

Preface

Somatic genome manipulation is revolutionizing medical and biological sciences. This has applications when the conventional sexual crossing approach cannot be used in breeding or genetic treatment of an individual organism. Examples can include gene or cell therapy of a person to correct disease, genetic improvement of vegetatively propagated plants, and genetic replacement of cytoplasm without significantly modifying the nuclear genome. The advantage of somatic genome manipulation is preservation of the genotype while improving select trait(s). Somatic genome manipulation is also an option for genetic improvement of seed propagated plants in overcoming issues of sexual incompatibility or infertility.

Our aim in writing this book was to bring together previously fragmented information on novel technologies in somatic genome manipulation. These technologies are developing quickly across a broad range of disciplines affecting humans and animals, plants, and microorganisms. This book represents the first attempt to assemble updated reviews, detailed protocols, and far-reaching applications in somatic genome manipulation. The chapters are written by 34 experts (physicians, professors, research scientists, research chairs, Doctors of Philosophy, Doctors of Medicine, etc.) on the topic with ready-to-use protocols that were originally developed or adapted from the literature in their laboratories. The book is divided into three major sections: I. Humans and animals; II. Plants; and III. General experimental and bioinformatic technologies.

Section I. Humans and Animals

Drug and gene delivery by electroporation is described for delivery of chemotherapeutic agents in cancer trials. This method (electrochemotherapy) may increase local effects on tumors, locally activate the immune system, or produce transgenic proteins followed by secretion to the systemic circulation. Gene therapy offers a relatively easier and less expensive strategy for therapy than pharmaceutical approaches, since DNA may be produced more easily than formulation of protein drugs; enabling a higher level of access to new potential pharmaceuticals.

Targeted porcine genome engineering with transcription activator-like effector nucleases (TALENs) enables precise editing (e.g., mutations or indels) or insertion of a functional transgenic cassette to user-designed loci without disturbing the general gene background of the individual. The three most promising approaches are reviewed, including TALENs, zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems using a model of genetically modified pigs.

Somatic gene therapy can also be effective using viral vectors. For example, lentivirus and adeno-associated virus-based technology has applications in clinical trials for treatment of inherited eye diseases, immunodeficiency syndromes, and hemophilia. The gene therapy community has succeeded in turning infectious agents into vehicles of therapeutics for treatment or amelioration of the disease phenotype, giving significant reassurance that gene therapy will become standard care for a number of individual disorders.

Nonviral gene delivery methods are also in use. The current status of nonviral gene transfer is reviewed, focussing on DNA and its mobilization. The major barriers to nonviral gene delivery are discussed and the potential of minicircle DNA, devoid of bacterial DNA, and the adaptation of DNA transposable elements for genomic gene insertion, are described. A short glimpse into current nonviral gene therapy trials, with particular focus on current attempts to treat cystic fibrosis, is also provided.

Stem cells, because of their nature, are currently considered the most suitable cells for cell therapy. Combining gene therapy with stem cell therapy provides an additional useful dimension to the use of stem cells for treatment. The potential use of gene-modified stem cells, in particular gene-modified mesenchymal stem cells (MSC), in therapy and the challenges facing their use in clinical practice are reviewed.

Transgenic animals, particularly genetically modified mice, have been instrumental in biomedical and genetic research. To facilitate translational research to humans, the development of larger species of transgenic animals is necessary. These have numerous possible applications including the development of higher quality production animals, creation of stem cells for tissue repair (therapeutic cloning), production of protein-based pharmaceuticals (animal pharming), creation of organ donors for xenotransplantation, and the creation of large animal models for biomedical research. Various techniques to produce genetically altered animals are reviewed.

Section II. Plants

Apomixis is the clonal production of a plant through seed; a naturally occurring trait. Studies of both naturally occurring apomicts and mutants of sexual species that mimic the component events of apomixes have revealed potential mechanisms

of control, the possible evolutionary origins of apomixes, and the impact it has had on the evolution of species and genomes. Apomixis leads to the formation of genetically uniform populations that can persist over many seedling generations. Important applications for agricultural crops are discussed.

The use of somatic embryogenesis in potato improvement programs is highlighted and discussed with emphasis on variants identified in cultivar Russet Burbank. Potato somatic embryogenesis is reviewed, including explant types, media components, effect of various growth regulators on the initiation and production of somatic embryos, and genes known to control somatic development.

The history of somatic hybridization use for modification of the cytoplasmic male sterility (CMS)-inducing *Ogura* radish cytoplasm and its application in hybrid seed production for *Brassica* crops is recounted. Highlights include cybrid production from early protoplast fusion experiments and identification of the mitochondrial gene causing CMS. This fascinating story fully explains the Ogu-INRA system, which is now widely used in agriculture.

Protoplast fusion has emerged as an exceptional breeding tool that has successfully produced a large number of intergeneric, intertribal, or interfamily somatic hybrids. The fusion of isolated protoplasts from somatic cells and regeneration of hybrid plants from the fusion products (somatic hybrids) allow combining of complete genomes of two desirable parents, irrespective of their taxonomic relationship. Procedures for protoplast fusion of potato to produce somatic hybrids are included, along with detailed lists of materials, and full descriptions of techniques.

Virus diseases inflict substantial economic losses to major crops by reducing yield and compromising quality. RNA silencing using, e.g., self-complementary hairpin RNA (hpRNA) or artificial microRNA (amiRNA), is an effective method to produce plants that are resistant to specific viruses. By targeting highly conserved viral sequences or several virus genes simultaneously using chimeric constructs, this method can counter multiple viruses and minimize any loss of viral resistance resulting from viral mutation. Due to public concerns about transgenic plant safety, a nontransgenic RNA silencing approach was used to directly deliver hpRNA into plant tissues to induce plant resistance to viruses.

Recent advances in genome engineering provide plant biologists with an important tool for understanding gene function and developing new traits. Three powerful techniques, including TALENs, ZFNs, and RNA-guided endonucleases (RGENs), have been developed for targeted DNA sequence modifications in plants. These sequence-specific nucleases create double-strand breaks (DSBs) in the genomic target sites that are primarily repaired by the nonhomologous end joining (NHEJ) or homologous recombination (HR) pathways, which can be employed to achieve targeted genome modifications such as gene mutations, insertions, replacements, or chromosome rearrangements. Considerable efforts have been made to understand the mechanisms governing gene targeting and to establish efficient DNA delivery systems to achieve precise gene targeting in plants.

Section III. General Experimental and Bioinformatic Technologies

Mitochondria are key players in cellular metabolism and energy production. Mitochondrial DNA mutations or rearrangements cause incurable neurodegenerative diseases in humans or cytoplasmic male sterility in plants, so manipulation of mitochondrial genetics is of particular relevance. The current challenge in the field is to define consensus biotechnological tools. Various procedures for manipulating mitochondrial genomes are reviewed and their promise discussed.

A wide range of laboratory techniques in somatic genome research are described, for research and training purposes, including: (1) in situ hybridization for studying tissue-specific gene expression; (2) mitochondrial visualization using rhodamine staining and confocal microscopy; (3) differential preparation of *Agrobacterium* Ti plasmid and binary plasmid using a noncommercial kit; (4) *Agrobacterium* binary plasmid DNA preparation using a commercial kit; (5) isolation of nuclei for DNA preparation; (6) chloroplast DNA extraction; (7) mitochondrial DNA extraction; (8) total DNA/RNA preparation; (9) enriched mitochondrial RNA preparation; (10) high-resolution DNA melting analysis for studying gene expression; and (11) transcriptome electrophoretic fingerprinting.

Bioinformatic analysis is critical for studies using huge amounts of DNA, RNA, and protein sequences. Various bioinformatics approaches developed or tested in the author's laboratory are described. These approaches include: (1) a statistical method for gene direction analysis, (2) some technical highlights for genome and chromosome base composition analysis, (3) some technical highlights on RNA polyadenylation site analysis; (4) allele comparison for protein domains, and (5) protein network analysis. Unsolved technical issues are highlighted and potential future research directions are discussed.

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Li, X.-Q.; Donnelly, D.J.; Jensen, Th.G. (Eds.)

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