Genetics and Environmental Mutagenesis Society

28th Annual Fall Meeting

"Deep Sequencing, Regulation and Cancer"

Friday, October 15, 2010

Sheraton Imperial Hotel & Convention Center
4700 Emperor Blvd
Durham, NC

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Dear GEMS Members and Guests,

Welcome to the 28th Annual Fall Meeting of GEMS. The theme of our meeting today is "**Deep Sequencing, Regulation and Cancer**", which is organized and chaired by Steve Little. Steve has done a truly outstanding job in planning and organizing today's meeting. The theme ties together many aspects of the science that GEMS was founded upon and have evolved today consistent with the scientific themes of our past meetings, especially over the past 2 years. Congratulations to Steve and our sincere thanks for all of his efforts on behalf of GEMS this past and this coming year. Your continued efforts in support will also by critical to the mission of GEMS.

Our society remains strong, scientifically viable, and serves an important role in our regional scientific community. Largely, our continued success is due to the efforts of a very talented and dedicated group of very active GEMS members, the Board of Directors (BOD). It has been a pleasure and a very rewarding experience to serve GEMS and work with the BOD. I encourage you; whether you are a long term or recent member, or a first time participate today, to continue to actively support GEMS. We welcome you to join us and support the scientific enterprise represented by this society. Please donate your time and talents, offer your suggestions for ways to improve, and encourage your colleagues to join and actively participate. With your help, GEMS will continue to be strong, relevant, and vital.

To our Corporate Sponsors, your support is vital to GEMS scientific programs each year. Your on-site participation today, offering products, services, and consultation, critical to our research is valuable. To participants here, I encourage you to visit the Sponsors exhibits and to discuss with them how they can help with your research needs. Also, a special appreciation and thanks goes to the NIEHS Division of Intramural Research Committee for Conferences and Distinguished Lectures, NIEHS has supported our meeting with a generous grant.

Finally, I thank you for the opportunity to serve as the President of GEMS this past year. It has been a rewarding experience for me, and I am honored to have been given the opportunity to help lead the Society. I take my leave today knowing that we have extraordinary leadership with Steve Little and the Board of Directors. Today, the BOD members of the "Class of 2008" depart, and new members have been elected. I congratulate Joel Myers, Carol Swartz, and JD Wilson for a job well done, and I encourage the new BOD members to be creative, take the necessary risks, and take us to the next step in growing and increasing the vitality of GEMS.

Sincerely,

Jef French
President, GEMS 2009-2010

President-Elect's Message

Dear Members and Guests,

I want to welcome everyone to what I believe will be an exciting meeting of the Genetics and Environmental Mutagenicity Society. The theme of this meeting is "**Deep Sequencing, Regulation and Cancer**". This meeting will feature both the presentation of new methodologies in genetics and molecular biology of cancer utilizing cutting edge technologies presented by established area researchers, as well as research by younger researchers in both new and traditional research areas.

The Deep Sequencing in the title refers to the multiple layers of genetic information, as well as the complete sequence information that is afforded by the High Throughput genetic sequencing technologies. This is made possible by the implementation of the much faster pyrosequencing method in new automatic sequencers. The usefulness of these new sequencers is evident in the formation of High Throughput Sequencing Centers at Duke University, UNC Chapel Hill and NC State University (which has a greater emphasis on plant genetics). **Piotr Mieczkowski**, the first speaker, will provide an overview of this new technology and associated methodologies. The greater speed of sequencing is just one advantage of the new sequencing technologies. The other is the simultaneous sequencing of epigenetic information and sequencing of all transcripts (and not just coding transcripts). This wider genetic coverage provides more information about the transcriptional regulatory control that could be relevant to understanding cancer pathologies. The second speaker, **Ian Davis**, will discuss genome wide transcriptional profiling in human cancers. The third speaker, **Jack Keene** will discuss new roles of RNA regulation in transcription, particularly post-transcriptional regulation.

I hope that the presentations today will give you a sense of the promise of High Throughput and Whole Genome sequencing which allows the sequencing of the entire DNA genetic sequence, both coding and non-coding regions. The noncoding genome holds many of the clues to understanding the regulation of dynamic biology such as cancer and developmental biology. I find it interesting that this week EMBL is holding a symposium on the Noncoding Genome, which provides additional evidence of timeliness of this topic. I look forward to seeing how future GEMS meetings will follow up on in this rapidly developing area of research.

Best Regards,

Steve Little
GEMS President-elect

GEMS

Genetics and Environmental Mutagenesis Society

"Deep Sequencing, Regulation and Cancer"

8:00 Registration and Continental Breakfast

8:45 Welcome: **Jef French, Ph.D.**, GEMS President

Speaker Introduction: **Stephen Little, M.Tox.**, GEMS President-Elect

9:00 Invited Speaker: **Piotr Mieczkowski, Ph.D**, University of North Carolina at Chapel Hill, High Throughput Sequencing Facility *"Next Generation Sequencing Tools"*

9:45 – 11:15 Poster Session & Visit Sponsors (Coffee)

11:15 Invited Speaker: **Ian Davis, M.D., Ph.D.**, UNC School of Medicine, Dept. of Pediatrics and Dept. of Genetics, *" Whole Genome Analysis of Transcription Regulation and Cancer "*

12:00 Lunch (short business meeting during lunch, election results, sponsors)

1:30-3:00 Selected presentations by Post-Doctoral Students, Technicians, and Students

1:30-1:45 S. Covo (Abstract T1)

1:45-2:00 R. Prasad (Abstract T2)

2:00-2:15 J. McNulty (Abstract T3)

2:15-2:30 Z. **Yin (Abstract T4)**

2:30-2:45 M. Kemp (Abstract T5)

2:45-3:00 S. Roberts (Abstract T6)

3:00 Invited Speaker: **Jack Keene, Ph.D** (Duke University Medical Center *"Ribonomics, Post-transcriptional RNA Operons and Cancer"*

4:00 Awards ceremony in reception area, followed by reception

GEMS gratefully acknowledges the financial support of the NIEHS Division of Intramural Research DSL Committee for Scientific Conferences in 2009

NIEHS
National Institute of Environmental Health Sciences

Piotr Mieczkowski, Ph.D

Director of the UNC High Throughput Sequencing Facility
Assistant Research Professor
Department of Genetics
The University of North Carolina at Chapel Hill
Chapel Hill, NC

Dr. Mieczkowski's scientific background is in the areas of DNA repair, recombination, replication, eukaryotic genome stability and DNA structural organization. Using standard genetics methodology, next generation sequencing and microarray technology he has been investigating various aspects of genome stability and DNA repair. As a postdoctoral fellow at UNC he was involved in construction of a microarray printer and developed methodology for analysis of copy number variances in model organisms and continued this methodolgy during his research at Duke University. In the early stages of the evolution of next generation sequencing, he became interested in developing this new technology for analysis of genome stability and mutagenesis. He has laid the groundwork for methodology and workflow used by UNC High Throughput Sequencing Facility and development of applications for mutagenesis research and has been a part of many research projects using next generation sequencing as a primary tool. As Director of UNC High Throughput Sequencing Facility he has experience in experimental design for large format sequencing projects using state of the art high throughput sequencing platforms (Genome Analyzer IIx , 454) including data analysis software.

Dr. Mieckiwski received his Ph.D. in Molecular Biology from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw, Poland. He was a Post – Doc in the Lab of Thomas Petes in the Department of Biology at the University of North Carolina in Chapel Hill. He was an Assistant Research Professor in the Department of Molecular Genetics and Microbiology at Duke University Medical Center in Durham.

Presentation abstract:

Next Generation Sequencing Tools

During past two years we have experienced rapid growth in the demand for next generation sequencing. Such a development proves that next generation sequencing has become an essential tool for genome analysis of human and model organisms. We have been gaining valuable experience along with technology development and are introducing new applications for instruments that we have at our disposal. We have learned a lot about weaknesses and advantages of the different sequencing platforms and their relevance to the desired scientific result.
-During this presentation I would like to give you an overview of several sequencing platforms and their performance in the context of different research applications.
-I will also describe the dynamic nature of infrastructure's growth that is associated with development of the core facility, allowing us to perform different types of sequencing experiments. ----Additionally, I will present plans for expansion of the facility – plans that will make possible accommodation of the new applications relevant to cancer research.
-I will also show you examples of large cancer-related projects that are using deep sequencing as a primary tool for investigation and are performed at UNC.

Ian Davis, M.D., Ph.D.

UNC School of Medicine,
Assistant Professor
Dept. of Pediatrics and Dept. of Genetics
The University of North Carolina at Chapel Hill
Chapel Hill, NC

Dr. Davis received his medical training at Northwestern University Medical School and a PhD in mammalian genetics from the University of Illinois at Chicago. As a graduate student with Lester Lau, he studied the regulation of orphan nuclear receptors. He completed his pediatric residency and chief residency at Children's Hospital Boston followed by pediatric hematology/oncology fellowship at Dana-Farber Cancer Institute and Children's Hospital Boston. As part of his postdoctoral studies with David Fisher, he studied the mechanisms of melanocytic differentiation in tumors other than melanoma. He remained a staff physician at Dana-Farber for four years prior to joining the Division of Hematology/Oncology in the Department of Pediatrics, the Department of Genetics and the Lineberger Comprehensive Cancer Center in 2006. Dr. Davis' lab explores the mechanisms of aberrant transcription in cancer. Using genome-wide strategies, the lab studies how chromatin structure and epigenetics influence oncogenic transcription to activate genes required for cancer development. Since arriving at UNC, Dr. Davis has received the V Foundation for Cancer Research Martin Abeloff, MD award and a Rita Allen Scholar Award.

Presentation Abstract

Whole Genome Analysis of Transcriptional Regulation in Cancer

Aberrant transcription is a common feature of human cancers. One mechanism to deregulate transcription is through translocation-induced gene fusions. How these tumor-specific hybrid genes result in oncogenic transcription unknown. Ewing sarcoma, a highly malignant bone and soft tissue of children and young adults, is marked by a recurrent translocation that joins segments of the EWS gene with the developmentally regulated transcription factor FLI1. Using a comparative genome-wide analysis of chromatin targeting by EWS-FLI and its normal parental proteins, we have dissected the molecular and cellular influences on transcriptional activity. We demonstrate that despite identical DNA binding domains, chimerism results in widespread mislocalization and a marked difference in transcriptional profile in the tumor cells. Whereas normal FLI1 protein targets enhancers and proximal promoters in combination with other transcription factors, oncogenic EWS-FLI1 binds repetitive elements that contain the core response element. We find that EWS-FLI interacts with chromatin that is typically found in a repressed, closed nucleosome configuration. The use of genomic technologies to study chromatin organization also revealed deletions in known and putative tumor suppressor genes.

Jack Keene, Ph.D.

Duke University Medical Center
Professor
Dept. of Molecular Genetics and Microbiology
Duke University
Durham, NC

JACK KEENE, Ph.D. is James B. Duke Professor of Molecular Genetics and Microbiology at Duke University Medical Center in Durham, North Carolina, where he served as chairman of the Department of Microbiology for ten years. He was Director of Basic Science for the Duke Comprehensive Cancer Center for eight years, and founded the Duke Center for RNA Biology in 1999. Prof. Keene is a graduate of the University of California, Riverside, and received his doctorate in Microbiology and Immunology from the University of Washington, Seattle, in 1974. After postdoctoral studies in molecular virology at the National Institutes of Health from 1974-78, he joined the faculty at Duke University Medical Center on January 1, 1979.

Since 1975, Professor Keene's research has concerned RNA regulation by RNA-binding proteins. His interest in RNA-protein interactions led the lab to derive the first genomic sequences at the termini of several RNA viruses, including VSV, rabies and Ebola. Keene identified genetic mutations responsible for the formation of defective interfering RNA viruses, as well as several small noncoding viral transcripts. Beginning in 1983, Prof. Keene's group derived cDNA clones encoding autoimmune proteins that are also RNA-binding proteins, including the La (SS-B), Ro (SS-A), U1-70K RNP and U1A, and studied their RNA binding properties. These studies led to the discovery of the RNA Recognition Motif (RRM) family of RNA-binding proteins, now one of the largest protein families in the human genome. His lab later derived cDNAs encoding ELAV/Hu RRM proteins and demonstrated that they bind and stabilize multiple early response gene transcripts. Novel methods devised in his laboratory in the 1990's allowed him to propose a new regulatory model of gene expression in eukaryotes based upon the organization and dynamics of messenger RNA subpopulations governed by RNA-binding proteins. The Keene lab demonstrated that these trans-acting factors coordinate the activities of functionally-related messenger RNAs in time and space, and orchestrate biological outcomes in neuronal and immune cells. Together with experiments performed by other researchers using yeasts, worms, flies, trypanosomes and mammals, Keene's concept of "Posttranscriptional RNA Operons and Regulons" has been broadly substantiated.

Presentation Abstract:

COMBINATORIAL mRNA COORDINATION BY RNA OPERONS AND REGULONS

Jack D. Keene
Duke University Medical Center
Durham, North Carolina USA

We study the targeting and dynamics of RNA in mammalian cells using neuronal differentiation and T cell activation as model systems. We developed immunoprecipitation of endogenous ribonucleoprotein complexes (RIP-chip and RIP-seq) for genome-wide applications of mRNA regulation using microarrays to quantify changes in RNA populations during growth and differentiation. We found that many sequence-specific RNA-binding proteins (RBPs) bind and regulate discrete subpopulations of functionally related mRNAs that are coordinated by multi-targeting of mRNAs (Tenenbaum et al., PNAS 2000). Moreover, while comparing several RBPs and their mRNA targets we noted that while each RBP gave a unique pattern of mRNA targets, some mRNAs were associated with more than one RBP suggesting that combinatorial interactions are key to mRNA coordination. Given that each type of mRNA has more than one copy, we proposed that each mRNA type participates in more than one functionally related group. These observations led to a model in which mRNAs that encode functionally related proteins are coordinated dynamically during cell growth and differentiation as post-transcriptional RNA operons or regulons (Keene, PNAS 2001; Keene and Tenenbaum, Mol Cell 2002). More recent findings from many labs have confirmed that multiple mRNAs are co-regulated by sequence-specific RBPs during splicing, export, stability, localization and translation (Keene, Nat Rev Gen 2007). However, while RBPs appear to coordinate many key decisions during cell growth and differentiation, the dynamic changes during RNA stability and translation are poorly studied on a global basis during these processes. I will discuss how quantitative probabilistic RNA dynamics can be used as a quantitative phenotype to query the drug-gene-disease-phenotype Connectivity Map in order to identify small molecule drugs affecting posttranscriptional regulatory networks during T-cell activation (Mukherjee et al., Mol Syst Biol 2009). In collaboration with Thomas Tuschl we have used the PAR-CLIP together with RIP-chip using the HuR RBP to examine mRNA targets that are functional and combinatorial with microRNAs. Time permitting, I will describe examples of RNA operons/regulons in yeast, fruit-fly, trypanosomes and mammalian cells, and their potential importance in processes such as the immune response, oxidative stress and disease.

T1

Sister chromatid cohesion prevents DNA damage-induced genome instability

Shay Covo, Amit K. Reddy, Dmitry A. Gordenin and Michael A. Resnick
National Institute of Environmental Health Sciences, RTP, NC

The sister chromatid cohesion (SCC) complex known as cohesin tethers sister chromatids and ensures faithful transmission of chromosomes to daughter cells. Cohesin is also involved in chromosome structure, maintenance, transcription and DNA repair. Consistent with these roles, mutations in cohesin are associated with cancer and developmental defects. Using a gene dosage approach developed in tetraploid yeast we recently reported that cohesin is present in limiting amounts and that reduced levels greatly enhance the risk of DNA damage-induced genome instability. This is because cohesin normally restricts repair to sister chromatids. Reduced cohesin level opens the genome to recombinational interactions between homologous chromosomes that can then lead to loss-of-heterozygosity. Given the importance of cohesin levels in genome stability, we have extended these findings to address the role of regulators of SCC. SCC is established during replication; however, it is positively regulated (*i.e.,* more cohesin) in response to DNA damage induced after replication. Contrary to the commonly held view that post-replication SCC protects against double strand break-induced genome instability, our recent results reveal a major role for the S-phase cohesin in preventing IR-induced recombination between homologues. Furthermore, this cohesin is also highly protective against interhomologue recombination induced by UV-lesions, which do not induce double-strand breaks. Overall, these results demonstrate that S-phase SCC is essential to maintenance of genome stability. Given that the sister chromatid cohesion process is sensitive to environmental perturbations we suggest that defects in S phase SCC are risk factors in human health in combination with DNA damage.

NOTES:

T2

Genotoxic Effects of Titanium Dioxide and Cerium Dioxide Nanoparticles in Human Respiratory Epithelial Cells

Raju Y. Prasad[1], K. Wallace[2], A.H. Tennant[2], A.D. Kligerman[2], C.F. Blackman[2], D.M. DeMarini[2]

[1] Student Services Contractor, Integrated Systems Toxicology Division, US EPA, RTP, NC 27711

[2] Integrated Systems Toxicology Division, US EPA, RTP, NC 27711

The nanomaterial industry has recently seen rapid growth, therefore, the risk assessment of human exposure to nanomaterials in consumer products is of paramount importance. The genotoxicity of nanomaterials is a fundamental aspect of hazard identification and regulatory guidance. However, reports do not always characterize adequately the particles and frequently has conflicting genotoxicity results. We investigated the genotoxicity of two titanium dioxide (Degussa P25 TiO_2, 27.5 nm, anatase/rutile mixture and Alfa Aesar TiO_2, 32 nm, anatase) and one cerium dioxide (NanoAmor CeO_2, 15-30 nm) nanomaterials on human tracheal epithelial cells (BEAS-2B) at concentrations of 10-150 µg/ml. Nanomaterials were prepared in two different treatment media: keratinocyte growth media (KGM) with 0.1% BSA, or with PBS with 0.6 mg/ml BSA plus 0.01 mg/ml of the surfactant DSPC (DM), to determine whether the degree of agglomeration impacts genotoxicity. Dynamic light scattering was used to determine the size of nanomaterials: in DM, size was significantly smaller than in KGM at all concentrations. Cytotoxicity was assessed by trypan blue dye exclusion and confirmed using Live/Dead® staining. Only Degussa P25 TiO_2 was cytotoxic at 150 µg/ml. To investigate genotoxicity, the comet assay and micronucleus test with kinetochore staining were performed. In KGM, the % tail DNA was increased significantly at all concentrations for both TiO_2 nanomaterials but not for CeO_2 nanomaterials. None of the nanomaterials induced micronuclei in KGM. We are currently investigating the genotoxic effects of these nanomaterials in DM. [Abstract does not necessarily reflect the policies of the U.S. EPA.]

NOTES:

T3

The Intra-S Checkpoint in Melanoma Cell Lines

John J. McNulty[1] and Marila Cordeiro-Stone[1,2]
[1]Dept.of Path. and Lab. Medicine ,UNC, Chapel Hill, NC 27599

Exposure to sunlight is associated with melanoma development. One common source of DNA damage is ultraviolet light (UV), the component of sunlight that damages DNA most efficiently. It is predicted that defects in the cellular capacity to sense and respond to DNA damage will increase the risk of cancer development. When cellular DNA is damaged checkpoints that delay the cell cycle are activated. The intra-S checkpoint inhibits new replicon initiation and slows down replication fork progression. We are examining a variety of melanocyte and melanoma cell lines to evaluate whether disruption of the intra-S checkpoint could have contributed to melanoma development. Cells are exposed to various fluences of UVC (254 nm), labeled with radioactive precursors, and analyzed by velocity sedimentation through alkaline sucrose gradients. DNA is distributed by size through each gradient, and the abundance of newly synthesized DNA provides a measurement of intra-S checkpoint function. Differences in abundance and phosphorylation status of proteins known to be involved in the intra-S checkpoint response are being examined by western blot analysis. We have found that seven of nine of the melanoma lines examined have replication profiles indicative of normal checkpoint response. Many of the genes required for the intra-S checkpoint are essential for survival and perhaps the intra-S checkpoint protects against accumulation of deleterious mutations that would compromise melanoma formation. Sponsored by PHS T32 ES07017 and RO1 ES015856

NOTES:

T4

RAP80 Plays a Critical Role in Maintaining Genomic Stability

Zhengyu Yin[1], Anton Jetten[1] . 1. Laboratory of Respiratory Biology, Division of Intramural Research, NIEHS, NIH, RTP, NC. 27909

The DNA damage response (DDR) coordinates activation of cell cycle checkpoints, apoptosis and DNA repair networks, to ensure accurate repair and genomic integrity. Phosphorylation of the histone H2A variant, referred as γ-H2AX, is one of the initial signaling events which sense DNA double strand breaks and is required for the subsequent recruitment of many DDR proteins to sites of DNA damage. Upon DNA damage events like ionizing irradiation (IR), ubiquitin interaction motif (UIM)-containing protein RAP80 binds to poly-ubiquitin chain of H2A and γ-H2AX, and mediates DNA repair events by recruiting DDR mediators and effectors. In this study, RAP80 knockout (KO) mice were generated and characterized. Although RAP80 KO mice are viable and there appears to be no major anatomic defect in mice less than 3 months old, mouse embryonic fibroblasts (MEFs) from RAP80 KO mice exhibited slower proliferation as well as higher percentage of premature senescence compared to wild type (WT) MEFs. RAP80 KO MEFs also showed increased spontaneous and IR-induced genomic instability, which led to prolonged G2/M cell cycle arrest. There is higher percentage of spontaneous γ-H2AX positive cells in RAP80 KO MEFs compared to WT MEFs; IR induces more nuclear fragmentation in RAP80 deficient MEFs than WT controls. Loss of RAP80 increased the sensitivity to IR *in vivo* and *in vitro*. Together, these data indicate that RAP80 plays an important role in maintaining genomic stability. Further studies to characterize the phenotype of the aged RAP80 KO mice and their potential susceptibility to carcinogens will help better understand the physiological role of RAP80 and its contribution in DDR and/or tumorigenesis under environmental stresses.

NOTES:

T5

DNA-PK is a Checkpoint Kinase 1 (Chk1)-kinase stimulated by bulky DNA adducts

Michael Kemp and Aziz Sancar, Department of Biochemistry & Biophysics, University of North Carolina School of Medicine. Chapel Hill, NC

Ultraviolet (UV) light from the sun and environmental chemicals such as benzo[a]pyrene induce the formation of bulky adducts in DNA, resulting in activation of signalling cascades that coordinate DNA repair and cell cycle progression in order to minimize mutagenesis. A critical regulator of this DNA damage response is the checkpoint kinase Chk1. Since the mechanism of its phosphorylation and activation by DNA damage is unclear, we developed an in vitro, cell-free system employing nuclear extract from XP-A cells and plasmid DNA containing bulky adducts formed by the UV mimetic *N*-acetoxy-2-acetylaminofluorene (NA-AAF). Using this novel system we observed significant enrichment for phosphorylated Chk1 in reactions containing DNA with bulky adducts. Importantly, damaged DNA-dependent Chk1 phosphorylation took place in the absence of nucleotide excision repair, DNA replication, or transcription. Through the use of several pharmacological and biochemical approaches, we identified the DNA-dependent protein kinase (DNA-PK) as the primary kinase responsible for Chk1 phosphorylation in this system. Consistent with these findings, purified DNA-PK displayed preferential binding to bulky adduct-containing DNA and directly phosphorylated recombinant Chk1. We conclude that DNA-PK is involved in the direct recognition of bulky adducts in DNA and can mediate phosphorylation and activate the important DNA damage response kinase Chk1.

NOTES:

T6

Localized Hyper-mutability Caused by Chronic Alkylation Damage to a Eukaryotic Genome

Steven A. Roberts[1], Yong Yang[1], Joan Sterling[1], Michael A. Resnick[1], Piotr Mieczkowski[2] and Dmitry A. Gordenin[1]
1. Laboratory of Molecular Genetics, NIEHS, NIH, DHHS, Research Triangle Park, NC

Mutations are responsible for the diversity of DNA sequences and underlie the process of evolution as well as many diseases. Usually mutations accumulate independently; however, most single base substitutions in coding sequences fail to alter significantly the protein's activity. Therefore, dramatic genetic consequences, like gene inactivation or generation of alleles with novel function, may require multiple closely-spaced mutations. We demonstrate here that mutation clusters occur in eukaryotic cells grown in the presence of a DNA damaging agent. Strains of *Saccharomyces cerevisiae* were developed that allow selection for forward mutations in two genes, *URA3* and *CAN1*. We found that chronic exposure to methyl methanesulfonate causes inactivation of both ORFs when they are adjacent (less than 1 kB apart) but not when separated by 85 kB, indicating that the dual inactivation occurs as a single localized event. To characterize these mutation clusters and the mechanisms that contribute to their formation, we sequenced the genomes of *ura3 can1* double mutants. Surprisingly, joint inactivation of *URA3* and *CAN1* is often accompanied by many neighboring mutations (up to 30) which compose clusters that span up to 250 kB and display densities as high as one per kB. Our results demonstrate a new pathway of damage-induced mutagenesis in which a localized impediment to repair of DNA damage, combined with error-prone translesion synthesis, leads to localized severe genetic alteration in one generation. This clustered mutagenesis pathway could allow rapid diversification and selective advantage in adaptive evolution as well as initiate genetic disease and cancer.

NOTES:

P1

Mechanisms of mutagenesis *in vivo* due to imbalanced dNTP pools. Amy L. Abdulovic, Dinesh Kumar, Thomas A. Kunkel, and Andrei Chabes, NIEHS, Research Triangle Park, NC

We have examined mechanisms by which dNTP pool imbalances induce genome instability in strains of *Saccharomyces cerevisiae* with three different amino acid substitutions in Rnr1, the large subunit of ribonucleotide reductase. These strains have different dNTP pool imbalances that correlate with elevated *CAN1* mutation rates, with both substitution and indel rates increasing by 10-fold to more than 300-fold. The locations of the mutations in a strain with elevated dTTP and dCTP are completely different from those in a strain with elevated dATP and dGTP, thus demonstrating that imbalanced dNTP pools reduce genome stability in a manner that is highly dependent on the nature and degree of the pool imbalance. Mutagenesis is enhanced despite the availability of proofreading and mismatch repair, the two major replication error correction mechanisms. The mutations can be explained by imbalanced dNTP-induced increases in dNTP misinsertion, DNA strand misalignment, and mismatch extension at the expense of proofreading. This implies that the relative dNTP concentrations measured in extracts are truly available to a replication fork in vivo. An interesting mutational strand bias is observed in one *rnr1* strain, suggesting that the S-phase checkpoint may selectively prevent formation of replication errors during leading strand replication..

NOTES:

P2

Bypass of UV photoproducts by DNA Polymerase zeta

Jana Stone, D. Kumar, C. Stith, S. Iwai, P. Burgers, A. Chabes and T. Kunkel,
NIEHS, NIH, Research Triangle Park, NC

Ultraviolet (UV) radiation from sunlight induces two major types of lesions in DNA, *cis-syn* cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (PPs). Both of these lesions block synthesis by the DNA polymerases that replicate the majority of the genome. Thus, if UV photoproducts are not removed from the DNA by repair processes prior to replication, they can cause replication forks to stall. DNA polymerase zeta (Pol ζ) is involved in translesion synthesis (TLS) past DNA adducts that stall replication forks. Biochemical studies have led to the suggestion that the role of Pol ζ in TLS of UV photoproducts is to extend primers created when another DNA polymerase inserts nucleotides opposite lesions. We demonstrate that *S. cerevisiae* Pol ζ has the capacity to bypass both major UV photoproducts *in vitro* without assistance from another DNA polymerase. In reactions permitting a single cycle of synthesis, both CPDs and (6-4) photoproducts are bypassed by a derivative of Pol ζ that contains a phenylalanine substituted for Leu979 at the polymerase active site of Rev3, the catalytic subunit of Pol ζ. TLS was observed in reactions containing dNTP concentrations present in normal yeast, and TLS efficiency was even greater when dNTP concentrations were increased to those induced by DNA damage *in vivo*. The increase in TLS efficiency conferred by the Phe substitution provides an explanation for the observation that yeast strains encoding L979F Pol ζ have elevated levels of UV-induced mutagenesis. Parallel TLS experiments demonstrate that at lower efficiency, wild-type Pol ζ can also bypass both UV photoproducts without assistance from another polymerase. The data are consistent with the possibility that Pol ζ may sometimes be the sole DNA polymerase involved in mutagenic TLS.

NOTES:

P3

Weighted sums approach to test for association of rare variants with continuous traits: Power comparisons with dichotomized traits on simulated models

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With recent advances in genotyping technology, many genetic studies are investigating the role of rare variants in complex traits via the Common Disease Rare Variant (CDRV) hypothesis. In this study, we modify the existing weighted sums test to test for excess of variants in the tails of quantitative traits. We call this approach the quantitative weighted sums test. Powers of this test are compared with dichotomizing the quantitative trait into cases and controls on a range of simulated models with varying parameters. The results show no significance difference for the power comparisons, although a reduction in power is seen for both the tests on small cohort sizes. The power of these tests can be improved for small cohorts by designing prior hypothesis. The simulations also show that the pooling of variants by some biological function improves the power of the test and decreases spurious association with random non-causal variants.

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P4

Dose-Response Evaluation of p53 and H2AX DNA Damage Response by Methyl Methanesulfonate, Etoposide, and Quercetin

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When cells encounter DNA damage, they respond by initiating cell cycle arrest, damage repair or apoptosis. How DNA damage pathways differentially respond to diverse DNA damage is not yet fully understood. The current study used the HT1080 human fibrosarcoma cell line, which expresses a wild-type p53, to examine time and dose dependent response of H2AX phoshporylation (pH2AX), total p53 and p53 phosphorylation (p-p53), with exposure to methyl methanesulfonate (MMS; methylating agent) etoposide (ETP; DNA/topoisomerase II binding chemotherapeutic) and quercetin (QUE; oxidative flavonoid). We found that 1 to 1000 μM MMS, 0.1 to 100 μM ETP, 1 to 100 μM QUE dose-dependently decreased HT1080 cell viability. Analysis of cell cycle by high throughput bioimaging demonstrated significant G0/G1 arrest at 10 μM QUE, while ETP induced M phase arrest at 1 μM. MMS, however, induced a G2/M arrest only at 10000 μM. Bioimaging and immunoblots showed that both ETP and QUE caused time- and dose-dependent increases in the DNA double strand break marker p-H2AX within 24 hr, while MMS had a marginal effect. ETP showed greatest ability to induce p53 and p53 phosphorylation at ser15 (p-p53), while MMS had the weakest effect. Results with this cell line are being compared to the lymphoblast AHH-1 cell line, which has been used in mutagenesis studies, in order to compare the dose-dependence of cellular repair response and increased mutation frequency. The preliminary data for AHH-1 cells suggest that AHH-1 cells, albeit less sensitive to those reagents, show comparable p53 and p-H2AX DNA damage response to HT1080 cells. In conclusion, these results suggest that ETP and QUE but not MMS cause DNA double strand breaks and those chemicals differentially activate DNA damage repair pathways.

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P5

Mechanisms Underlying Sunlight-Induced Skin Carcinogenesis

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The primary risk factor for skin cancer development is solar ultraviolet (UV) radiation, an established environmental carcinogen that induces DNA damage. Because of differences in energy, types of DNA damage, dose, and skin penetration, it is difficult to assess the contributions of different UV wavelengths in promoting carcinogenesis. Previous research has suggested that the most prevalent and mutagenic UV-induced photolesion is the cyclobutane pyrimidine dimer (CPD), and that UVA (320-400nm) causes more mutations per CPD than do UVB (280-320nm) or UVC (200-280nm). One potential explanation could be that UVA inhibits efficient repair of CPD or that UVA inhibits DNA damage checkpoints that allow time for DNA repair prior to replication. To examine this hypothesis, nucleotide excision repair (NER) of CPD and the activation of the intra-S phase checkpoint were examined in normal human keratinocytes (K2) or fibroblasts (NHF1), following exposure to UVA or UVC. Results show that although UVA induced higher mutation frequency than UVC in fibroblasts, no apparent differences in the rate of CPD repair were observed in K2 or NHF1 exposed to UVA or UVC. Intra-S phase checkpoint activity was assessed by examining the degree of Chk1 phosphorylation and the extent to which UVA or UVC inhibited replicon initiation and DNA elongation. Results showed that for a given CPD density, UVA and UVC activated the intra-S phase checkpoint to similar extents. These data suggest that the increased mutational burden incurred by UVA, as compared to shorter wavelengths, is not due to alterations to NER or the intra-S phase checkpoint.

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P6

Discovery of novel micro-RNAs in the Rat transcriptome

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Micro-RNAs (miRNAs) are post-transcriptional regulators and play a significant role in the normal functioning of eukaryotic cells. Aberrant expression of miRNA is known to be associated with many diseases including cancer, heart disease and psychological disorders. Recently, microRNAs have emerged as effective biomarkers of disease and toxic injury. This has generated a great deal of interest in identification and characterization of novel miRNAs in genomes of various species. In this study, we use glucocorticoid-induced apoptosis of lymphocytes as a model system along with deep sequencing technology to investigate hormone dependent changes in miRNA expression. We use RNA preparation from untreated and glucocorticoid treated Rat primary thymocytes and employ small RNA sequencing protocol along with the Illumina-Solexa deep sequencing platform to obtain millions of short sequence reads. The sequencing data is analyzed using a comprehensive bioinformatics approach to calculate changes in expression of both previously annotated as well as potentially novel miRNAs. For novel miRNA discovery, we use a multi-step computational approach based on miRNA prediction algorithms using sequence features, secondary structure prediction and machine learning methods. Our results indicate that a large number of known miRNAs are down regulated up on hormone treatment. In addition, we find a set of potentially novel miRNAs in the Rat transcriptome whose expression also changes due to hormone treatment. Quantitative PCR validation agrees with our bioinformatics analyses for a set of putative novel miRNA loci, thus providing further evidence for the presence of novel miRNAs in the Rat transcriptome.

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P7

Oxidative stress and p38 MAPK activation as toxicity mechanisms of silver nanoparticles in soil nematode *Caenohabditis elegans*

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We have examined mechanisms by which dNTP pool imbalances induce genome instability in strains of *Saccharomyces cerevisiae* with three different amino acid substitutions in Rnr1, the large subunit of ribonucleotide reductase. These strains have different dNTP pool imbalances that correlate with elevated *CAN1* mutation rates, with both substitution and indel rates increasing by 10-fold to more than 300-fold. The locations of the mutations in a strain with elevated dTTP and dCTP are completely different from those in a strain with elevated dATP and dGTP, thus demonstrating that imbalanced dNTP pools reduce genome stability in a manner that is highly dependent on the nature and degree of the pool imbalance. Mutagenesis is enhanced despite the availability of proofreading and mismatch repair, the two major replication error correction mechanisms. The mutations can be explained by imbalanced dNTP-induced increases in dNTP misinsertion, DNA strand misalignment, and mismatch extension at the expense of proofreading. This implies that the relative dNTP concentrations measured in extracts are truly available to a replication fork in vivo. An interesting mutational strand bias is observed in one *rnr1* strain, suggesting that the S-phase checkpoint may selectively prevent formation of replication errors during leading strand replication..

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P8

Altered DNA Methylation Patterns in Individuals with Arsenicosis

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Inorganic arsenic (iAs) is a documented carcinogen and an environmental toxicant, poisoning tens of millions of people worldwide through drinking water. Individuals exposed chronically to iAs are susceptible to arsenicosis, or arsenic poisoning. Such exposure is associated with the development of skin lesions and in some cases skin cancer. One of the proposed mechanisms of iAs-induced disease is altered gene regulation via epigenetic modes of action such as DNA methylation. Here we set out to identify differentially methylated genomic regions associated with arsenicosis in humans exposed to iAs via their drinking water. Lymphocyte DNA of sixteen individuals from the arsenicosis endemic region of Zimapan, Mexico was analyzed. Of these individuals, half showed signs of arsenicosis with skin lesions. All samples were analyzed using a Methylated CpG Island Recovery-Chip assay. The methylomes of two individuals selected with or without skin lesions were subsequently compared at single nucleotide resolution using Reduced Representation Bisulfite Sequencing. These combined analyses assess differential methylation at more than 1 million CpG sites within ~14, 000 CpG islands. Mapping CpG sites to islands and performing comparative analysis resulted in the identification of genes with differential methylation patterns associated with arsenicosis. These genes are enriched for their involvement in cancer-associated pathways such as p53 and NF-kB. These data demonstrate the significant effects of iAs on the epigenome and suggest that changes in DNA methylation patterns may serve as biomarkers of adverse health effects associated with iAs exposure.

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P9

The Consequences of Incorporating Ribonucleotides into DNA

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The presence of ribonucleotides in DNA alters helical parameters to create one form of alternatively structured DNA. In reactions containing dNTP and rNTP concentrations present *in vivo*, *S. cerevisiae* DNA polymerases α, δ, and ε incorporate rNMPs into DNA during DNA synthesis *in vitro* (PNAS 107:4949). Quantification of rNMPs incorporated, and extrapolations based on the roles of these three polymerases in replication, suggests that rNMPs could be the most common of all non-canonical nucleotides introduced into the nuclear genome. To determine if ribonucleotides are indeed incorporated into DNA during DNA replication *in vivo*, we substituted Leu or Gly for Met644 in the active site of Pol ε. Compared to wild type Pol ε, rNMP incorporation *in vitro* is 3-fold lower for M644L Pol ε and 11-fold higher for M644G Pol ε. This order is re-capitulated *in vivo* in yeast strains lacking RNase H2. Compared to control strains, an RNase H2-deficient *polε-M644G* mutant progresses more slowly through S-phase, has elevated dNTP pools and generates 2-5 base pair deletions in repetitive sequences at a high rate. The data indicate that rNMPs are incorporated during replication *in vivo*, that they are removed by RNase H2-dependent repair, and that defective repair results in replicative stress and genome instability via strand misalignment. Additional studies are underway to investigate genetic control of ribonucleotide incorporation and repair, as well as how unrepaired rNMPs in DNA are converted into mutations. The results are providing novel information regarding a largely unexplored aspect of DNA repair and genome instability.

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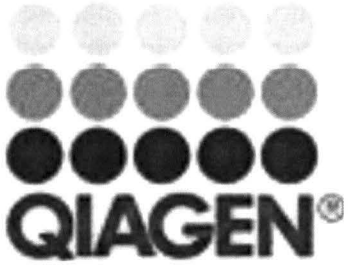

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