Genome Stability

Written by an international team of experts, Somatic Genome Variation presents a timely summary of the latest understanding of somatic genome development and variation in plants, animals, and microorganisms. Wide-ranging in coverage, the authors provide an updated view of somatic genomes and genetic theories while also offering interpretations of somatic genome variation. The text provides geneticists, bioinformaticians, biologist, plant scientists, crop scientists, and microbiologists with a valuable overview of this fascinating field of research.

Both helicases and deaminases are enzymes that play an important role in maintaining genomic stability and immune responses. Errors in duplicating DNA can result in genomic instability, leading to various human diseases, such as cancer, immune system disorder, muscle dystrophy, and neurodegenerations. Thus, maintaining genomic integrity is vital to the normal growth of cells and to human health. Maintenance of genome integrity during S phase depends in part on the activity and regulation of the replication enzymes, including replicative helicase called the MCM complex to preserve the genetic information. In contrast, deliberate errors are introduced during maturation of the immune system by modifying enzymes called deaminases. The cells response to different forms of damage is fundamental to its ability to repair itself when challenged by environmental or chemical insults. The goal of this proposal is to investigate mechanisms of genome integrity in cells using a combined genetic, biochemical, and structural approaches. These studies are highly relevant to understanding the development of cancer, including leukemias and breast cancer.

Hyperthermophilic archaea (HA) have evolved cellular mechanisms for adapting to extreme environmental conditions. Little, however, is understood about how these organisms repair or tolerate DNA damage in order to maintain genome stability. Thus, the aim of this dissertation was to elucidate strategies employed by these organisms to maintain genomic integrity by utilizing HA Sulfolobus acidocaldarius as a model species. Five studies were conducted which include (i) investigating cellular responses to chemically-induced damage by cisplatin, (ii) determining the function of genes implicated in UV photoproduct repair, (iii) verifying the functions of an S. acidocaldarius translation synthesis polymerase (TLS) Dbh, (iv) examining the role of TLS bypass in tolerating abasic lesions, and (v) investigating the effect of promoter strength on mutagenesis in reporter gene lacS. The chemical cisplatin was utilized to evaluate cellular sensitivity and genetic effects to bulky-adducts. This project involved experiments that were technically challenging and therefore the results were very preliminary. However, the data suggested that S. acidocaldarius has the ability to repair or tolerate large numbers of cisplatin damage. Additionally, there was no evidence that cisplatin-damage was mutagenic, and the process of homologous recombination seemed to be unaffected by cisplatin-induced damage. Studies regarding UV photoproduct repair analyzed disruptant mutants, Phr and Uvde, and showed that the photolyase protein homologue Phr is required for the repair of UV damage in the presence of light, while the UV-endonuclease UVDE homologue did not seem to play a critical role in dark-repair of UV damage. TLS Dbh polymerase disruption mutants were used to determine the role of this polymerase in DNA damage sensitivity and in replication accuracy of spontaneous mutations in a selectable gene pyrE. The absence of Dbh did not seem to have an affect on the sensitivity of the cells to chemicals, however the mutation spectrum sampled from Dbh- mutants versus Dbh+ strains did show significant differences in the types and positions of mutations occurring in these strains. The role of TLS bypass in tolerating abasic lesions was determined by introducing a site-specific abasic lesion into the genome and selecting recombinants that successfully tolerated the damage. Genotyping techniques were then used to identify which base was present at the site of the damage. The results suggest that Dbh does not play a prominent role in bypassing abasic lesions. Finally, to examine how the rate of transcription may affect mutagenesis, mutation rates and the specific activity for reporter gene lacS, found in different genetic contexts and under the control of different promoters, was measured. The results suggested that stronger promoters regulating lacS in S. acidocaldarius yielded higher mutation rates. The results are consistent with the phenomenon of transcription-associated mutagenesis (TAM), where highly transcribed genes have been observed to yield higher mutation rates. In conclusion, the data obtained from these experiments have brought insight into various DNA damage repair and tolerance mechanisms, while setting the foundation for future experiments.

Effect of Calorie Restriction on the Genome Stability in DNA Repair Defective Cells

Mobile Genetic Elements in Cellular Differentiation, Genome Stability, and Cancer

Genome Stability and Human Diseases

in Animals, Plants, and Microorganisms

DNA Mismatch Repair, Genome Stability and Tumorigenesis

The human genome, as with the genome of most organisms, is comprised of various types of mobile genetic element derived repeats. Mobile genetic elements that mobilize by an RNA intermediate, include both autonomous and non-autonomous retrotransposons, and mobilize by a "copy and paste" mechanism that relies of the presence of a functional reverse transcriptase activity. The extent to which these different types of elements are actively mobilizing varies among organisms, as revealed with the advent of Next Generation DNA sequencing (NGS). To understand the normal and aberrant mechanisms that impact the mobility of these elements requires a more extensive understanding of how these elements interact with molecular pathways of the cell, including DNA repair, recombination and chromatin. In addition, epigenetic based-mechanisms can also influence the mobility of these elements, likely by transcriptional activation or repression in certain cell types. Studies regarding how mobile genetic elements interface and evolve with these pathways will rely on genomic studies from various model organisms. In addition, the mechanistic details of how these elements are regulated will continue to be elucidated with the use of genetic, biochemical, molecular, cellular, and bioinformatic approaches. Remarkably, the current understanding regarding the biology of these elements in the human genome, suggests these elements may impact developmental biology, including cellular differentiation, neuronal development, and immune function. Thus, aberrant changes in these molecular pathways may also impact disease, including neuronal degeneration, autoimmunity, and cancer.

Most bacterial infections can be correlated to contamination of consumables such as food and water. Upon contamination, boil water advisories have been ordered to ensure water is safe to consume, despite the evidence that heat-killed bacteria can induce genomic instability of exposed (intestine) and distal cells (liver and spleen). We hypothesize that exposure to components of heat-killed Escherichia coli O157:H7 will induce genomic instability within animal cells directly and indirectly exposed to these determinants. Mice were exposed to various components of dead bacteria such as DNA, RNA, protein or LPS as well as to whole heat-killed bacteria via drinking water. Here, we report that exposure to whole heat-killed bacteria and LPS resulted in significant alterations in the steady state RNA levels and in the levels of proteins involved in proliferation, DNA repair and DNA methylation. Exposure to whole heat-killed bacteria and their LPS components also leads to increased levels of DNA damage.

DNA damage response (DDR) is a term that includes a variety of highly sophisticated mechanisms that cells have evolved in

safeguarding the genome from the deleterious consequences of DNA damage. It is estimated that every single cell receives tens of thousands of DNA lesions per day. Failure of DDR to properly respond to DNA damage leads to stem cell dysfunction, accelerated ageing, various degenerative diseases or cancer. The sole function of DDR is to recognize diverse DNA lesions, signal their presence, activate cell cycle arrest and finally recruit specific DNA repair proteins to fix the DNA damage and thus prevent genomic instability. DDR is composed of hundreds of spatiotemporally regulated and interconnected proteins, which are able to promptly respond to various DNA lesions. So it is not surprising that mutations in genes encoding various DDR proteins cause embryonic lethality, malignancies, neurodegenerative diseases and premature ageing. The importance of DDR for cell survival and genome stability is unquestionable, but how the sophisticated network of hundreds of different DDR proteins is spatiotemporally coordinated is far from being understood. In the last ten years ubiquitin (ubiquitination) and the ubiquitin-relative SUMO (sumoylation) have emerged as essential posttranslational modifications that regulate DDR. Beside a plethora of ubiqutin and sumo E1-activating enzymes, E2-conjugating enzymes, E3-ligases and ubiquitin/sumo proteases involved in ubiquitination and sumoylation, the complexity of ubiqutin and sumo systems is additionally increased by the fact that both ubiquitin and sumo can form a variety of different chains on substrates which govern the substrate fate, such as its interaction with other proteins, changing its enzymatic activity or promoting substrate degradation. The importance of ubiquitin/SUMO systems in the orchestration of DDR is best illustrated in patients with mutations in E3-ubiquitin ligases BRCA1 or RNF168. BRCA1 is essential for proper function of DDR and its mutations lead to triple-negative breast and ovarian cancers. RNF168 is an E3 ubiquitin ligase, which creates the ubiquitin docking platform for recruitment of different DNA damage signalling and repair proteins at sites of DNA lesion, and its mutations cause RIDDLE syndrome characterized by radiosensitivity, immunodeficiency and learning disability. In addition, recently discovered the ubiquitin receptor protein SPRTN is part of the DNA replication machinery and its mutations cause early-onset hepatocellular carcinoma and premature ageing in humans. Despite more than 700 different enzymes directly involved in ubiquitination and sumoylation processes only few of them are known to play a role in DDR. Therefore, we feel that the role of ubiquitin and the ubiquitin-related SUMO in DDR is far from being understood, and that this is the emerging field that will hugely expand in the next decade due to the rapid development of a new generation of technologies, which will allow us a more robust and precise analyses of human genome, transcriptome and proteome. In this Research Topic we provide a comprehensive overview of our current understanding of ubiquitin and SUMO pathways in all aspects of DDR, from DNA replication to different DNA repair pathways, and demonstrate how alterations in these pathways cause genomic instability that is linked to degenerative diseases, cancer and pathological ageing.

CCDC6 Negatively Modulates the Phosphatase Activity PP4c in DNA Damage Response, Maintaining the Genome Stability

Book Review Issue

The Influence of a Human Repetitive DNA on Genome Stability

Mechanisms of DNA Recombination and Genome Rearrangements: Intersection Between Homologous Recombination, DNA Replication and DNA Repair

This volume presents forty-two methods and protocols to analyze diverse aspects of genome instability. Chapters detail mutagenesis and repair, methods to quantify and analyze the properties of DNA double-strand breaks, profile replication, replication proteins strand-specifically, genome instability, fluorescence microscopic techniques, and genomic and proteomic approaches. Written in the highly successful Methods in Molecular Biology series format, chapters include introductions to their respective topics, lists of the necessary materials and reagents, step-by-step, readily reproducible laboratory protocols, and tips on troubleshooting and avoiding known pitfalls. Authoritative and cutting-edge, Genome Instability: Methods and Protocols aims to provide a comprehensive resource for the discovery and analysis of the proteins and pathways that are critical for stable maintenance of the genome.

Genetic instability is a hallmark of cancer. A commonly observed class of genetic alterations in tumor cells is termed gross chromosomal rearrangements (GCRs) that encompass translocations, large-scale interstitial deletions and chromosome arm deletions. To identify novel GCR regulators, the budding yeast, Saccharomyces cerevisiae, was used as a model system. Using unbiased large-scale and candidate approaches, I have identified at least ten novel regulators of chromosome stability. Firstly, from a small screen of putative genome regulators, I identified the GCR suppressor, ELG1. Further study of this gene showed that Elg1 forms an alternative replication factor C complex that interacts with PCNA with a possible role in Okazaki fragment maturation. Secondly, I executed a systematic genome-wide screen for GCR suppressors utilizing the non-essential haploid yeast deletion mutants and SGA methodology. From this comprehensive screen, I identified nine novel GCR suppressors including ZIP1, RML2, BUD16, WSS1, SLX8, HEX3, RAD5, RMI1 and ESC2. Initial characterization of WSS1, ESC2, and SLX8/HEX3 suggests that some of these genes may affect DNA repair and/or DNA replication. Thirdly, I characterized the highly conserved vitamin B6 enzyme, pyridoxal kinase (Bud16/Pdxk), which I found to be a potent suppressor of GCRs. This enzyme plays a critical role in maintaining adequate levels of pyridoxal-5-phosphate (PLP) or vitamin B6, which prevents the formation of DNA lesions. Through the identification of various regulators involved during S phase, this work has expanded our current knowledge of DNA replication, DNA repair and chromosome stability. In the long-term, efforts to identify GCR suppressors from the budding yeast may lead to the identification of human homologs, which may function as 'genome caretakers' in human cancers.

Since the establishment of the DNA structure researchers have been highly interested in the molecular basis of the inheritance of genes and of genetic disorders. Scientific investigations of the last two decades have shown that, in addition to oncogenic viruses and signalling pathways alterations, genomic instability is important in the development of cancer. This view is supported by the findings that aneuploidy, which results from chromosome instability, is one of the hallmarks of cancer cells. Chromosomal instability also underpins our fundamental principles of understanding tumourigenesis: It thought that cancer arises from the sequential acquisition of genetic alterations in specific genes. In this hypothesis, these rare genetic events represent rate-limiting ‘bottlenecks’ in the clonal evolution of a cancer, and pre-cancerous cells can evolve into neoplastic cells through the acquisition of somatic mutations. This book is written by

international leading scientists in the field of genome stability. Chapters are devoted to genome stability and anti-cancer drug targets, histone modifications, chromatin factors, DNA repair, apoptosis and many other key areas of research. The chapters give insights into the newest development of the genome stability and human diseases and bring the current understanding of the mechanisms leading to chromosome instability and their potential for clinical impact to the reader.

Heterochromatin and Genome Stability

From Virus to Human Application

A High-throughput Screen for Novel Genes Involved in Maintaining Genome Stability and the DNA Damage Response Pathway in Saccharomyces Cerevisiae

Cancer and Ageing

Trypanosoma Brucei BRCA2 in the Regulation of Genome Stability and DNA Repair

Genome Stability: From Virus to Human Application, Second Edition, a volume in the Translational Epigenetics series, explores how various species maintain genome stability and genome diversification in response to environmental factors. Here, across thirty-eight chapters, leading researchers provide a deep analysis of genome stability in DNA/RNA viruses, prokaryotes, single cell eukaryotes, lower multicellular eukaryotes, and mammals, examining how epigenetic factors contribute to genome stability and how these species pass memories of encounters to progeny. Topics also include major DNA repair mechanisms, the role of chromatin in genome stability, human diseases associated with genome instability, and genome stability in response to aging. This second edition has been fully revised to address evolving research trends, including CRISPRs/Cas9 genome editing; conventional versus transgenic genome instability; breeding and genetic diseases associated with abnormal DNA repair; RNA and extrachromosomal DNA; cloning, stem cells, and embryo development; programmed genome instability; and conserved and divergent features of repair. This volume is an essential resource for geneticists, epigeneticists, and molecular biologists who are looking to gain a deeper understanding of this rapidly expanding field, and can also be of great use to advanced students who are looking to gain additional expertise in genome stability. A deep analysis of genome stability research from various kingdoms, including epigenetics and transgenerational effects Provides comprehensive coverage of mechanisms utilized by different organisms to maintain genomic stability Contains applications of genome instability research and outcomes for human disease Features all-new chapters on evolving areas of genome stability research, including CRISPRs/Cas9 genome editing, RNA and extrachromosomal DNA, programmed genome instability, and conserved and divergent features of repair

Mismatch repair (MMR) systems correct mismatches formed during DNA replication and genetic recombination and as the result of DNA damage. MMR is initiated by a conserved family of MutS (MSH) and MutL (MLH) homolog proteins. MLH1 is unique among these proteins because it is also required for high levels of crossing over which are necessary for the proper disjunction of homologous chromosomes during the first meiotic division.

Together, these studies characterize additional roles for domains of Sgs1 and Exo1 that are not entirely understood as well as their roles in combination with DNA damage checkpoints, and repair pathways that are necessary for maintaining genome stability.

Maintaining Genome Stability at the Tandemly Repeated RDNA Locus

Functional Roles of Nucleases in DNA Metabolism and Genome Stability

Maintaining Genome Stability in Saccharomyces Cerevisiae

The Genome Stability is Preserved by a Stress Response Protein: CCDC6

DNA Repair and Recombination

"Genome Stability: DNA Repair and Recombination describes the various mechanisms of repairing DNA damage by recombination, most notably the repair of chromosomal breaks. The text presents a definitive history of the evolution of molecular models of DNA repair, emphasizing current research. The book introduces the central players in recombination. An overview of the four major pathways of homologous recombinational repair is followed by a description of the several mechanisms of nonhomologous end-joining. Designed as a textbook for advanced undergraduate and graduate students with a molecular biology and genetics background, researchers and practitioners, especially in cancer biology, will also appreciate the book as a reference"--Provided by publisher.

Proper DNA replication and well-timed cell cycle progression are vital to the normal functioning of a cell. Precise coordination between these mechanisms' constituent proteins ensures their processivity while safeguarding against DNA damage. The Ctf4 protein is a central member of the replication fork and links the replicative MCM helicase and polymerase [alpha]-primase. In addition, it has been implicated as a member of a complex that promotes replication fork stability, the Fork Protection Complex (FPC). This investigation represents the first phenotypic analysis of the function of the Ctf4 protein within a multicellular organism model. We show that Ctf4 interacts with Polymerase [alpha], MCM2, Psf1, and Psf2. We also demonstrate that knockdown of this central replication fork component via a GAL4-UAS RNAi system results in a lower frequency of mitosis due to an S-phase delay, endoreplication defects, as well as mitotic bridging in early embryonic development.

Mechanisms of DNA Recombination and Genome Rearrangements: Intersection between Homologous Recombination, DNA Replication and DNA Repair, Volume 601, the latest release in the Methods in Enzymology series, continues the legacy of this premier serial with quality chapters authored by leaders in the field. Homologous genetic recombination remains the most enigmatic process in DNA metabolism. The molecular machines of

recombination preserve the integrity of the genetic material in all organisms and generate genetic diversity in evolution. The same molecular machines that support genetic integrity by orchestrating accurate repair of the most deleterious DNA lesions, however, also promote survival of cancerous cells and emergence of radiation and chemotherapy resistance. This two-volume set offers a comprehensive set of cutting edge methods to study various aspects of homologous recombination and cellular processes that utilize the enzymatic machinery of recombination. The chapters are written by the leading researches and cover a broad range of topics from the basic molecular mechanisms of recombinational proteins and enzymes to emerging cellular techniques and drug discovery efforts. contributions by the leading experts in the field of DNA repair, recombination, replication and genome stability documents cutting edge methods

Genome Instability and Transgenerational Effects

“The” Role of Exonuclease-1 and Its Interaction Partners in Genome Stability

Maintaining Genome Stability: The Role of Helicases and Deaminases

MicroRNA Signatures in Plant Genome Stability and Genotoxic Stress

DNA Replication, the Cell Cycle and Genome Stability

ABSTRACT Maintaining genome integrity is indispensible for cells to prevent and limit accruement of deleterious mutations and to promote viable cell growth and proliferation. Cells possess a myriad of mechanisms to detect, prevent and repair incurred cellular damage. Here we discuss various proteins and their accompanying cellular pathways that promote genome stability. We first investigate the NEDD8 protein and its role in promoting homologous recombination repair via multiple Cullin E3 ubiquitin ligases. We provide specific mechanisms through which, UBE2M, an E2 conjugating enzyme, neddylates various Cullin ligases to render them catalytically active to degrade their substrates by the proteasome. We show that CUL1, CUL2 and CUL4 are important in regulating various steps in the DNA damage response. Our data indicates that UBE2M and the neddylation pathway are important for genome stability. Our second topic discusses the role of the USP1- UAF1 deubiquitinating enzyme in promoting homologous recombination. We show that USP1-UAF1 interact with and stabilize RAD51AP1 (RAD51- Associated Protein 1). RAD51AP1 has previously been reported to promote homologous recombination by facilitating recombinase activity of RAD51, an essential protein involved in homologous recombination repair. We show that USP1, UAF1 and RAD51AP1 depletion leads to genome instability. Our data demonstrates the importance of these proteins in promoting genome integrity via homologous recombination.
RPA is a highly conserved, heterotrimeric ssDNA binding protein with a ubiquitous role in all DNA transactions involving ssDNA intermediates. RPA promotes resection at DSBs to facilitate HR and abrogation of this function has severe consequences. Defective RPA can lead to the formation of secondary structures and impair loading of homology search proteins such as Rad52 and Rad51. Using a chromosomal end-joining assay, we demonstrate that hypomorphic rfa1 mutants exhibit elevated frequencies of MMEJ by up to 350-fold. Biochemical characterization of RPAt33 and RPAt48 complexes show these mutants are compromised for their ability to prevent spontaneous annealing and the removal of secondary structures to fully extend ssDNA. These results demonstrate that annealing between MHs defines a critical control to regulate MMEJ repair. Therefore, RPA bound to ssDNA intermediates shields complementary sequences from annealing to promote error-free HR and prevents repair by mutagenic MMEJ, thereby preserving genomic integrity.

Genome Stability: DNA Repair and Recombination describes the various mechanisms of repairing DNA damage by recombination, most notably the repair of chromosomal breaks. The text presents a definitive history of the evolution of molecular models of DNA repair, emphasizing current research. The book introduces the central players in recombination. An

Transposons and the Dynamic Genome

Promoting Genome Stability Via Multiple Dna Repair Pathways

Maintenance of Genome Stability and Breast Cancer: Molecular Analysis of DNA Damage-Activated Kinases

Roles for the Saccharomyces Cerevisiae MLH1 Mismatch Repair Gene in Genome Stability and Genetic Recombination

Effects of a Highly Repetitive Endogenous Ty1 Retrotransposon on Genome Stability

The maintenance of genomic stability is beneficial for the survival of an individual cell and crucial for cancer avoidance. Cells invest huge resources to maintain genomic stability, and cancer cells undergo an array of genetic changes to escape these barriers. The initiation of carcinogenesis represents a mutational event, which in most instances occurs despite functional DNA damage response (DDR) mechanisms. This dissertation aims to study the mechanisms of chromosomal instability in tumor development. We intend to investigate the role of CCDC6, a gene frequently rearranged with RET and with genes other than RET in several tumors, in the signal transduction pathway activated by DNA damage. Remarkably, in most cancers harboring CCDC6 gene fusions, the product of the normal allele is supposed to be functionally impaired or absent. In this work, we have explored the consequences of CCDC6 gene product loss or inactivation in the carcinogenetic process. We report that CCDC6 is a stress response protein that sustains DNA damage checkpoint and contributes to genome stability maintenance by modulating PP4c activity directed toward the pH2AX dephosphorylation in response to DNA damage.

Genome stability of every species depends on complex interaction of predefined and environmentally induced genetic and

epigenetic states. Predefined states consist of chromatin structure and cell metabolic processes such as DNA repair, radical scavenging and cell signalling, whereas induced states depend on interactions with the environment. Organisms are able to respond to a changing environment by various alterations in their somatic cells as well as in their germline and progeny. In this book, we will describe various phenomena associated with the maintenance of genome stability. These include genetic and epigenetic responses to various stresses in exposed cells and organisms, bystander and, bystander-like effects, transgenerational changes in genome stability and stress tolerance in bacteria, plants and animals.

The work outlined in this dissertation focuses on two distinct areas that are important for genome stability. Both areas focus on DNA repair pathways that require the action of nucleases, specifically Exonuclease 5 and Ribonuclease H2. First, I describe the biochemical and molecular characterization of the novel Exonuclease 5 family of enzymes from S. cerevisiae, S. pombe, and humans. The Exo5 family consists of bi-directional single-strand DNA specific exonucleases that all contain an iron-sulfur cluster as a structural motif and all have various roles in DNA metabolism. In the Saccharomycetales order that includes the budding yeast, S. cerevisiae, Exo5 is a mitochondrial protein that is essential for mitochondrial genome maintenance. In an unrelated yeast species, Schizosaccharomyes pombe, Exo5 is important for both nuclear and mitochondrial DNA metabolism. The human ortholog is important for nuclear genome stability, and for DNA repair. The work outlined in Chapter II of this Dissertation establishes Exo5 as a protein that is important for DNA metabolism. The second area of study outlined in Chapters III and IV is related to the phenomenon of ribonucleotide incorporation into the genome by replicative polymerases, and these chapters focus on the enzymes that remove these noncanonical nucleotides. Ribonucleotides are incorporated into DNA by the replicative DNA polymerases at frequencies of about 2 per kb, which makes them by far the most abundant form of potential DNA damage in the cell. Their removal is essential for restoring a stable intact chromosome. In Chapter III, I present a complete biochemical reconstitution of the ribonucleotide excision repair (RER) pathway with enzymes purified from Saccharomyces cerevisiae. I highlight the requirement for RNase H2 in the process of RER and investigate the redundancies at different steps of repair. Also outlined in this dissertation is the dissection of the different functions of RNase H2 in RER and in the removal of RNA-loops in DNA, and implications for genome instability in human diseases that are affected for these activities. Chapter IV of this dissertation discusses work on an alternative pathway for ribonucleotide removal from the genome by Topoisomerase I. In S. cerevisiae, deletion of rnh201, the catalytic subunit of RNase H2, results in the persistence of ribonucleotides remain in the genome, which leads to ~100-fold increase in the frequency of 2-5 bp deletions at di-nucleotide repeat sequences. These deletions are dependent on topoisomerase I (Top1) activity. Here we present an in vitro reconstitution of the mechanism of Top1-dependent deletions at di-nucleotide repeat sequences and a mechanism for Top1-initiated removal of ribonucleotides outside of the context of these repeat sequences in S. cerevisiae. Top1 attack at a ribonucleotide leads to the formation of a 2', 3' cyclic phosphate terminated ssDNA nick, followed by subsequent formation of a Top1-cleavage complex (Top1-cc) upstream of the 2', 3' cyclic phosphate. If the ribonucleotide is in the context of a di-nucleotide repeat, there can be realignment of the DNA allowing for religation and release of Top1, leading to a 2-nucleotide deletion. If the ribonucleotide resides outside a repeat sequence, the realignment is not possible and a different pathway must repair the Top1-cc. Tdp1-dependent repair of Top1-cc requires prior proteolytic processing of the Top1-cc before it can be removed leaving a 3'-phosphate that can be removed by Tpp1, Apn1, or Apn2 forming a substrate suitable for repair by DNA polymerase [delta], FEN1 and DNA ligase.

Exploring Pathways that Maintain Genome Stability by Preventing the Formation of Gross Chromosomal Rearrangements

Influence of Pathogenic Bacterial Determinants on Genome Stability of Exposed Intestinal Cells and of Distal Liver and Spleen Cells

Somatic Genome Variation

Cellular Responses that Preserve Genome Stability in Saccharomyces Cerevisiae

The Role of SGS1 and EXO1 in the Maintenance of Genome Stability

DNA double strand breaks (DSBs) can cause massive genome instability. In eukaryotes, multiple pathways exist to resolve DNA DSBs. One major repair pathway, homologous recombination (HR), utilizes homologous DNA templates to mediate repair. The presence of repetitive elements in the template DNA used for repair can lead to mutagenic repair. Prior studies in Saccharomyces cerevisiae have observed gross chromosomal rearrangements (GCRs) mediated by repetitive DNA from the Ty1 retrotransposon family. Ty1 retrotransposons are highly repetitive sequence elements located throughout the S. cerevisaie genome. In this work, we utilize an endogenous Ty1 element to investigate Ty1-mediated GCRs. We show that the presence of an endogenous high copy repeat sequence in a nonessential chromosome arm increases the rate of GCR formation involving that chromosome arm by nearly 400 fold. Almost all GCRs isolated in this study were mediated by this high copy repeat sequence and ranged from simple monocentric nonreciprocal translocations to nonreciprocal translocations involving template switches to breakage-fusion-bridge (BFB) events. Additionally, despite different mutational defects, the nonreciprocal translocation phenotype appeared to be the predominant GCR phenotype. By adapting a previously developed PCR technique known as multiplex ligation-dependent amplification (MLPA) to study the distribution profiles of GCRs formed in both wild type and mutant strains, we identify ectopic high copy repeat target hotspots. These hotspots mediate the majority of the observed recombinations in wild type strains and may act as fragile sites in the genome. Surprisingly, we find examples of high copy repeat-mediated nonreciprocal translocations in a mutant deficient for RAD52, an important HR gene. Finally, we demonstrate that loss of a histone H3 lysine residue 56 (H3K56) acetylator leads to an increased frequency of aneuploidy that occurs simultaneously with observed GCR events. Taken together, the results indicate Ty1 repeat sequences greatly increase genome instability and that the mechanisms causing increased instability can be further explored using our adapted MLPA technique.

Resistance (R) genes, a key factor in determining the resistance of plants, have been shown often to be highly allelic entities existing in duplicated regions of the genome. This characteristic suggests that R-gene acquisition may have arisen through frequent genetic rearrangements as a result of transient, reduced genome stability. Tabacco plants transgenic for a recombination construct exhibited reduced genome stability upon infection with a virulent pathogen (tobacco mosaic virus). The reduced genome stability manifested as an increase in recombination events in the transgene. Such increases were observed following a virulent pathogen attack. This increase in recombination was shown to be systemic and was observed prior to systemic viral movement suggesting the presence of a systemic recombination signal. Further molecular analyses revealed that specific R-gene loci experience a large frequency of rearrangements following a virulent pathogen encounter. The possible targeting of instability to R-gene regions may be controlled through epigenetic

processes, in particular, DNA methylation.
The ATR (ATM and Rad3-Related) kinase is essential to maintain genomic integrity. ATR is recruited to DNA lesions in part through its association with ATR-interacting protein (ATRIP), which in turn interacts with the single-stranded DNA binding protein RPA (Replication Protein A). In this study, a conserved checkpoint protein recruitment domain (CRD) in ATRIP orthologs has been identified by biochemical mapping of the RPA binding site in combination with NMR, mutagenesis and computational modeling. Mutations in the CRD of the yeast ATRIP ortholog Ddc2 disrupt the Ddc2-RPA interaction, prevent proper localization of Ddc2 to DNA breaks, sensitize yeast to DNA damaging agents, and partially compromise checkpoint signaling. These data demonstrate that the CRD is critical for localization and optimal DNA damage responses. However, the stimulation of ATR kinase activity by binding of TopBP1 to ATRIP-ATR can occur independently of the interaction of ATRIP with RPA. Our results support a multi-step model for ATR activation that requires separable localization and activation functions of ATRIP.
Special Issue
The Effect of Pathogens on Plant Genome Stability
DNA Damage Checkpoint Pathways and the Maintenance of Genome Stability in C. Elegans
Genome Stability
Roles for CSM4 During Meiotic Recombination and the Effect of Genetic Background on DNA Repair
This volume gives an overview on mobile DNA and how such contradiction to the obligatory stability of genomes can be understood. Obviously, an understanding can only be achieved by cutting deeply into the evolutionary history of life.
To ensure the faithful transmission of genetic material to each progeny cell, DNA replication has to be accomplished faithfully and the duplicated chromosomes have to be distributed equally to the daughter cells. My dissertation focuses on the MSA1 gene, which functions as a transcription factor to facilitate the DNA replication during S-phase, and HSK3, which functions in the DASH complex to ensure the proper segregation of chromosomes in mitosis. DRC1 is isolated as a genomic suppressor of dpb11-1 and forms the initiator complex with Dpb11 that facilitates the recruitment of DNA polymerase to origins. The drc1-1 mutant shows sensitivity to the replication inhibitor, hydroxyurea. In the first study, we identify a cell cycle regulated transcription factor, MSA1, as a suppressor of drc1-1 . MSA1 overproduction also suppresses the temperature sensitivity of dpb11-1 and pol2-12 (the catalytic subunit of DNA polymerase [varepsilon]). Conversely, msa1 deletion exacerbates the mutant phenotypes of both drc1-1 and dpb11-1 and msa1 deletion alone results in a delay in S phase entry, which suggests a positive role for MSA1 in DNA replication. MSA1 represents a new cell cycle regulated gene important for S phase entry.
Drosophila Ctf4 is Essential for Genome Stability and Normal Cell Cycle Progression
Replication Protein A in the Maintenance of Genome Stability
Methods and Protocols
Mechanisms of Genome Stability in the Hyperthermophilic Archaeon Sulfolobus Acidocaldarius
Ubiquitin and Ubiquitin-Relative SUMO in DNA Damage Response