Preface

"Advances in Genetics Research" presents original research results on the leading edge of genetics discovery. Each article has been carefully selected in an attempt to present substantial research results across a broad spectrum. In this continuing series compilation, the authors present and discuss the development of multifunctional plasmids for diverse biotechnological applications; fragile x syndrome-associated language phenotypes; study of the fmr1 gene structure among women with ovarian dysfunction from the Basque country; chromatin structure and epigenetics; the evolution of mammalian x chromosomes and x chromosome inactivation; modifying effect of smoking on the association between SLE and the genetic polymorphisms involved in ROS production; therapeutic approaches to fragile x syndrome; genetic diversity and phylogeny of wild specie teosinte within genera *Zea*.

Chapter 1 – A bewildering variety of plasmids have been developed for a wide array of purposes in biotechnology and biomedical research. These purposes include transient and stable protein expression, mutation, recombination-mediated nuclear gene knockout, promoter analysis, RNA stability, targeted RNA degradation, and gene therapy. Each plasmid has been optimized for only one of these functions, and consequentially, convenient restriction enzyme sites surround only one genetic element. However, an optimal expression system often requires the use of different genetic elements that are rarely found on the same plasmid (such as differing promoters or eukaryotic antibiotic resistance), and exchange of these elements between plasmids is difficult. The authors retrofitted pcDNA3 (an archaic but well-validated and useful plasmid from Stratagene, Inc.) to facilitate the exchange of various genetic elements by inserting useful restriction endonuclease sites at the borders of these elements. With the retrofitted plasmid, called pc3.5, the authors can exchange not only the ectopically expressed gene of interest but also promoters or polyadenylation sites of the gene of interest, as well as the eukaryotic antibiotic resistance gene, its promoter or polyadenylation sequences. The authors have used derivatives of these plasmids for transient and stable gene expression, promoter analysis and induction, and mesenchymal stem cell engineering. Retrofitting plasmids is a simple process than can be applied to a wide variety of plasmid or viral vector systems to diversify and optimize their utility.

Chapter 2 – Language impairment is an integral part of Fragile X syndrome (FXS), the most common inherited cause of intellectual disability, and the most common single gene disorder associated with autism. Genetic alterations, most frequently expansion of an intragenic CGG repeat, that affect the expression of the Fragile X Mental Retardation Gene

(FMR1) result in deficiency or aberrant functions of the RNA-binding FMRP protein that regulates the stability, transport, storage, and translation of a range of brain-expressed mRNAs and, significantly, those involved in synaptic development. As a consequence of these functional deficiencies, FXS subjects demonstrate aberrant neuronal circuits and reduced brain network connectivity and display weaknesses across a range of cognitive and language domains with considerable individual and genderdependent variations that are also shaped by environmental influences.

Chapter 3 – Primary ovarian insufficiency (POI) is an ovarian dysfunction defined as irregular menses and elevated gonadotrophin levels before or at the age of 40 years. Several genes have been reported as having significance in POI but the Fragile X Mental Retardation 1 (FMR1) is one of the most important genes associated with it. FMR1 intermediate (35-54 CGGs) and premutation (55-200 CGGs) alleles have been related with the development of this condition. A group of 68 women with ovarian dysfunction of unknown aetiology and 47 control women from the Basque Country was analyzed. Two important factors implicated in the stability of the CGG repeat were evaluated: the length of the repeat and the AGG interspersion pattern. In relation to the number of CGG repeats, the frequency of alleles with ≥35 CGG repeats (intermediate and premutation alleles) was statistically higher in the whole patient group (14.71% vs. 0%). The patient group was also divided into three categories based on their ovarian condition: group 1, irregular cycles, reduced fecundity and FSH levels <10 IU/L; group 2, irregular cycles reduced fecundity and FSH levels ≥10 IU/L and group 3, amenorrhea for at least 4 consecutive months and FSH levels ≥10 IU/L. In the three subgroups the frequency of alleles with ≥35 CGG was also statistically higher than in controls. Regarding the CGG repeat internal structures, the data showed that many of the intermediate and premutation alleles found in the whole patient group and in the three subgroups appeared to have two interruptions (70%), the first AGG located in the 10^{th} position (75%) and more than 15 CGG at the 3' end (83.3%). Interestingly, among these alleles the predominant structure was 9+9+n, indicating a loss of AGG interruptions at the 3' end. Therefore, the data showed that among patients the alleles were more unstable and that this instability influencing the FMR1 expansion might be related with the development of an ovarian dysfunction.

Chapter 4 – The term "chromatin" was first referred to the darkly stained DNA in the nucleus in early 1880s. Over the past century, much meaning has been added to the term chromatin with regard to its structure, organization and function. During the last two decades the field of chromatin has evolved in an incredible pace with the utilization of high throughput techniques. These comprehensive studies reveal that chromatin is no longer a static structure but is changing dynamically in order to regulate nuclear processes including DNA replication and gene transcription. The dynamic nature of chromatin is ruled by several epigenetic mechanisms such as nucleosome remodelling, histone post-translational modifications and DNA methylation. Recent studies in the chromatin field provide insights to the detailed mechanisms underlying the regulation of gene expression by structural and chemical changes in chromatin. This chapter reviews recent advances in chromatin research and epigenetic mechansisms, intending to provide a comprehensive and detailed understanding on how chromatin structure is organized and dynamically modified in order to regulate gene expression.

Chapter 5 – For many years it was thought that the X chromosome in all three major lineages of mammals (monotremes, marsupials and eutherians) had a shared evolutionary

history. However, monotreme and marsupial genome projects and the employment of molecular cytogenetic techniques have enabled the evolutionary history of mammalian X chromosomes to be more accurately traced. This research revealed that monotremes have a complex sex chromosome system consisting of multiple X chromosomes that share no homology to the X chromosome of therian (marsupial and eutherian) mammals. Therian sex chromosomes arose from a pair of autosomes with a subsequent addition to the X chromosome having occurred in the eutherian lineage.

The evolution of mammalian sex chromosomes has led to large, gene-rich X chromosomes and smaller, gene-poor Y chromosomes, which is thought to create an imbalance in transcriptional output between the X chromosome and autosomes in males that, requires dosage compensation. This hypothesis has only been thoroughly interrogated since the advent of microarray and RNA-sequencing technologies but the findings of these studies remain controversial. However, it is more generally accepted that the dosage imbalance for X-borne genes between the sexes requires some form of compensation; otherwise females would be expressing twice as much of an X-linked gene as their male counterparts. Compensation for this imbalance was deemed essential, as an extra copy of autosomal genes is usually deleterious. It has been generally accepted that in eutherian mammals, this is achieved by transcriptionally silencing one X chromosome in female somatic cells. Fifty years of research has uncovered many of the features of this remarkable epigenetic phenomenon. However, research into X chromosome inactivation in marsupials and monotremes has lagged far behind that of their eutherian counterparts, largely due to the limited knowledge of genic content of their X chromosomes. Fortunately, genome sequencing of the platypus and several marsupial genomes has alleviated this problem and enabled such research to forge ahead at a great pace. This has led to a number of seminal findings and new hypotheses regarding the evolution of X chromosome inactivation.

Chapter 6 – Exposure to reactive oxygen species (ROS) via cigarette smoking is thought to contribute to the development of systemic lupus erythematosus (SLE). ROS increase immunogenicity of DNA, LDL and IgG, generating ligands for which autoantibodies show higher avidity. To evaluate modifying effect of the genetic polymorphisms in ROS production on the association of cigarette smoking with SLE risk could be important for understanding of the pathogenesis of SLE. The relationship of the genetic polymorphisms of cytochrome P450 (*CYP*) *1A1* rs4646903 and glutathione S-transferase (*GST*) *M1* deletion, N-acetyltransferase 2 (*NAT2*) and tumor necrosis factor receptor superfamily, member 1B (*TNFRSF1B)* rs1061622 to SLE risk with attention to interaction with cigarette smoking were investigated. *CYP1A1* rs4646903 and *NAT2* genotypes were significantly associated with SLE risk. The allele (genotype) presumed to increase the risk of SLE is designated as the "at-risk" allele (genotype). The multiplicative interaction between the four genetic polymorphisms and smoking was far from significant. As for the four genetic polymorphisms, smokers with "at-risk" allele (genotype) had a significantly higher risk of SLE than never smokers with no "at-risk" allele (genotype). The observed high ORs were attributed largely to the effect of ever-smoking, however. There were significant additive interactions between smoking and any one of the following: *CYP1A1* rs4646903, *NAT2* or *TNFRSF1B* rs1061622. Specifically, about 50 % of the excess risk for SLE in smokers with the "at-risk" genotype was due to the additive interaction. Testing replication in different populations is an important step and additional studies are warranted to confirm interaction between the genetic polymorphisms associated with ROS production and smoking suggested in Japanese samples.

Chapter 7 – Fragile X syndrome (FXS) is the most frequent form of inherited intellectual disability. It is characterized by cognitive, physical and behavioral features that overlap with some common neurological and psychiatric abnormalities, including autism spectrum disorders. FXS is caused by a triplet expansion that inhibits expression of the FMR1 gene, whose product (FMRP) regulates the translation of a number of proteins involved in receptor signaling and spine morphology in the brain. Understand the FMRP function has open the way for rational treatment designs that could potentially reverse many of the neurobiological and behavioral changes observed in FXS. During the past decade, intense efforts have been focused on the development of specific FXS treatments in order to improve behavioral and cognitive problems in these patients.

Two approaches are presently being considered for a substantial treatment of the FXS patients: a) reactivation of the affected gene and b) compensating for the lack of FMRP. As result, an enormous quantity of information is now available related with epigenetic modulators, glutamatergic system, GABAergic system, GSK3β inhibitors, NMDA antagonists and other FMRP downstream targets. Among the behavioral problems, symptoms to treat are aggression, anxiety, seizures and attention deficit. The educational stimuli have also been beneficial in facilitating the development of self-care and social and adaptive behaviors. Together, these treatments can raise the quality of life for both patients and their families. This chapter summarizes the recent studies about treatments targeted to FXS.

Chapter 8 – Maize (*Zea mays* subsp. *mays*) is a member of the grass family *Poaceae* (*Gramineae*), together with many other important agricultural crops. The wild species commonly known as teosintes are the closest relatives of maize. Teosintes are wild grasses with a native distribution area from Mexico to Nicaragua (Mesoamerican region) and are an important genetic resource for maize improvement. Teosinte from Nicaragua (*Zea nicaraguensis*) is to a large extent an unutilized genetic resource and its properties in terms of desirable traits, such as water-lodging adaptation and disease resistance have to be phenotypically and genetically characterized. This chapter includes results from cytological, genetic, morphological and phylogenetic studies. Cytogenetic studies were based on C-banding techniques. Microsatellite (SSR) markers and DNA sequences of cpDNA regions were used for genetic diversity and phylogenetic studies respectively, whereas morphological studies involved various quantitative morphological traits. The C-banding pattern revealed that *Z. nicaraguensis* is more similar to *Z. luxurians* than other teosintes and cultivated maize. Among the Meso-American teosintes, *Z. diploperennis, Z. perennis* and *Z. nicaraguensis* showed the highest values in the number of rare and unique alleles and the results also indicated that the gene flow between *Z. nicaraguensis* and *Z. luxurians* has been more frequent than between other teosintes in the Mesoamerican region. The morphological characterization revealed that the traits of the number of lateral branch nodes bearing ears, the glume width, the number of tiller nodes bearing ears and the number of tillers per plant were the most important in discriminating between taxa and the principal component analysis grouped many traits around the *Z. nicaraguensis, Z. luxurians* and *Z. mays* subsp. *huehuetenangensis*. Finally, the variation among cpDNA sequences need more analysis to give a definitive phylogenetic resolution among the five *Zea* species, but even so the author's results support the idea that *Z. nicaraguensis* could be treated as a separate species distinct from *Z. luxurians*.