BIOGRAPHICAL SKETCH

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NAME: Gao, Yang

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

POSITION TITLE: Assistant 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)	END DATE MM/YYYY	FIELD OF STUDY
University of Science and Technology of China, Hefei, Anhui	BS	05/2007	Life Science
Iowa State University, Ames, IA	PHD	05/2013	Biochemistry

A. Personal Statement

The overarching goal of my career is to illustrate the structural and mechanistic basis of DNA replication. My proposed research aims to address the fundamental questions related to genomic DNA replication. Specifically, I will combine *in vitro* reconstitution and biochemical, biophysical and structural biological techniques to investigate operation principles of replisomes from bacteriophage T7 and mitochondria. I was educated as a structural biologist and biochemist since the beginning of my career. I employed various techniques to characterize structure and mechanism of proteins in glucose metabolism during my Ph. D. My journey on molecular complexes in DNA metabolism started since my first postdoctoral position in 2013. In the past, I have adapted diffusion-based time-resolved crystallography to directly observe DNA synthesis in crystallo and identified a transiently bound metal ion essential for DNA synthesis. In addition, I have biochemically reconstituted replisome from bacteriophage T7 and determined around 20 cryo-electron microscope structures of T7 helicase on single-stranded DNA and helicase-polymerase complexes for leading and lagging strand DNA synthesis. Our structures illustrated the molecular mechanisms of helicase translocation and helicase-polymerase coupling during DNA replication. My past work has led to 25 publications, including two first-author publications in journal Science. Besides my scientific achievement, I have accumulated extensive experience on mentoring students and managing a research laboratory. I have trained a handful of undergraduate students during my Ph. D. and postdoc and published three research articles with the undergraduate trainees as the first or co-first authors. After setting up my lab at Rice for three years, I have built a research team with one technician, two postdoctoral fellows, five graduate students and five undergraduate students. Moreover, Rice university is located within the multi-institutional Texas Medical Center. We have easy access to various instruments including cryo-electron microscope and many experts related to the project. In summary, my past experience and publication records demonstrated my qualification and the research environment at Rice are well-suited for proposed work. A complete list of my publication can be found in my bibliography https://www.ncbi.nlm.nih.gov/myncbi/yang.gao.3/bibliography/public/.

1. Gao Y, Yang W. Different mechanisms for translocation by monomeric and hexameric helicases. Curr Opin Struct Biol. 2020 Apr;61:25-32. PubMed Central PMCID: PMC7156327.
2. Yang W, Seidman MM, Rupp WD, Gao Y. Replisome structure suggests mechanism for continuous fork progression and post-replication repair. DNA Repair (Amst). 2019 Sep;81:102658. PubMed Central PMCID: PMC7467748.
3. Gao Y, Cui Y, Fox T, Lin S, Wang H, de Val N, Zhou ZH, Yang W. Structures and operating principles of the replisome. Science. 2019 Feb 22;363(6429) PubMed Central PMCID: PMC6681829.
4. Gao Y, Yang W. Capture of a third Mg^{2+} is essential for catalyzing DNA synthesis. Science. 2016 Jun 10;352(6291):1334-7. PubMed Central PMCID: PMC6320252.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019 -	Assistant Professor, Rice University, Houston, TX
2014 - 2019	Postdoctoral Fellow, National Institute of Health (NIH), Bethesda, MD
2013 - 2014	Postdoctoral Fellow, Iowa State University, Ames, IA

Honors

2023 - 2026	American Cancer Society Research Scholar Award, American Cancer Society
2021 - 2026	Maximizing Investigators' Research Award (MIRA) for Early Stage Investigators, National Institutes of Health
2019 - 2023	CPRIT Award for First-Time Tenure Track Faculty Member, Cancer Prevention and Research Institute of Texas
2013 - 2013	Teaching Excellence Award, Graduate College, Iowa State Univ.

C. Contribution to Science

1. Structural basis of replisome operation. Since DNA was established as the genetic material and the DNA double-helical structure was proposed, the mechanism of DNA replication has been a central subject in molecular biology research. However, the structural basis of DNA replication was not resolved due to the complexity of DNA replication. To determine replisome structures, I reconstituted the simplest replisome from Bacteriophage T7 and adapted the cutting-edge cryo-electron microscope technique. By solving nearly 20 structures of T7 replisomes on its relevant DNA substrates, I illustrated the mechanism of helicase translocation, helicase-leading strand polymerase coupling and primase-lagging strand polymerase coordination (publication "d"). In traditional textbook model of DNA replication, helicase was always placed at the replication fork for unwinding the parental DNA followed by DNA synthesis by DNA polymerases. With structural and biochemical methods, we domenstrated that it is the polymerase, but not the helicase, that directly contact and unwind the parental DNA in bacteriophage DNA replication (publication "a"). Based on the first structure of a replisome, I consolidated many years of biochemical data and pointed out the potential mechanisms of replication coupled repair (publication "c"). In addition, I summarized mechanisms of different helicases specialized in different processes of DNA metabolism (publication "b"). In summary, my work provided a scaffold in understanding structural basis of DNA replication.

    a. Lo CY, Gao Y. DNA Polymerase-Parental DNA Interaction Is Essential for Helicase-Polymerase Coupling during Bacteriophage T7 DNA Replication. Int J Mol Sci. 2022 Jan 25;23(3) PubMed Central PMCID: PMC8835902.
    b. Gao Y, Yang W. Different mechanisms for translocation by monomeric and hexameric helicases. Curr Opin Struct Biol. 2020 Apr;61:25-32. PubMed Central PMCID: PMC7156327.
    c. Yang W, Seidman MM, Rupp WD, Gao Y. Replisome structure suggests mechanism for continuous fork progression and post-replication repair. DNA Repair (Amst). 2019 Sep;81:102658. PubMed Central PMCID: PMC7467748.
    d. Gao Y, Cui Y, Fox T, Lin S, Wang H, de Val N, Zhou ZH, Yang W. Structures and operating principles of the replisome. Science. 2019 Feb 22;363(6429) PubMed Central PMCID: PMC6681829.

2. Mechanism of metal ion dependent DNA synthesis. DNA polymerases perform highly accurate genomic DNA replication with the assistance of divalent metal ions. It was proposed for decades that two metal ions were required and sufficient for polymerase catalysis. However, the conclusion was mainly based on static crystal structures of polymerases prepared under inhibitory conditions and the dynamic catalytic process was not directly visualized. I investigated the polymerase catalysis with a novel diffusion-based time-resolved crystallography method. By collecting over a hundred crystal structures during polymerase catalysis under different conditions, I for the first time proved that DNA polymerase with well aligned DNA, dNTP and two metal ions are not adequate for the chemical reaction (publication "d"). Only upon the arrival of a third metal ion the reaction proceeds. The third metal ion is present transiently and not directly coordinated by the polymerase, explaining why it escaped from detection for decades. The deprotonation

of 3'-OH of primer end is a key step in initializing DNA synthesis. Through mutagenesis and chemical modification of DNA, we observed that DNA synthesis reaction could proceed through multiple deprotonation pathways and no single general base is strictly required (publication "c"). Moreover, we identified metal ion binding induced primer alignment as a key step in polymerase misincorporation (publication "a"). My finding not only illustrated the chemical mechanism of polymerase reaction but also revolutionized how people think about enzyme catalysis. We suspect that the transient bound metal ions play essential roles in all divalent metal ion dependent enzymes in nucleic acid metabolism. In addition, we have collaborated with XPose Inc. to search for novel polymerase inhibitors with structure-based fragment screening (publication “b”).

a. Chang C, Lee Luo C, Gao Y. In crystallo observation of three metal ion promoted DNA polymerase misincorporation. Nat Commun. 2022 Apr 29;13(1):2346. PubMed Central PMCID: PMC9054841.
b. Wilson DM, Duncton MAJ, Chang C, Lee Luo C, Georgiadis TM, Pellicena P, Deacon AM, Gao Y, Das D. Early Drug Discovery and Development of Novel Cancer Therapeutics Targeting DNA Polymerase Eta (POLH). Front Oncol. 2021;11:778925. PubMed Central PMCID: PMC8653755.
c. Gregory MT, Gao Y, Cui Q, Yang W. Multiple deprotonation paths of the nucleophile 3'-OH in the DNA synthesis reaction. Proc Natl Acad Sci U S A. 2021 Jun 8;118(23) PubMed Central PMCID: PMC8201771.
d. Gao Y, Yang W. Capture of a third Mg^{2+} is essential for catalyzing DNA synthesis. Science. 2016 Jun 10;352(6291):1334-7. PubMed Central PMCID: PMC6320252.

3. Allosteric communication mechanisms of Mre11-Rad50 (MR) complex. MR complex play pivot roles in DNA double-strand break. The MR complex contains endo- and exo-nuclease activity to initialize DNA recession, ATPase activity to drive DNA translocation, and a several hundred Angstrom long coiled-coil domain that can potentially bridge different DNA strands. How different activities are coordinated within MR complex are not well understood. I have worked with Dr. Nelson at Iowa State University for nine months and characterized the allosteric communication of MR complex from bacteriophage T4. I showed that the nuclease activities of Mre11 is autoinhibited by its C-terminal domain and Rad50 binding to the C-terminal domain of Mre11 relieves its autoinhibition (publication “d”). I participated in studying how the long coiled-coil domain mediate MR complex function (publication “c”). In addition, I collaborated with two undergraduate students and investigated the allosteric communication with evolutionary analysis (publication “b”) and characterized the role of Rad50 C-terminus unstructured region in DNA binding (publication “a”). Collectively, my work revealed the working mechanism of the highly conserved MR complex.

a. Streff HE, Gao Y, Nelson SW. Functional evaluation of the C-terminal region of bacteriophage T4 Rad50. Biochem Biophys Res Commun. 2020 May 28;526(2):485-490. PubMed PMID: 32238267.
b. Gao Y, Meyer JR, Nelson SW. A network of allosterically coupled residues in the bacteriophage T4 Mre11-Rad50 complex. Protein Sci. 2016 Nov;25(11):2054-2065. PubMed Central PMCID: PMC5079247.
c. Barfoot T, Herdendorf TJ, Behning BR, Stohr BA, Gao Y, Kreuzer KN, Nelson SW. Functional Analysis of the Bacteriophage T4 Rad50 Homolog (gp46) Coiled-coil Domain. J Biol Chem. 2015 Sep 25;290(39):23905-15. PubMed Central PMCID: PMC4583041.
d. Gao Y, Nelson SW. Autoinhibition of bacteriophage T4 Mre11 by its C-terminal domain. J Biol Chem. 2014 Sep 19;289(38):26505-26513. PubMed Central PMCID: PMC4176212.