotic cell biology [1]. For understanding the molecular mechanism of DNA repair in eukaryotes, yeast was chosen as a model system to study the role of radiation-repair (*rad*) genes during the cell division cycle [2]. Plasmids containing various RAD-lacZ gene fusions were integrated into the chromosome of haploid yeast cells, and the integrated strains were examined for the expression of beta-galactosidase after treatments with ultraviolet radiation (UV) or 4-nitroquinoline-1-oxide (NQO). It was found that the functions controlled by RAD genes are essential for normal meiosis and DNA replication and recombination [3]. Thus, geneticists can easily manipulate yeast gene expression and study the resulting phenotypic effects. RAD51 gene of yeast is a homolog of RecA gene of *E. coli*. RecA catalyzes repair and recombination and induces SOS response in *E. coli*. SOS system was first described by Miroslav Radman after the distress signal "SOS" in the Morse alphabet (three dots, three dashes, three dots) (https://www.sciencedirect.com/topics/neuroscience/sos-response).

Transfection experiments are carried out in studying the role of viruses in causing diseases. Viruses cause a variety of infectious diseases, and various infections have been linked to atherosclerosis and coronary heart disease [4]. By preparing cDNA and subtraction cDNA libraries from explant and transformed cells, the mechanism of transformation and the role of viruses were studied in causing atherosclerosis [5, 6]. Viruses can precipitate various conditions, including coronary heart diseases and chronic kidney diseases. Understanding the mechanisms could lead to the new areas of investigation and overall cellular pathophysiology.

Gene expression is regulated from DNA to RNA transcription and further to the posttranscriptional modifications of the protein. The expression and regulation of insulin-like growth factor-binding proteins (IGFBPs) -1, -2, -3, and -4 mRNAs in purified rat Leydig cells and their biological effects were studied. The actions of insulin-like growth factors (IGFs) are modified by IGFBPs. IGFBPs may potentiate or inhibit the effects of IGFs on cell growth and protein synthesis. Leydig cells are found in the testis, and the major physiological role of Leydig cells is to produce androgens required for spermatogenesis and development of male characteristics. The testis is a dynamic tissue, containing many cell types that produce a variety of compounds that affect Leydig cell function. The pituitary dependency of IGFBP mRNA expression in Leydig cells was studied by isolating and culturing rat Leydig cells from normal male Sprague Dawley rats (50-day-old) and from 50-day-old rats 5 days after hypophysectomy. The results revealed that on hypophysectomy, IGF-I, IGFBP-2, IGFBP-3, and IGFBP-4 mRNA expressions were reduced in Leydig cells, suggesting that their expressions were pituitary dependent. It was also shown that multiple species of IGFBPs are expressed in Leydig cells and that IGFBPs modify the effect of IGF-I on Leydig cell function [7]. Another good example of gene regulation and expression is the process of erythropoiesis. The erythroid cells exhibit patterns of gene expression that direct a precise ensemble of proteins required for cellular structure and function. Erythroblasts decrease in size, cells produce large

amounts of hemoglobin, and membranes undergo reorganization and enucleation. Erythropoiesis yields a highly specialized cell type, the mature erythrocyte to meet the organismal needs of increased oxygen-carrying capacity [8].

The cloning of a gene and studying its expression are an important part of biotechnology. It leads to understanding of the functions of the gene in the living organisms and by modifying the cloned gene, could produce novel compounds and new functions in the cells or the living organisms.

A gene was cloned that was differentially expressed in normal adult rat Leydig cells and whose expression was inhibited by *human chorionic gonadotropin* (hCG) but was induced by interferon-gamma (IFN-γ). DNA sequence analysis identified this gene as rat IFN-γ-inducible protein 10 (IP-10), a member of the CXC chemokine superfamily of proinflammatory cytokines. It was found that high levels of IP-10 messenger RNA (mRNA) were constitutively expressed in freshly isolated and primary cultured Leydig cells and may have paracrine and autocrine effects on testicular function [9]. The effects of overexpression of cytokine-responsive gene-2 (CRG-2) (systematic name CXCL10, also known as interferon-γ-inducible protein 10 (IP-10)) on MA-10 mouse were studied in Leydig tumor cell steroidogenesis and proliferation. Chemokines have been implicated in tumor growth, angiogenesis, metastasis, and the host immune response to malignant cells. CXCL10 is a potent chemokine expressed predominantly by macrophages and Leydig cells in the testis. CXCL10 belongs to a large family of chemotactic cytokines, now termed "chemokines," that are expressed in diverse cell types wherein they regulate innate and adaptive immune responses [10]. Chemokines are chemotactic factors and growth regulators, which exert their effects through seven transmembrane domain or G protein-coupled receptors [11, 12]. CXCL10 binds to CXCR3 receptor (a G protein-coupled receptor) and acts via Giα protein. The purpose of this study was to determine the effects of overexpression of CXCL10 by transfection experiments in MA-10 cells on cell growth, CXCR3 expression, progesterone biosynthesis, and steroidogenic acute regulatory protein (StAR D1, a key regulatory factor in steroidogenesis) gene expression. The complete CXCL10 cDNA was cloned in a mammalian expression vector with the CMV promoter, pcDNA3.1D/V5-His-TOPO, and its expression was confirmed with rat CXCL10 antibody and V5 antibody. Results showed large amounts of CXCL10 protein secreted in the medium in the CXCL10 transfectants by Western blotting. The production of CXCL10 mRNA ranged from 30- to 50-fold more ($n = 6$) in the transfected cells than the control cells, as determined by semiquantitative and real-time RT-PCR. 8-Br-cAMP downregulated CXCL10 mRNA expression and stimulated CXCR3 mRNA expression. Transfection of MA-10 cells with CXCL10 decreased cAMP-induced progesterone synthesis from 38.5 ± 1.7 ng/ml ($1.5 \times 10^5$ cells/ml) in control cells to 23.2 ± 1.5 ng in transfected cells ($p < 0.01$). 8-Br-cAMP (0.2 mM)-induced StAR D1 mRNA was decreased 30–40% by transfection with CXCL10. Interestingly, overexpression of CXCL10 induced the expression of its receptor CXCR3 gene, as determined by RT-PCR and fluorescence-activated cell sorter (FACS) analysis. Transfection of CXCL10 also significantly decreased [$^3$H]thymidine incorporation into DNA. These data suggested that CXCL10 inhibited StAR D1 expression, decreased progesterone synthesis, and inhibited cell proliferation. CXCL10 has the potential to be used in gene therapy for prostate cancer due to its antiangiogenic effect and its inhibitory effect on steroidogenesis [13]. CXCL10 may be potentially useful in the treatment of prostate cancer. The experiments were designed to study the effects of overexpression of CXCL10 in human prostate LNCaP cells on CXCR3 gene expression and inhibition of cell proliferation. LNCaP cells were transiently transfected with CXCL10 cDNA in pIRES2-EGFP vector. CXCL10, CXCR3, prostate-specific antigen (PSA), and *glyceraldehyde 3-phosphate dehydrogenase* (G3PDH) mRNA levels were determined by semiquantitative conventional and quantitative real-time RT-PCR, and

protein expression was determined by fluorescence-activated cell sorting (FACS). The expression of CXCL10 was markedly enhanced in the transfected cells at mRNA and protein levels in the cells. Overexpression of CXCL10 inhibited cell proliferation of the transfected cells by 30–40% in serum-limited medium (1% FCS in RPMI1640 medium) and decreased PSA production. CXCR3 expression was significantly induced by the overexpression of CXCL10 as determined by RT-PCR and FACS. These results indicated that CXCL10 inhibited LNCaP cell proliferation and decreased PSA production by upregulation of CXCR3 receptor. In conclusion, overexpression of CXCL10 may be potentially useful in the gene therapy of prostate cancer [14].

The identification of unknown organisms is carried out on genetic bases, whereas the molecular and chemical characteristics provide the complementary information. The DNA barcoding is one of the technologies for species classification and identification of unknown organisms. DNA barcoding involves tissue sample collection, DNA isolation, amplification of the isolated DNA, sequencing, and analyses of the results in order to identify the organism. The goal of DNA barcoding is to identify biological specimens using a short specific region of DNA. For example, a short fragment (658 bp) of mitochondrial gene cytochrome c oxidase I (COI) DNA from a single individual when amplified by polymerase chain reaction (PCR) was 100% successful in correctly identifying 200 closely allied species of Lepidoptera specimens [15]. Stoeckle and Thaler [16] pointed out that DNA barcode differences within animal species are usually much less than the differences among species, making it generally straightforward to match unknowns to a reference library. The barcodes could provide new insights and innovations into the evolution and diversification of life.

PCR is a technology to amplify a specific piece of DNA or gene and is used for analyses of genetic diseases, genetic fingerprinting, monitoring microbiological quality of food, detection and diagnosis of infectious diseases, and for many other uses. PCR is used to create copies of DNA for introduction into host organisms such as *Escherichia coli* in genetic engineering and to amplify stretches of genetic material for Sanger sequencing. PCR is used in archaeology, to identify human or animal remains, including insects trapped in amber, and to track human migration patterns; degraded DNA samples may be able to be reconstructed during the early cycles of PCR. PCR can be used to differentiate between similar organisms or work out relationships between different species (https://www.xxpresspcr.com). PCR technique was developed by Kary Mullis and his group in the 1980s, and in 1993, Kary Mullis and Michael Smith were awarded Nobel Prize in Chemistry for their work. The PCR technique involves the synthesis of a new strand of DNA complementary to the template strand using DNA polymerase and a set of primers. Since then a lot of progress has been made in this technique. Real-time PCR has been developed that monitors the progression of the amplification in real time by using fluorescent signaling as the amplification occurs [17].

Using PCR technology, ribosomal RNA (rRNA) 16S–23S interspace region sequence variability in bacterial species was studied [18]. Two distinct genetic clusters within the species *Bacillus subtilis* and *Bacillus atrophaeus* were selected, and it was concluded that *B. atrophaeus* is distinct genetically from *B. subtilis* subgroups represented by W23 and 168, respectively. This was the first study to make sequence comparisons at the genome, strain, and species level for rRNA interspace region. Considerations of this issue will be important in using ribosomal space region (RSR) methodology to differentiate other closely related bacterial species.

Fox et al. [19] identified *Brucella* spp. by RSR PCR molecular technology and chemical characteristics as complementary information in the differentiation of closely related organisms. PCR products were unique to brucellae, allowing them to be readily distinguished from other gram-negative bacteria (including *Bartonella*

spp. and *Agrobacterium* spp.). Carbohydrate profiles differentiated *B. canis* from the other three *Brucella* species due to their absence of the rare amino sugar quinovosamine. PCR of the rRNA region was useful in identification of the genus *Brucella*, while carbohydrate profiling was capable of differentiating *B. canis* from the other *Brucella* species. Species differentiation can also be achieved by determination of approximate size of PCR products of intergenic spacer region (ISR) of 16S–23S rRNA based upon their relative electrophoretic mobility on agarose gels [20].

One of the major properties of genes is their expression pattern. Notably, genes are often classified as tissue specific or housekeeping [21]. Tissue-specific gene expression experiments in adult male rat (55- to 65-day-old) tissues revealed that glucose transporter 8 (GLUT8) was expressed predominantly in the testis, in smaller amounts in the heart and kidney, and in negligible amounts in the liver and spleen. GLUT8 mRNA was found to be highly expressed in crude interstitial cells, Leydig cells, and testicular and epididymal germ cells. In prepubertal rat (20-day-old) tissues, GLUT8 expression was comparatively much lower than in the adult rat tissues. By comparative reverse transcriptase PCR (RT-PCR), human chorionic gonadotropin (hCG) caused dose- and time-dependent increases of GLUT8 mRNA levels. hCG and IGF-I had synergistic effects on GLUT8 mRNA and protein expression. GLUT1 and GLUT3 were also found to be expressed in Leydig cells. However, neither GLUT1 nor GLUT3 was affected by treatments with hCG, IGF-I, or hCG and IGF-I combined. The addition of murine interleukin-1α (mIL-1α; 10 ng/ml), murine tumor necrosis factor-α (mTNF-α; 10 ng/ml), and murine interferon-γ (mIFN-γ; 500 U/ml) separately or in combination decreased hCG-induced GLUT8 mRNA levels significantly. In conclusion, GLUT8 mRNA in Leydig cells was positively regulated by hCG and IGF-I and downregulated by cytokines, mIL-1α, mTNF-α, and mIFN-γ. These results indicated that hCG, growth factors, and cytokines affect Leydig cell steroidogenesis by modulating GLUT8 expression [22]. Another example of tissue-specific gene expression is that in liver tumorigenesis, it has been shown that pyrroline-5-carboxylate reductase (PYCR1) gene is upregulated and the inhibition of PYCR1 may be a novel therapeutic strategy to target liver tumor cells [23].

This book provides deeper understanding of the recent progresses in biotechnology and genetic engineering contributing to synthetic biology and biological processes. The readers will appreciate the significance of the diverse nature of the articles and the eminence of the authors covered in this book. I hope this book gives you enough information on very specific topics and inspires you to further explore and re-invent the subjects in synthetic biology—a new interdisciplinary science.

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