Yair Dorsett

Curriculum Vitae

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Date of Birth: March 8, 1978

Place of Birth: Oak Ridge, Tennessee

Citizenship: USA and IL

Education:
2000, B.A. Biology. The Colorado College, Colorado Springs, Colorado

2002, M.A. Biology. New York University, New York, New York

2008, Ph.D. The Rockefeller University, New York, New York.
Field: Cancer Immunology. Advisor: Michel Nussenzweig

Research Experience:
Summer 2000 – Postgraduate Research, Rockefeller University, New York. PI: Michel Nussenzweig. Role of dendritic cells in immune response - in vivo targeting of antigen to dendritic cells by recombinant antibody specific for the DEC receptor.

2001-2002 – Masters Research, New York University, Biology Department. PI: Gloria Corruzzi. Identification of cis promoter elements that react to light, nitrogen or carbon in plants by DNA sequence and microarray analysis of genes that respond to these stimuli.

2002 – 2005 - Graduate Research, Rockefeller University, New York. PI: Thomas Tuschl. Research: Mechanisms of RNA interference in mammalian germ cells and Drosophila.

2005 – 2008 - Ph.D. Thesis Research, Rockefeller University, New York. PI: Michel Nussenzweig. Role of AID and microRNA-155 in *c-myc-IgH* translocations.

2008 – 2009 - Postdoctoral Research, Rockefeller University, New York, PI: Michel Nussenzweig. Role of microRNA-155 in regulation of AID and *IgB* to *IgH* and *c-myc* to *IgH* translocations.

2009 – 2015, Postdoctoral Fellow and Staff Scientist, Washington University School of Medicine. PI: Barry Sleckman. Role of H2AX and 53BP1 in non-homologous end joining and development of a novel assay for the identification of DNA end structures at single nucleotide resolution.

2015 – present, Associate Research Scientist, The Jackson Laboratory for Genomic Medicine. PI: George M. Weinstock. Development of methods for defining the microbiome and investigation of how the microbiome influences the development of Multiple Sclerosis.

Fellowships and Awards:

1/1/09 – 12/31/11, Department of Health and Human Services Public Health Service Ruth L. Kirschstein National Research Service Award.

1/1/13 - 12/31/13, American Cancer Society Postdoctoral Fellowship.

Teaching Experience:

2000 – 2002, New York University, General Biology Laboratory.

Website:

http://www.researchgate.net/profile/Yair_Dorsett2
https://www.ncbi.nlm.nih.gov/sites/myncbi/1BkwIjXJbZGAR/bibliography/51999401/public/?sort=date&direction=ascending

Invited Lectures:

October 2008, 24th Annual Tolmach Symposium and 4th Annual Siteman Cancer Center DNA Repair Program Symposium on Radiation Biological Sciences. Washington University School of Medicine (lecture given).

August 2015, Zing Genomic Integrity Conference, Cairns, Australia (lecture not given).

Poster Presentations:

1. 2007, A role for AID in c-myc-IgH translocations. DNA Repair Symposium, Spain - Summer 2007.

2. 2007, A role for AID in c-myc-IgH translocations. Rockefeller Symposium

3. 2008, MircoRNA-155 suppresses AID mediated c-myc – IgH translocations. Cold Spring Harbor Immunology Symposium

Patents:

1. US Patent 7,739,053. June 15, 2010
Palenchar, P., Shasha, D., Chou, M., Rejali, M., Dorsett, Y., Kouranov, A., Coruzzi, G. System and process of determining a biological pathway based on a treatment of a biological specimen.

Experience:

Molecular Cloning: Construct generation, 5' RACE and clone mutagenesis.

Other Nucleic Acid techniques: Southern blotting (genomic & PCR), northern blotting, microRNA northern, DNA isolation and RNA isolation.

Protein work: Nuclear and cytoplasmic protein fractionation, sucrose gradient fractionation of miRNPs after radiolabelling of miRNA antisense oligo, western blotting, immunoprecipitation and protein expression / isolation from bacteria on ion exchange columns.

Mouse Work: Mouse dissection, mouse husbandry, BAC construction for gene targeting.

Cell Culture Work: Cell isolation by magnetic beads, FACS, lentivirus and retrovirus production/collection/infection, cell line generation, nucleofection and transfection.

Enzyme Usage/Knowledge: Restriction enzymes, cell culture expression of RAG, AID, Zinc finger nuclease and CRISPR - Cas9 to generate DNA double stranded breaks and design of Cas9 gRNAs.

PCR: DNA oligo purification, PCR, qRT-PCR, bisulphite PCR, Tdt-mediated PCR and LM-PCR.

Sequencing library preparation: MinIon, PacBio, MiSeq, 454 and S5 Ion Torrent.

Writing Experience: Grant, paper and proposal writing and editing.

Developed & Adapted methods: Formation of long concatemers from template sequence for generating consensus sequences on the MinIon sequencing platform, Urea agarose heat denaturing gel and identifying individual DNA end structures at single nucleotide resolution from a heterogeneous population of DNA end structures (Hairpin-Capture of DNA End Structures (HCoDES).

Publications:

1. Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J.V., Steinman, R.M., and Nussenzweig, M.C. (2001) Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. ***J Exp Med*** *194*, 769-779.

2. Dorsett, Y., and Tuschl, T. (2004) RNA interference. ***McGraw-Hill Yearbook Of Science & Technology***. 292-295.

3. Dorsett, Y., and Tuschl, T. (2004) siRNAs: applications in functional genomics and potential as therapeutics. ***Nat Rev Drug*** *3*, 318-329.

4. Meister, G., Landthaler, M., Dorsett, Y., and Tuschl, T. (2004) Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. ***RNA*** *10*, 544-550.

5. Meister, G., Landthaler, M., Patkaniowska, A., Dorsett, Y., Teng, G., and Tuschl, T. (2004) Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. ***Mol Cell*** *15*, 185-197.

6. Dorsett, Y., Robbiani, D.F., Jankovic, M., Reina-San-Martin, B., Eisenreich, T.R., and Nussenzweig, M.C. (2007) A role for AID in chromosome translocations between c-myc and the IgH variable region. ***J Exp Med*** *204*, 2225-2232.

7. Dorsett, Y., McBride, K.M., Jankovic, M., Gazumyan, A., Thai, T.H., Robbiani, D.F., DiVirgilio, M., San-Martin, B.R., Heidkamp, G., Schwickert, T.A., Eisenreich, T., Rajewsky, K., Nussenzweig. M.C. (2008) MicroRNA-155 Suppresses Activation-Induced Cytidine Deaminase-Mediated *Myc-Igh* Translocation**.** ***Immunity*** *28*, 630-638.

8. Robbiani, D.F., Bothmer, A., Callen, E., Reina-San-Martin, B., Dorsett, Y., Difilippantonio, S., Bolland, D.J., Chen, H.T., Corcoran, A.T., Nussenzweig, A., Nussenzweig, M.C. (2008) Activation Induced Deaminase is required for the chromosomal breaks in *c-myc* that lead to *c-myc/IgH* translocations. ***Cell*** *135*, 1028-1038.

9. Sernández, IV., de Yébenes, VG., Dorsett, Y., Ramiro, AR. (2008). Haploinsufficiency of activation-induced deaminase for antibody diversification and chromosome translocations both in vitro and in vivo. ***PLoS One*** *3*, e3927.

10. Jankovic, M., Robbiani, D.F., Dorsett, Y., Eisenreich, T., Xu, Y., Tarakhovsky, A., Nussenzweig, A., Nussenzweig, M.C. (2010). Role of the translocation partner in protection against AID-dependent chromosomal translocations. ***PNAS*** *107*, 187-192.

11. Helmink, B.A., Tubbs, A.T., Dorsett, Y., Bednarski, J.J., Walker, L.M., Feng, Z., Sharma, G.G., McKinnon, P.J., Zhang, J., Bassing, C.H., Sleckman, B.P. (2011). H2AX prevents CtIP-mediated DNA end resection and aberrant repair in G1-phase lymphocytes. ***Nature*** *469*, 245-249.

12. Gapud EJ, Dorsett Y, Yin B, Callen E, Bredemeyer A, Mahowald GK, Omi KQ, Walker LM, Bednarski JJ, McKinnon PJ, Bassing CH, Nussenzweig A, Sleckman BP. (2011). Ataxia telangiectasia mutated (Atm) and DNA-PKcs kinases have overlapping activities during chromosomal signal joint formation. ***PNAS*** *108*, 2022-2027.

13. Lee BS, Gapud EJ, Zhang S, Dorsett Y, Bredemeyer A, George R, Callen E, Daniel JA, Osipovich O, Oltz EM, Bassing CH, Nussenzweig A, Lees-Miller S, Hammel M, Chen BP, Sleckman BP. (2013). Functional intersection of ATM and DNA-dependent protein kinase catalytic subunit in coding end joining during V(D)J recombination. ***Mol. Cell Biol*** *33*(18):3568-79.

14. Tubbs AT, Dorsett Y, Chan E, Helmink B, Lee BS, Hung P, George R, Bredemeyer AL, Mittal A, Pappu RV, Chowdhury D, Mosammaparast N, Krangel MS, Sleckman BP. 2014. KAP-1 promotes resection of broken DNA ends not protected by γ-H2AX and 53BP1 in G1-Phase Lymphocytes. ***Mol Cell Biol*** *34*(15):2811-21.

15. Dorsett Y, Zhou Y, Tubbs AT, Chen BR, Purman C, Lee BS, George R, Bredemeyer AL, Zhao Jiang-yang, Weinstock ES, Weinstock GM, Reyes A, Oltz ME, Dorsett D, Misulovin Z, Payton JE, Sleckman BP. 2014. HCoDES Reveals Chromosomal DNA End Structures with Single-Nucleotide Resolution, ***Molecular Cell*** *56* (6):808-818.

Additional HCoDES sequence analysis and potential applications: - 2015

The DNA ends generated by double-strand breaks are normally protected from nucleolytic processing that inhibit NHEJ and facilitate aberrant repair. Many tumors that prove resistant to chemotherapy have mutations in the factors that protect DNA ends, including the H2AX histone variant and 53BP1 repair protein. To understand how these proteins protect DNA ends and prevent aberrant repair, we developed a new method to characterize the *in-vivo* structures and DNA methylation status of individual broken ends. This method, called HCoDES (hairpin capture of DNA end structures), uses high-throughput sequencing to define these mutagenic DNA end structures with single nucleotide resolution. Yanjiao Zhou (my wife and collaborator in the laboratory of George M. Weinstock), wrote an extensive sequence analysis pipeline as well as a suite of R programs for visualizing and interpreting the wealth of information produced by this assay, (**Figures 2 & 3**).

Application of this novel approach to double stranded DNA breaks generated by either a Zinc finger nuclease, CRISPR-Cas9 or the RAG recombinase- in G1 phase arrested B lymphocytes deficient for H2AX or 53BP1, revealed extensive DNA end processing. Unexpectedly, this aberrant processing gave rise to both 3' and 5' overhangs, and generated de-novo double-stranded breaks near the site of the original break (**Figure 2)**. Differences in the frequency and distributions of DNA end structures in lymphocytes deficient for H2AX and 53BP1 further suggest that they play distinct roles in DNA end protection and facilitating NHEJ. The DNA end structures revealed by these studies are likely key intermediates in the formation of chromosomal translocations observed in immune tumors and those that develop resistance to chemotherapy. The DNA end structures revealed by this aberrant nucleolytic processing was surprising in that the normal nucleolytic processing that occurs during S phase to prime DNA synthesis during repair by homologous recombination is believed to generate only long 3' overhangs. Surprisingly, the HCoDES assay also revealed that in Artemis -/- cell lines (Artemis is a nuclease that opens DNA hairpins), aberrant DNA end processing had decreased kinetics and concomitantly formed hairpin intermediates at DNA ends during DNA end processing, suggesting that Artemis facilitated DNA end resection by opening hairpin intermediates (data not shown).

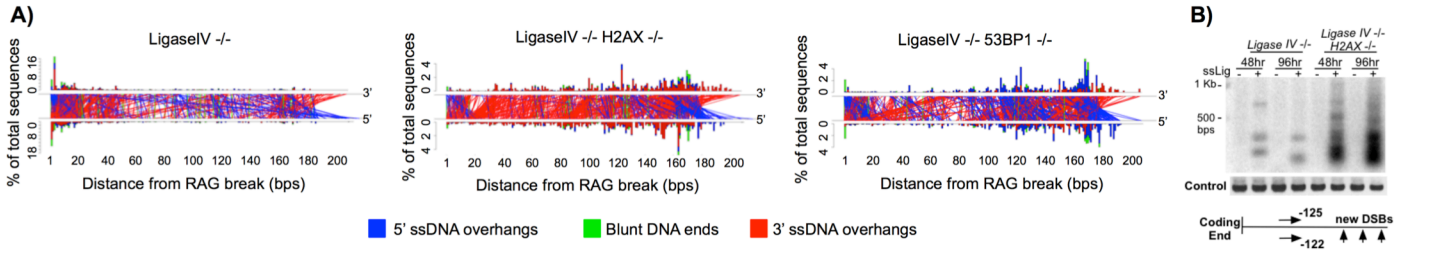

Figure 2. HCoDES detects aberrantly processed DNA ends at single nucleotide resolution. A) Segments plots depicting DNA end structures of unrepaired RAG breaks generated at a chromosomal recombination substrate in (Ligase IV -/- cells) and aberrantly resected RAG breaks (Ligase IV -/- H2AX -/- cells and LigaseIV -/- 53BP1-/- cells). Each graph contains sequences from three independent PCR reactions conducted with primers 200 bps away from the RAG break. Barplots on the top and bottom of each graph, indicate the percent of total sequencing reads with a DNA end at that particular nucleotide position, for the top and bottom strands, respectively. The colors of each of the bars indicate what proportion the ends are part of 5' overhangs (blue), 3' overhangs (red) or blunt ends (green). The lines connecting the top and bottom strands represent specific DNA end structures, with blue, red and green lines representing 5' overhangs, 3' overhangs and blunt ends respectively. **B)** PCR southern of HCoDES conducted with primers that face away from the RAG DSB (Coding End) generated at a chromosomal recombination substrate. Primer (arrows) distances from the DSB indicated in bps. New DSBs are more abundant and accumulate overtime only in cells undergoing resection (*Ligase IV -/- H2AX -/-*).

Our HCoDES approach also resulted in the unexpected finding that DNA methylation may protect unrepaired endogenous RAG DSBs from nucleolytic processing (not shown). These observations also suggested that once a DNA end is aberrantly processed to form a 5' overhang, that 5' overhang is demethylated only on the 5' end-bearing strand, and that this allows for 5' to 3' exonucleolytic resection and the formation of 3' overhangs (**Figure 3A & B**). Furthermore, our data shows that the demethylation of 5' overhangs depends on CtIP (**Figure 3C**), a transcription factor/nuclease that promotes this aberrant nucleolytic processing as well as the normal 5' to 3' exonucleolytic resection that takes place during homologous recombination in S phase. These observations provide important clues as to how altered DNA methylation in cancer may influence genomic stability in response to breaks generated by chemotherapy.

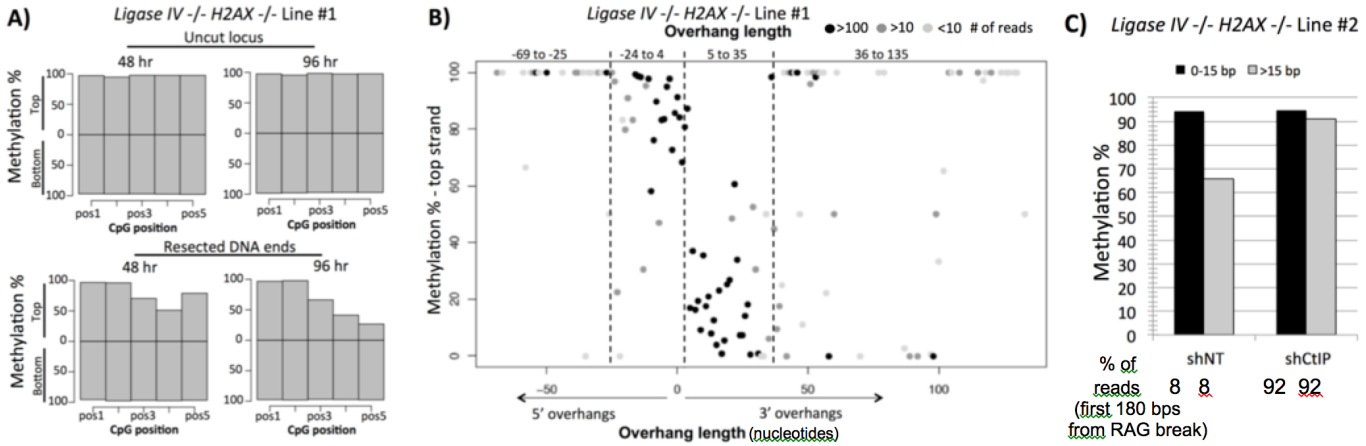

Figure 3. Resected DNA ends generated at endogenous Jk1 gene segments at the Kappa light chain locus are demethylated on the 5' end-bearing strand in a CtIP dependent manner. A) Resected DNA ends are demethylated on the top strand**.** Bar plot showing overall percent methylation at each of the five CpG positions observed with primers positioned 180 bps of the RAG break for the top and bottom strands. Top panels indicate methylation status of uncut Jk1 gene segments at 48 and 96 hrs post formation of RAG DSBs in G1 phase arrested cells as determined by HCoDES conducted on restriction enzyme digested DNA that cleaves only the uncut locus. Bottom panels indicate methylation status of unrepaired and resected DNA ends. While the uncut locus is fully methylated on top and bottom strand (even in cycling cells-not shown), resected DNA ends are demethylated on the top strand overtime. **B)** Methylation status of DNA ends depends on DNA end structure. The percent methylation of the CpG's remaining on the top strand (Y axis) after 48 hrs of nucleolytic resection was plotted by overhang length in nucleotides (X axis). Each dot represents a unique DNA end structure. Grey scale (top of scatter plot) is used to represent the sequencing read abundance of unique DNA end structures. Since our data indicates that 5' overhangs undergo 5' to 3' exonucleolytic resection overtime (not shown), this data suggests that exonucleolytic resection requires the demethylation of the 5' end-bearing strand. **C)** CtIP promotes DNA demethylation. Percent methylation of the CpG's remaining on the top strand (up to 3) for 5' ends resected less than 15 bp (black bars) and 5' ends resected more than 15 bps (grey bars) with (shCtIP) and without (shNT) CtIP knockdown. CtIP knockdown inhibits 5' to 3' resection (not shown) as well as demethylation of the top strand for aberrantly resected DNA ends. Consistent results were obtained for DNA ends in a separate cell line and in a *Ligase IV-/-* cell line.

The current HCoDES technology and bioinformatic analysis developed for these studies are broadly applicable to the study of DNA repair mechanisms in other cell types and processes including the processing of DNA nicks (ssLigase also catalyzes "trans" ligation reactions) or viral DNA ends upon infection. Furthermore, HCoDES could be used to assay chromatin structure when combined with nuclease accessibility assays or assays that measure GpC methyltransferase accessibility. Besides helping to answer biological questions, this assay has other practical approaches, such as enriching for sequences of interest.

Technological development and research: Summer 2015 – present.

In the laboratory of George M. Weinstock at The Jackson Laboratory for Genomic Medicine, I worked with Lei Chen to develop and utilize a method for generating long concatemers of full-length PCR amplicons in-order to employ consensus sequencing on the MinIon sequencing platform to increase accuracy. The increased accuracy would allow for the accurate identification of unique sequences from heterogeneous samples. We developed this approach utilizing full-length PCR amplicons of bacterial 16S ribosomal RNA genes so that heterogeneous bacterial communities may be accurately profiled using the MinIon as well as to test the capabilities of several different iterations of this rapidly developing technology. We initially generated and analyzed data from an artificially generated bacterial community and then moved on to utilizing real world samples that have extensive bacterial heterogeneity. This work demonstrated that sequencing accuracy could be dramatically increased after five copies of full-length 16S (97%) and up to ~99% with 10 copies, with accuracy increasing even further with additional copy number (**See Figure 4**).

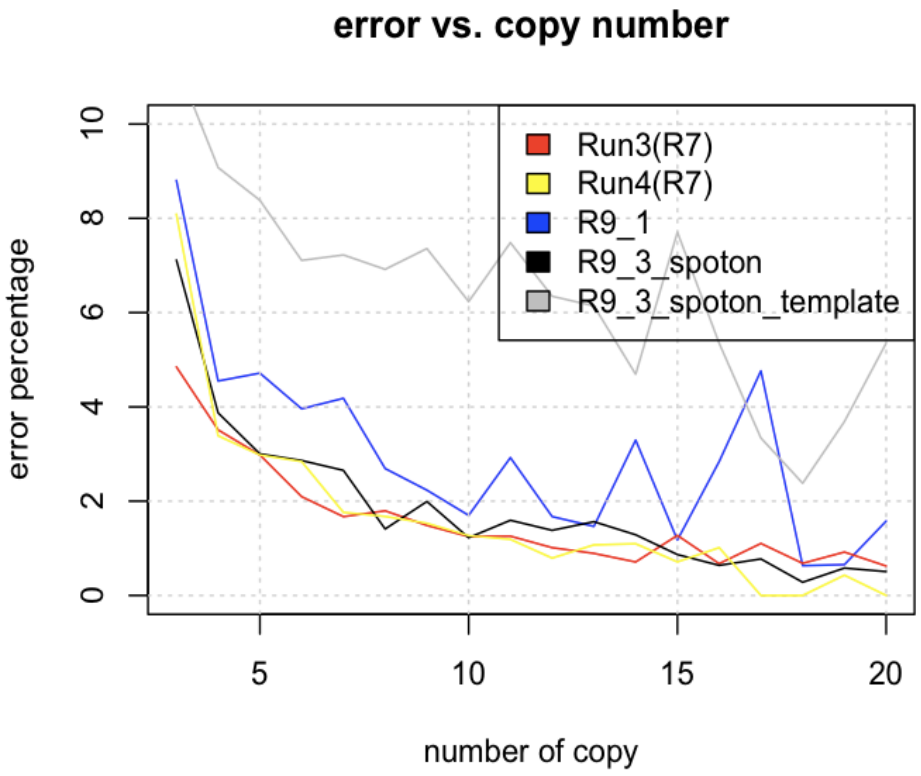

Figure 4. Graph illustrates that generating consensus sequences from increasing number off full length 16S amplicons decreases sequencing error rate of individual reads for several versions of MinIon chemistry. All consensus sequences were generated from 2D reads (2D reads are consensus sequences generated from sequencing the top and bottom strand of the same DNA molecule) with the exception of the "R9_3_spoton_template" sample, which represents the accuracy of only the first strand entering the pore (template strand). Lines represent the mean error rate and MinIon chemistry versions are Indicated by sample name.

I have also worked towards developing novel approaches for extracting DNA from microorganisms in the hope of identifying novel microorganisms and developing inexpensive approaches for rapid DNA isolation. I was given the opportunity to test a novel enzyme cocktail developed as part of the extreme microbiome project which aims to identify bacteria that are difficult to identify using traditional extraction approaches. The sequencing data indicated that the enzyme cocktail greatly aided the identification of gram-positive bacteria, permitting the identification of rare bacteria as well as those that are refractory to identification utilizing traditional approaches.

I also worked to optimize the amplification of the v1-v2 bacterial 16S ribosomal RNA genes for sequencing on the new Ion-Torrent S5 platform. Sequence analysis by others in our group indicated that the unidirectional sequencing approach of this platform produced ~5 fold greater number of high quality reads in the forward orientation then the reverse orientation and that the spectrum of the highly abundant bacteria was similar to that observed when sequencing v1-v3 amplicons on the MiSeq platform from the same biological sample.

Furthermore, I have also worked with others to generate sequencing reads on the 10X Genomics Chromium system from heterogeneous microbial DNA samples to better ascertain the true complexity of human bacterial community structure by utilizing the systems immense number of unique barcodes. Extensive analysis by members our laboratory of the preliminary sequencing data suggests that the development and adjustment of the current wet lab protocol will allow us to attain this goal.

These experiments will serve as a basis for the implementation of novel technological designs and ideas.

Professional References

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