Preface

In this book, the authors present current research in the study of DNA replication and mutation, including connections between BLM and the Fanconi anemia pathway in the repair of replication fork damage; the mutagenic potential of methacrylates used in restorative dentistry; DNA mutation of the progranulin gene in familial frontotemporal lobar degeneration; effect of DNA polymerase a-inhibitor dehydroaltenusin on DNA replication in cultured cells; gel-based methods of DNA-binding zinc II complexes in the detection of DNA mutations and DNA mutations and genetic coding.

Chapter I - The authors DNA is continuously subjected to damage-causing endogenous and exogenous factors, resulting in lesions ranging from interstrand crosslinks to double strand breaks. These and other types of DNA damage encumber the S-phase of the cell cycle, as damaged template DNA can pose a problem for replication fork progression. Stalled replication forks can lead to fork collapse, causing additional DNA damage in the form of double strand breaks. Mammalian cells have developed checkpoint mechanisms that sense and repair DNA damage while maintaining replication fork stability. The process of replication fork restart is complex and involves multiple pathways depending on the lesion type. Initiation of these checkpoints results in cell cycle arrest and consequent stabilization of the replication fork in order to prevent collapse. Repair of damage is believed to occur via translesion synthesis (TLS) or homologous recombination, however the exact mechanism through which these pathways act to maintain genomic stability and accomplish repair of DNA lesions remains unknown. This chapter will highlight pathways required for attempt to describe and evaluate current research into the area of resumption of replication fork stalls in mammalian cells. In this reviewthe authors will focus on the role of helicases and endonucleases in the restart of damaged replication forks, with a particular

emphasis on interplay between BLM helicase and components of the Fanconi anemia (FA) pathway of DNA repair.

Chapter II - DNA damage is a prerequisite for DNA mutation, so the studies on DNA-damaging potential of environmental and medical chemicals are justified. Methacrylates are used in the polymer form as composite restorative materials in dentistry. However, the process of polymerization, led in situ, is always incomplete and the polymers can release monomers into the oral cavity and the pulp, from where they can migrate into the bloodstream reaching virtually all organs. The local concentration of the released monomers can be high enough to induce adverse biological effects. Genotoxicity of methacrylate monomers is of a special significance due to potential serious phenotypic consequences, including mutations and cancer, and a long latency period. In the present work, the authors investigated genotoxicity of a mixture of monomers of model methacrylate composite consisting of 45% 2-hydroxyethyl methacrylate and 55% bisphenol A-diglycidyl dimethacrylate (w/w) (HEMA/Bis-GMA) in human gingival fibroblast by measuring DNA damage and repair, apoptosis and cell cycle. The mixture displayed the ability to damage cellular DNA as measured by the alkaline and neutral comet assay indicating the presence of DNA single-strand breaks, alkali-labile sites and double-strand breaks in DNA. The ability of the composite to induce DNA double-strand breaks, the most serious DNA damage, was confirmed by pulsed field gel electrophoresis. This compound oxidatively modified the DNA bases, as checked by two DNA repair enzymes: endonuclease III and formamidopyrimidine-DNA glycosylase. HEMA/Bis-GMA induced apoptosis and disturbed the cell cycle, increasing the fraction of the cells in the G2/M checkpoint. The results obtained indicate that the HEMA/Bis-GMA model methacrylate composite, which can be considered as a representative for methacrylate-based dental restorations, displays a broad spectrum of genotoxicity and so – a high mutagenic potential. Therefore, new formulations of materials should be created with the emphasis on the decrease of their mutagenic potential.

Chapter III - Frontotemporal lobar degeneration (FTLD) with transactive response (TAR) DNA-binding protein of 43kDa (TDP-43) proteinopathy (FTLD-TDP) is a neurodegenerative disease characterized by variable neocortical and allocortical atrophy principally affecting the frontal and temporal lobes. Histologically, there is neuronal loss, microvacuolation in the superficial cortical laminae, and a reactive astrocytosis. A variety of TDP-43 immunoreactive changes are present in FTLD-TDP including neuronal cytoplasmic inclusions (NCI), neuronal intranuclear inclusions (NII), dystrophic neurites (DN) and, oligodendroglial inclusions (GI). Many cases of familial FTLD-TDP are caused by DNA mutations of the progranulin (GRN) gene. Hence, the density, spatial

patterns, and laminar distribution of the pathological changes were studied in nine cases of FLTD-TDP with GRN mutation. The densities of NCI and DN were greater in cases caused by GRN mutation compared with sporadic cases. In cortical regions, the commonest spatial pattern exhibited by the TDP-43 immunoreactive lesions was the presence of clusters of inclusions regularly distributed parallel to the pia mater. In approximately 50% of cortical gyri, the NCI exhibited a peak of density in the upper cortical laminae while the GI were commonly distributed across all laminae. The distribution of the NII and DN was variable, the most common pattern being a peak of NII density in the lower cortical laminae and DN in the upper cortical laminae. These results suggest in FTLD-TDP caused by GRN mutation: 1) there are greater densities of NCI and DN than in sporadic cases of the disease, 2) there is degeneration of the cortico-cortical and cortico-hippocampal pathways, and 3) cortical degeneration occurs across the cortical laminae, the various TDP-43 immunoreactive inclusions often being distributed in different cortical laminae.

Chapter IV - In a screening for selective inhibitors of eukaryotic DNA polymerase (pol) species, dehydroaltenusin extracted from a fungus (*Alternaria tennuis*) was found to be an inhibitor of DNA replicative pol α. This compound inhibited only mammalian pol α, and did not influence the activity of other DNA replicative pols such as pols δ and ε, but also showed no effect even on pol α activity from other vertebrate, fish, or plant species. Dehydroaltenusin also had no effect on other DNA metabolic enzymes tested. The inhibitory effect of dehydroaltenusin on mammalian pol α was dose-dependent with an IC_{50} value of 0.5 μM. This effect was 10-fold stronger than that of aphidicolin, a well-known potent inhibitor of eukaryotic DNA replicative pols α, δ and ε. The inhibitory mode of dehydroaltenusin for mammalian pol α activity was competitive with the DNA template-primer and non-competitive with the nucleotide substrate. This compound inhibited the proliferation of a human cervix carcinoma cell line, HeLa, with an LD_{50} value of 38.0 μM, by arresting cells at S-phase, and preventing the incorporation of thymidine into the cells. Dehydroaltenusin increased cyclin E and cyclin A levels, and induced cell apoptosis. Selective inhibitors of DNA replicative pol α, such as dehydroaltenusin, might provide novel markers for the development of anti-proliferative drugs. NIH3T3 cells that took up dehydroaltenusin by hypotonic shift, that is, the transient exposure of cultured cells in hypotonic buffer to small molecules that cannot penetrate cells, also showed inhibition of cell growth. At a low concentration (10 μM) of dehydroaltenusin, DNA replication was inhibited and several large foci of replication protein A (RPA) were found. Furthermore, when dehydroaltenusin

was incubated with aphidicolin, RPA foci were not observed in cells. In summary, these findings suggest that dehydroaltenusin blocks DNA replication through pol α inhibition, and generates single-stranded DNA, resulted in uncoupling of leading strand and lagging strand synthesis. Dehydroaltenusin could be useful as a "molecule probe" for pol α research in DNA replication. The role of pol α function in DNA replication is discussed.

Chapter V - Detection of single-nucleotide polymorphisms (SNPs) or mutations in the human genome can contribute to the establishment of genetic linkages, the diagnosis of inherited diseases, and the selection of rational remedies. Several procedures have been developed to facilitate high-throughput detection of SNPs and mutations, but these usually require expensive apparatus and the services of skillful analysts, making it difficult for most clinical researchers and physicians to obtain useful data on SNPs and mutations. The establishment of a reliable and cost-effective detection method that uses conventional laboratory equipment is therefore desirable, particularly in medical applications. Here, the authors introduce two methods based on polyacrylamide gel electrophoresis (PAGE) of DNA-binding zinc(II) complexes that are suitable for detecting mutations. The first method, known as Zn^{2+}–cyclen/PAGE, is based on a difference in the mobility in PAGE of mutant DNA compared with that of nonmutated DNA of the same chain length. This difference in mobility results from binding of the Zn^{2+}–cyclen complex (cyclen = 1,4,7,10-tetraazacyclododecane) to thymine bases, which results in a decrease in the total charge and a local conformational change in the target DNA. The Zn^{2+}–cyclen/PAGE method, when combined with polymerase chain reaction (PCR)-based heteroduplexing, permits visualization of heteroduplex bands on PAGE gels and allows screening for SNPs and mutations. The second method is known as Zn^{2+}–Phos-tag/PAGE. This is based on a difference in the mobility of a phosphorylated DNA fragment compared with that of its nonphosphorylated analogue containing an identical numbers of base pairs when they are subjected to PAGE on a phosphate-affinity gel containing an immobilized polyacrylamide-bound dizinc(II) complex phosphate-binding tag molecule, Zn^{2+}–Phos-tag {Phos-tag = 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olate}. The Zn^{2+}–Phos-tag/PAGE method, when combined with an allele-specific PCR method using a 1:1 mixture of 5'-phosphate-labeled and nonlabeled allele-specific primers, permits the separation of the less mobile 5'-phosphate-labeled PCR product from its more-mobile nonlabeled counterpart, and permits the determination of a genotype as heterozygotic or homozygotic. Here, the authors review some applications of gel-based methods based on zinc(II) complexes, and the authors

compare their resolving power in separating mutant DNA with that of conventional gel-based electrophoresis techniques.

Chapter VI - There is a need to understand how biological organization at one level translates into biological organization at the next level. Biology encompasses numerous levels from nanometers to meters, from atoms and macromolecules to organisms and populations of individuals. Here the authors shall consider the DNA mutations at the level of single nucleotides and their impact at the higher level of codons, sets of synonymous codons and their symmetries linked to tRNA-aminoacylation and degeneracy in the genetic code. The authors review will be restricted to DNA mutations, which are either single-base deletions known to be highly deleterious within open reading frames or base substitutions, which are transitions (substitutions of a pyrimidine by another pyrimidine or substitutions of a purine by another purine) or transversions. Concepts such as coevolution, minimization of the effects of errors or metrics which found so far little use in the natural sciences, can be successfully applied to understand the connections between biological properties at these molecular and macromolecular levels.

Chapter VII - The parasites belonging to the family Trypanosomatidae (order Kinetoplastida) are among the most primitive eukaryotes. Some trypanosomatids are the etiologic agents of neglected human pathologies such as South American and African trypanosomiasis and leishmaniasis. As a consequence of their ancient phylogenetic position, nuclear DNA replication in trypanosomatid protozoa shows conserved and non-conserved features. DNA replication in trypanosomatids initiates nearly simultaneously in the nucleus and in the genetic material of the single mitochondrion (or kinetoplast), suggesting that DNA synthesis is coordinately regulated in both organelles. In eukaryotes, nuclear DNA replication is preceded by assembly of the pre-replication complex, which is coordinated by the Origen Recognition Complex (ORC). However, in trypanosomatids, the pre-replication complex differs from other eukaryotes and is similar to *Archaea*. All of these parasites contain only one protein that recognizes the replication origins and is found in the nucleus throughout the cell cycle, which suggests that it is not involved in the control of replication initiation. In the S phase, DNA replication starts at these origins and, in trypanosomes, occurs mainly at the nuclear periphery. In *Leishmania* spp., from the beginning up to mid S phase, replication sites are spread throughout the nuclear space to form subnuclear foci of active DNA replication. From mid-to-late S phase, replication is restricted to sites at the nuclear periphery. Few nuclear DNA polymerases have been described in trypanosomatid protozoa, although putative members of all polymerase families are found in their genomes. Structural and functional analyses indicate that most

of these polymerases are highly conserved, with some of them being involved in polymerization and the repair of DNA damage. Although there are no descriptions of DNA polymerase δ in these protozoa, one of this protein's partners, proliferating cell nuclear antigen (PCNA), is found in the nucleus throughout the cell cycle. Trypanosomatid PCNA forms distinct subnuclear foci in the S phase, whereas its distribution is more diffuse in the G2/M phase and in post-mitotic phase cells. This finding suggests that there may be phase-specific regulation of PCNA in the cell cycle. DNA replication in trypanosomatid telomeres is terminated by the action of telomerase. The biochemical properties of the trypanosomatid enzyme are conserved and resemble those described in other eukaryotes. *Leishmania* telomeres replicate late in S phase and at the beginning of G2 phase the chromosomes cluster at the nuclear periphery. Telomerase co-localizes with telomeres from the late S to G2 phases. These observations point to the existence of replication factories in trypanosomatids, the importance of which will be reviewed and discussed in this chapter.