BIOGRAPHICAL SKETCH

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NAME: David Jeruzalmi

eRA COMMONS USER NAME (credential, e.g., agency login): DJERUZALMI

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Cincinnati, Cincinnati, OH	B.S.	06/1987	Chem./Biochem.
Yale University, New Haven, CT	M. Phil	05/1989	Mol.Biophys/Biochem.
Yale University, New Haven, CT	Ph. D.	05/1994	Mol.Biophys/Biochem.
Yale University, New Haven, CT	Post-doc	08/1996	Protein Crystallography
The Rockefeller University, New York, NY	Post-doc	06/1998	Protein Crystallography

A. Personal Statement

My research group is broadly interested in the molecular mechanisms that underlie the faithful transmission of genetic information. We are currently focused on two areas, the machinery associated with DNA replication initiation and 2) nucleotide excision repair. A large body of work has described the associated complexes and details of how they operate. And yet, something as fundamental as a three-dimensional image of the active entity in various stages of operation remains elusive. The goal of research in my laboratory is to provide a structural view of these "machines" and, by concomitant application of biochemical approaches, to provide a fundamental understanding of the underlying mechanisms. We are working with eukaryotic and bacterial entities, with the idea that parallel analysis will enable extraction of fundamental principles. Control of DNA replication initiation and genome integrity are central to cell growth and are frequently damaged in cancer. Understanding the structures of the associated protein complexes will spur development of new therapeutics.

In twenty years, the PI has mentored 105 post-doctoral scientists, doctoral, master's, undergraduate and high school students (male: 40, female: 65, underrepresented groups: 15, and 1 disabled student). Many trainees have gone on to academic positions, or medical/graduate school on completion of their training.

To enable undergraduate students, and especially those from CCNY and CUNY community colleges, to engage in research, Jeruzalmi organizes three undergraduate research programs: 1) an NSF sponsored Research Experiences for Undergraduates (REU) program titled: "REU in Biochemistry, Biophysics, and Biodesign (B^3)" as PI, 2) the CUNY and CCNY-sponsored Opportunities in Research and Creative Arts (ORCA), and 3) the NSF-supported STEM-Communities Research Program for CCNY undergraduates (PI: Joe Barba). Each program embeds undergraduates (from within and without CCNY) in a summer immersion research experience. Research is supplemented with workshops, seminars, and field trips to enable students, especially those from under-represented and under-resourced groups, to develop a research mindset that will motivate pursuit of advanced degrees. These programs center the following themes: 1) engaging undergraduates from under-represented groups in faculty research, 2) lowering barriers for students from under-resourced backgrounds to participation in research, and 3) improving graduation rates. Between 2017 and 2022, the B^3-REU program trained 52 fellows (32 women and 20 men; 20 from groups underrepresented in science; ~50% from under-resourced backgrounds; ~20% from community colleges; ~60% from outside of CCNY). Between

2016 and 2022, the ORCA program hosted 143 CCNY undergraduates from all academic disciplines at CCNY. Self-assessment surveys document student gains. The PI is also working with the CCNY Initiative to Promote Academic Success in STEM (CiPASS) on many of the same goals as above. The PI recently convened the CCNY-wide Committee on Undergraduate Research and Experiential Learning (CUREL) to present program offerings to undergraduate students in a unified manner. Notably, Professors Li and Jeruzalmi's efforts with ORCA were recognized in 2022 by the CCNY Provost's Award for Pedagogical and Curricular Innovation.

Jeruzalmi partners with three New York City public high schools to engage students (grades 9-12) in STEM: 1) The New Explorations into Science, Technology, and Math (NEST+M) High School, 3) the Leaders High School, and 3) Bronx High School of Science. ~72% of students in the NYC public school system are from economically disadvantaged backgrounds, and ~66% are Hispanic or black. Jeruzalmi's involvement consists of a) hosting high-school interns, b) visits to career days at high schools, c) tours of science facilities at CCNY, e) judging science fairs, and f) collaborations with teachers on course development. Since 2013, Jeruzalmi has made 24 visits to high schools or hosted groups at CCNY.

Ongoing Projects

2022 – 2026	National Science Foundation; co-Principal Investigator; "RaMP: On-Ramp to the Molecular Machine Shop: Postbaccalaureate Training in Biochemistry, Biophysics, and Biodesign" (DBI #2216654);
2021 – 2025	National Science Foundation; Principal Investigator; "Telling Right from Wrong in the Genome: Molecular Machinery of the Bacterial Nucleotide Excision Repair Pathway" (MCB #2114509);
2019 - 2023	National Science Foundation; Principal Investigator; Research Experience for Undergraduates (REU), "Research and Training in Biochemistry, Biophysics and Biodesign (B^3) for Undergraduates" (DBI #1852496);
2018 - 2024	National Science Foundation; Principal Investigator; "Molecular Mechanisms of Bacterial Helicase Assembly and Activation at a Replication Origin" (MCB #1818255);

Completed Projects

2017 - 2019	New York State Department of Health; Principal Investigator; "Laying the Foundation for Combination Therapy Against Breast Cancer: Analysis of the Human Shelterin-DNA Complex" (DOH01-Rowley-2015-00111);
2016 - 2019	National Science Foundation; Principal Investigator; Research Experience for Undergraduates (REU), "Research and Training in Biochemistry, Biophysics and Biodesign (B^3) for Undergraduates" (DBI #1560384);
2015 – 2018	Silicon Mechanics, Inc.; Principal Investigator; "A High-Performance Computer Cluster to Support Inquiry in Basic and Applied Science, Medicine, Biotechnology, and the Humanities at City College of New York and the City University of New York." (SM-2015-289297);
2013 - 2018	National Science Foundation; Principal Investigator (MCB #1330528); "The Bacterial Nucleotide Excision Repair Pathway: Structure and Mechanism"

B. Positions, Scientific Appointments, and Honors

Positions, Scientific Appointments:

Sept 1, 2020 – present	Visiting Professor, Universita del Piedmonte Orientale
June 4, 2012 – present	Professor Department of Chemistry, City College of New York.
July 1, 2011 – June 30, 2012,	Visiting Professor Dept. of Molecular and Cellular Biology,
July 1, 2006 – June 30, 2011,	Associate Professor Dept. of Molecular and Cellular Biology,
July 1, 2002 – June 30, 2006,	Assistant Professor Dept. of Molecular and Cellular Biology, Harvard University.

	Harvard University.
	Harvard University.
Feb1, 2002 – June 30, 2002,	Special fellow, laboratory of Prof. Mike O'Donnell
Feb1, 2002 – June 30, 2002,	Research associate, laboratory of Prof. John Kuriyan Dept. of Molecular and Cell Biology, The University of California at Berkeley.
June 1, 1998 – Jan 31, 2002,	Research associate, laboratory of Prof. John Kuriyan Laboratory of Molecular Biophysics, The Rockefeller University. Laboratory of DNA Replication, The Rockefeller University.

Honors:

October 2022	City College Provost's Award for Pedagogical and Curricular Innovation
May 2018	Faculty Service Award, The City College of New York
July 2003	Kimmel Scholar Award, The Sidney Kimmel Foundation for Cancer Research
Nov 2002	Smith Family Award for Excellence in Biomedical Research.
June 1987	High Honors, Dept of Chemistry, The University of Cincinnati
June 1987	B.S in Chemistry, *summa cum laude*, The University of Cincinnati
March 1987	National Science Foundation pre-doctoral fellowship, honorable mention.
May 1986	Phi Beta Kappa (Delta of Ohio).
1983-1987	Albert Voorheis Academic Scholar, The University of Cincinnati

Membership in Professional Societies:

- American Assoc for the Advancement of Science, American Chemical Society, American Crystallographic Association

Community Service Related to Professional Work:

- Reviewer for Nature, Cell, Molecular Cell, Structure, Proceedings of the National Academy of Sciences, Biochemistry, Proteins: Structure, Function and Bioinformatics, Nucleic Acids Research, FEBS Journal.
- Panelist, Various programs in the Biology Division, National Science Foundation.
- Grant reviewer for The National Institutes of Health, The Department of Defense, The Medical Foundation, Boston, MA.

C. Contributions to Science

1) Jeruzalmi, D., Yurieva, O., Zhao, Y., Young, M., Stewart, J., Hingorani, M., O'Donnell, M., and Kuriyan, J. (2001) Mechanism of Processivity Clamp Opening by the Delta Subunit Wrench of the Clamp Loader Complex of E. coli DNA Polymerase III. *Cell*, 106, 417-428.

The discovery that the ring-shaped sliding clamps of bacterial chromosomal replicases bind to DNA via topology, rather than chemistry immediately raised the question of how a closed protein ring is loaded onto DNA, a long polymer, with no ends. Subsequent work revealed that a large ensemble (clamp loader) employed ATP to open the ring and place it on DNA. One of the loader subunits ('delta-wrench') was shown to be the major point of contact between loader and clamp, but the mechanism of opening was unknown. Further, the isolated wrench subunit could open the ring, but this activity was somehow repressed in the intact loader, unless ATP was present.

In looking at the catalytic cycle executed by the clamp loader, I decided to aim for two structures that could be leveraged to shed light on understanding the entire cycle. The first was the structure of the wrench bound to the clamp. This work showed that the wrench-clamp interface was composed of two sub-sites, one was involved in opening the clamp (by changing the structure to render it incapable of closing) and the second provided affinity to the interaction. Subsequent biochemical analysis lent support to our model (1). Unexpectedly, we found evidence that the sliding clamp stored spring tension in the closed ring, and we suggested a role for this feature in the loading reaction.

In work published at the same time, I determined the structure of the complete bacterial clamp loader (see below and (2)). This effort revealed the structure of the wrench in the context of the loader (determined without nucleotide). Comparison of the wrench in two contexts revealed why ring-opening was suppressed by the loader in the absence of nucleotide. Modeling considerations suggested the structural changes that would have to occur for a productive interaction with the clamp.

1. Indiani C, O'Donnell M. Mechanism of the delta wrench in opening the beta sliding clamp. J Biol Chem. 2003 Oct. 10;278(41):40272–81.
2. Jeruzalmi D, Odonnell M, Kuriyan J. Crystal structure of the processivity clamp loader gamma complex of E. coli DNA polymerase III. Cell. 2001 Aug. 24;106(4):429–41.

2) Jeruzalmi, D., O'Donnell, M., and Kuriyan, J. (2001) Crystal Structure of the Processivity Clamp Loader Gamma Complex of E. coli DNA Polymerase III. *Cell*, 106, 429-441.

The clamp loader (gamma complex) is an AAA+ ATPase that uses the energy of ATP binding to open the ring-shaped sliding clamp and place it on DNA. The clamp loader is a sub-assembly of DNA Polymerase III (Pol III), the chromosomal replicase in bacteria, and is composed of five subunits (gamma, delta, deltaprime, chi and psi). The ring-shaped clamp enables processive DNA synthesis by Pol III. At the time, the architecture of the clamp loader, the role of nucleotide, and interactions with clamp and primer-template were not well understood.

Our structure of the bacterial clamp loader revealed, for the first time, its stoichiometry and overall architecture. Our work also provided insights into the function of ATP, the mechanism of clamp opening (together with another structure (1)) and suggested the location of primer-template DNA. The loader adopted a pentameric ring-like arrangement made up of three gamma subunits, one delta and deltaprime subunit (chi and psi were not included in the structure). The carboxy-terminal domains of these subunits associated into a circular collar from which the amino-terminal ATP binding domains hung loosely. The ATP binding domains were deployed asymmetrically around the ring and one of the sites was blocked by a neighbor. We speculated that ordered changes in the nucleotide binding sites might accompany clamp loading.

This work and the structure of the 'delta-wrench' bound to the clamp (1), showed the wrench in two contexts. The first, as part of the loader, represented a conformation that did not bind the ring. In the second structure, the 'delta-wrench'-clamp interface revealed the mechanism of ring opening. Superimposing the two structures revealed why the nucleotide-free loader could not bind clamp and suggested the changes that would have to occur for productive interaction. Subsequent biochemical and structural analysis carried these ideas further (2,3).

1. Jeruzalmi D, Yurieva O, Zhao Y, Young M, Stewart J, Hingorani M, et al. Mechanism of processivity clamp opening by the delta subunit wrench of the clamp loader complex of E. coli DNA polymerase III. Cell. 2001 Aug. 24;106(4):417–28.
2. Johnson A, O'Donnell M. Ordered ATP hydrolysis in the gamma complex clamp loader AAA+ machine. 2003 Apr. 18;278(16):14406–13.
3. Bowman GD, O'Donnell M, Kuriyan J. Structural analysis of a eukaryotic sliding DNA clamp-clamp loader complex. Nature. 2004 Jun. 17;429(6993):724–30.

3) Pakotiprapha D., Inuzuka Y., Bowman B., Moolenaar, Goosen G., Jeruzalmi D*., Verdine G*. (2008) Crystal Structure of *Bacillus stearothermophilus* UvrA Provides Insight into ATP-modulated Dimerization, UvrB Interaction and DNA Binding. *Mol Cell*. 29, 122-33. *co-corresponding authors

Nucleotide excision repair (NER) is distinguished by its ability to process divergent damaged DNA structures. Here, we focus on the bacterial NER pathway whose early stages are implemented by three proteins, UvrA, UvrB and UvrC. The UvrA•UvrB (AB) complex scans the genome for damaged DNA. Once located, damaged DNA is stably bound by UvrA within the AB complex. A major reorganization then occurs in which UvrA is lost from the ensemble, and concomitantly, UvrB localizes to the lesion. Finally, additional events lead to excision of the damage by the UvrC nuclease, and repair of the resulting single-stranded gap. Prior to our structural study, neither the architecture of UvrA, nor how it interacts with UvrB or DNA were known.

Our study came to three major findings. First, the architecture of UvrA dimer, and the organization of its four ABC-style (ATP Binding Cassette) ATP binding sites were revealed. Second, the structure revealed the DNA binding surface. Third, biochemical analysis revealed the UvrB binding site. Moreover, the presence of two

such binding sites suggested that the UvrA dimer bound two UvrB molecules, not one as previously envisioned (our structure of the UvrA•UvrB complex verified this arrangement (1,2)).

The significance of the structure of UvrA grew exponentially in light of the UvrA•UvrB structure (1). We noted that the conformation of the UvrA dimer was significantly different in the two structures, with important implications for the DNA binding surface. The shape of the DNA binding surface in isolated UvrA is open, shallow and wide. By contrast, the corresponding surface in UvrA from the UvrA–UvrB complex comprises a deep and narrow channel and represents a closed state. To accommodate both sets of findings, we propose that interconversion between the two forms of UvrA is a significant feature of the genome scanning process.

1. Pakotiprapha D, Samuels M, Shen K, Hu JH, Jeruzalmi D. Structure and mechanism of the UvrA–UvrB DNA damage sensor. Nat Struct Mol Biol. Nature Publishing Group; 2012 Feb. 5;:1–9.
2. Pakotiprapha D, Jeruzalmi D. Shape and Composition of the UvrA–UvrB DNA Damage Sensor Inferred from Small-Angle X-ray Scattering. Proteins, Structure, Function, and Bioinformatics, under revision, 05-15-2012.

4) Pakotiprapha D, Samuels M, Shen K, Hu JH, Jeruzalmi D. Structure and mechanism of the UvrA–UvrB DNA damage sensor. Nat Struct Mol Biol. Nature Publishing Group; 2012 Feb. 5;:1–9.

The UvrA•UvrB (AB, ~400 kDa) complex is the DNA damage sensor in bacterial nucleotide excision repair (NER). To understand damage discrimination by the AB complex, and handoff of lesion DNA from UvrA to UvrB during NER, we determined the structure of the complete AB complex, and carried out supporting studies. The structure revealed three major findings. First, the AB structure shows that the UvrA DNA binding groove, previously observed in an open tray-like conformation (1) which binds lesion DNA, can also exist in a closed conformation with a narrow, deep groove that is predicted to bind native B-form DNA *only*. This finding forces consideration of models in which discrimination of native from lesion-containing DNA during genome scanning involves interconversion of UvrA between two (i.e. open and closed) conformations rather than differences in free energy of binding alone within a single static structure.

Second, the structure defined for the first time the disposition of UvrB within the complex. Two UvrB molecules are present at opposite ends of the UvrA dimer, ~80 Å away from the lesion. This arrangement implies that UvrB must travel to the lesion, once UvrA has been evicted from the complex. Translocation is likely mediated by UvrB's single-stranded DNA translocase activity.

Third, a conserved domain (signature II) of UvrA mediates a nexus of contacts between UvrA, UvrB, and DNA. Moreover, in a new UvrA structure, absence of nucleotide at the adjacent ATP site is correlated with rotation of this domain into a conformation that weakens the interaction with UvrB. We propose that this transition serves to evict UvrA from the UvrA-UvrB-lesion ternary complex, leaving UvrB behind on the DNA. Taken together, these findings provide new information about the three early stages of NER, and lead to an integrated view of how these stages could progress.

1. Pakotiprapha D, Inuzuka Y, Bowman BR, Moolenaar GF, Goosen N, Jeruzalmi D, et al. Crystal structure of Bacillus stearothermophilus UvrA provides insight into ATP-modulated dimerization, UvrB interaction, and DNA binding. Mol Cell. 2008 Jan. 18;29(1):122–33. PMCID: PMC2692698.

5) Chase, Jillian, Andrew Catalano, Alex J Noble, Edward T Eng, Paul Db Olinares, Kelly Molloy, Danaya Pakotiprapha, et al. "Mechanisms of Opening and Closing of the Bacterial Replicative Helicase." *eLife* 7 (December 24, 2018): 1822. doi:10.7554/eLife.41140.

Assembly of bacterial ring-shaped hexameric replicative helicases on single-stranded (ss) DNA requires specialized loading factors. However, mechanisms implemented by these factors during opening and closing of the helicase, which enable and restrict access to an internal chamber, are not known. Here, we investigate these mechanisms in the *Escherichia coli* DnaB helicase·bacteriophage l helicase loader (lP) complex. We show that five copies of lP bind at DnaB subunit interfaces and reconfigure the helicase into an open spiral conformation that is intermediate to previously observed closed ring and closed spiral forms; reconfiguration also produces openings large enough to admit ssDNA into the inner chamber. The helicase is also observed in a restrained inactive configuration that poises it to close on activating signal, and transition to the translocation state. Our findings provide insights into helicase opening, delivery to the origin and ssDNA entry, and closing in preparation for translocation.

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