BIOGRAPHICAL SKETCH

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NAME: PATEL, SMITA S

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

POSITION TITLE: Distinguished 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
Bombay University, Bombay	BS	05/1981	Physics and Chemistry
Indian Institute of Techonology (IIT), Bombay	MS	06/1983	Chemistry
Tufts University, Boston, MA	PHD	12/1988	Chemistry
Pennsylvania State University, State College, PA	NIH training grant	12/1991	Fidelity of replicative DNA polymerase using transient state kinetics

A. Personal Statement

We study the mechanisms of helicases and polymerases involved in essential biological processes such as DNA replication, transcription, and innate immunity. With my training in physical and bioorganic chemistry and enzymology, I bring unique chemical and quantitative perspectives to problems in biology. I was trained as an organic chemist in David Walt's lab, which developed fluorescent-based technology for next-generation sequencing (co-founder of Illumina). I used various spectroscopy and fluorescent methods to analyze small and large molecules. I learned transient state kinetics from Kenneth Johnson, a world expert in rapid kinetic methods (President of KinTek Corp). My lab has developed new enzymological approaches to measure the reactions of processive nucleic acid enzymes that move on DNA or RNA. These approaches combine equilibrium, transient state kinetics, and computational kinetic modeling and provide fine mechanistic details to build quantitative frameworks to understand the reactions of replication, transcription, and pathogen recognition. We are highly skilled in all aspects of molecular biology, protein expression/purification/modification, DNA and RNA handling to produce high-quality reagents for enzymology and crystallography. We have assembled an excellent team of collaborators that complement our ensemble measurements with single molecule kinetics (optical tweezers and TIRF microscopy) and structural analysis (crystallography and cryo-EM/image analysis). Our current basic research on mitochondrial enzymes and RIG-I helicase has applications in developing therapies for mitochondrial diseases, viral infections, and autoimmunity. I am committed to teaching and training students to prepare them for research in biomedical sciences and becoming leaders. I teach enzymology to first-year Medical and Master’s students, and I am the co-director of the 8-credit core course for first-year graduate students in the multidisciplinary Molecular Biosciences Program at Rutgers University. I have trained 10 undergraduates, 20 graduate students, and 10 post-docs currently working in science-related areas in medicine, industry, federal labs, or academia.

As applicable, all applicants may include details on ongoing and completed research projects from the past three years that they want to draw attention to within the personal statement, Section A.

1. Johnson LC, Singh A, Patel SS. The N-terminal domain of human mitochondrial helicase Twinkle has DNA-binding activity crucial for supporting processive DNA synthesis by polymerase γ. J Biol Chem. 2023 Jan;299(1):102797. PubMed Central PMCID: PMC9860392.
2. Schweibenz BD, Devarkar SC, Solotchi M, Craig C, Zheng J, Pascal BD, Gokhale S, Xie P, Griffin PR, Patel SS. The intrinsically disordered CARDs-Helicase linker in RIG-I is a molecular gate for

RNA proofreading. EMBO J. 2022 May 16;41(10):e109782. PubMed Central PMCID: PMC9108607.

3. Martinez SE, Singh A, De Wijngaert B, Sultana S, Dharia C, Vanbuel H, Shen J, Vasilchuk D, Patel SS, Das K. Assembly and Cryo-EM structure determination of yeast mitochondrial RNA polymerase initiation complex intermediates. STAR Protoc. 2021 Jun 18;2(2):100431. PubMed Central PMCID: PMC8044712.
4. Ramachandran A, Basu U, Sultana S, Nandakumar D, Patel SS. Human mitochondrial transcription factors TFAM and TFB2M work synergistically in promoter melting during transcription initiation. Nucleic Acids Res. 2017 Jan 25;45(2):861-874. PubMed Central PMCID: PMC5314767.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 -	Distinguished Professor, Rutgers - RWJ Medical School, Piscataway, NJ
2020 - 2021	Co-Organizer, FASEB meeting on Helicases: Structure, Function and Roles in Human Disease
2019 -	Editorial Board, Biophysical Journal
2013 - 2022	Professor, Rutgers-RWJMS, Piscataway, NJ
2011 - 2015	Editorial Board, JBC
2005 - 2007	Gender Equity Program Mentor, CUNY
2002 -	Ad hoc Reviewer, NSF, ACS, NIH MGA, Eureka, Pathway to Independence, MSFE, PCMB, Special Emphasis panel,
2002 - 2013	Professor, UMDNJ-RWJMS, Piscataway, NJ
1998 - 2002	Associate Professor, UMDNJ-RWJMS, Piscataway, NJ
1997 - 2002	Member, NIH Biochemistry Study Section
1996 - 1998	Associate Professor, The Ohio State University, Columbus, OH
1992 - 1996	Assistant Professor, The Ohio State University, Columbus, OH

Honors

2017	Biomedical Research Exemplar, P.I. Program at Washington University School of Medicine
2015	Board of Trustees Award for Excellence in Research, Rutgers University
2014	Excellence in Research Award, New Jersey Health Foundation
2013	Outstanding Medical Research Scientist Award for Basic Biomedical Research , Edward J. Ill Excellence in Medicine Awards
2010	Research Award for Basic Sciences, UMDNJ Foundation
2009	Master Educator Guild, UMDNJ-RWJMS
2007	MERIT Award, NIH
2007	Antoine Saugrain Award and Lecture, Chemistry and Biochemistry at Hunter College
2005	Invited honorary speaker for Frontiers in Biology, Stanford University Biochemistry Graduate Students
1995	Junior Faculty Research Award, American Cancer Society
1989	NIH Postdoctoral Fellowship, NIH
1985	DuPont Fellowship for Academic Excellence, Tufts University
1983	Silver Medalist, IIT Bombay, India

C. Contribution to Science

1. MECHANISM AND STRUCTURE OF REPLICATIVE RING-SHAPED HELICASES We discovered the ring-shaped hexameric structure of T7 helicase and were the first to demonstrate the helicase ring

binds DNA in the central channel. This mode of DNA binding is now recognized as a general feature of ring-shaped helicases. The structure raised many immediate questions, and the one that intrigued me the most was the mechanistic basis for directional translocation and the order of nucleotide hydrolysis around the ring. Using mutant poisoning, transient state kinetics, and computational kinetic modeling with two different ring helicases, T7 and Rho, we showed that helicases employ a sequential mechanism of catalysis to efficiently move on ssDNA and unwind dsDNA. Using similar approaches, we are now investigating the mechanism of human mitochondrial DNA helicase, Twinkle.

a. Egelman EH, Yu X, Wild R, Hingorani MM, Patel SS. Bacteriophage T7 helicase/primase proteins form rings around single-stranded DNA that suggest a general structure for hexameric helicases. Proc Natl Acad Sci U S A. 1995 Apr 25;92(9):3869-73. PubMed Central PMCID: PMC42063.
b. Hingorani MM, Washington MT, Moore KC, Patel SS. The dTTPase mechanism of T7 DNA helicase resembles the binding change mechanism of the F1-ATPase. Proc Natl Acad Sci U S A. 1997 May 13;94(10):5012-7. PubMed Central PMCID: PMC24622.
c. Adelman JL, Jeong YJ, Liao JC, Patel G, Kim DE, Oster G, Patel SS. Mechanochemistry of transcription termination factor Rho. Mol Cell. 2006 Jun 9;22(5):611-21. PubMed PMID: 16762834.
d. Johnson DS, Bai L, Smith BY, Patel SS, Wang MD. Single-molecule studies reveal dynamics of DNA unwinding by the ring-shaped T7 helicase. Cell. 2007 Jun 29;129(7):1299-309. PubMed Central PMCID: PMC2699903.

2. FUNCTIONAL COORDINATION BETWEEN HELICASE, POLYMERASE, AND PRIMASE We discovered the mechanistic basis for coupling helicase and polymerase at the fork junction. The helicase is a poor unwindase; we showed that an actively synthesizing T7 DNA polymerase stimulates the helicase. When coupled, the helicase and polymerase create an efficient combined motor that moves in steps of one ATP/bp to unwind and copy the DNA strand. We demonstrated that cooperativity for leading strand synthesis requires physical proximity of helicase and polymerase at the leading strand fork junction. On the other hand, we found little cooperativity between primase and helicase during lagging strand synthesis. Our studies showed that helicase does not pause during primer synthesis, contrary to published results, and single molecule FRET kinetics demonstrated a priming loop between helicase and primase.

a. Singh A, Pandey M, Nandakumar D, Raney KD, Yin YW, Patel SS. Excessive excision of correct nucleotides during DNA synthesis explained by replication hurdles. EMBO J. 2020 Mar 16;39(6):e103367. PubMed Central PMCID: PMC7073461.
b. Sun B, Pandey M, Inman JT, Yang Y, Kashlev M, Patel SS, Wang MD. T7 replisome directly overcomes DNA damage. Nat Commun. 2015 Dec 17;6:10260. PubMed Central PMCID: PMC4703881.
c. Nandakumar D, Pandey M, Patel SS. Cooperative base pair melting by helicase and polymerase positioned one nucleotide from each other. Elife. 2015 May 13;4 PubMed Central PMCID: PMC4460406.
d. Pandey M, Patel SS. Helicase and polymerase move together close to the fork junction and copy DNA in one-nucleotide steps. Cell Rep. 2014 Mar 27;6(6):1129-1138. PubMed Central PMCID: PMC4010093.

3. MECHANISM OF TRANSCRIPTION BY SINGLE SUBUNIT RNA POLYMERASES Parallel with T7 helicase and polymerase studies, my lab studies the enzymology of single-subunit T7 RNA polymerase. Our goal has been to delineate the biochemical pathway of transcription initiation by quantifying the multi-step process and understanding how a single subunit enzyme can carry out promoter-specific transcription during initiation and promoter-independent transcription during elongation. To achieve this level of understanding, we developed new ensemble and single molecule methods that measured promoter DNA binding, melting, DNA bending, the rates of RNA extension,

initial bubble collapse, promoter release, and transition into elongation, as well as the rate constants of nucleotide addition during elongation. We demonstrated that T7 RNAP undergoes sequential rotational and scrunching changes during initiation to accommodate the growing RNA:DNA hybrid and then a concerted conformational change in DNA and RNAP to make the final transition into elongation. The concepts and methods developed through these studies are generally applicable and greatly aid our research of the related mitochondrial enzymes. The mitochondrial RNAPs are homologous to phage T7 RNAP. However, unlike T7 RNAP, the mtRNAPs rely on transcription factors for promoter-specific initiation. We showed that the initiation factor directly interacts with the promoter DNA to induce the bending and melting of the promoter. We have captured the promoter conformational changes during transcription initiation steps using smFRET and protein-DNA changes using cryoEM structures.

a. De Wijngaert B, Sultana S, Singh A, Dharia C, Vanbuel H, Shen J, Vasilchuk D, Martinez SE, Kandiah E, Patel SS, Das K. Cryo-EM Structures Reveal Transcription Initiation Steps by Yeast Mitochondrial RNA Polymerase. Mol Cell. 2021 Jan 21;81(2):268-280.e5. PubMed Central PMCID: PMC7855493.
b. Sohn BK, Basu U, Lee SW, Cho H, Shen J, Deshpande A, Johnson LC, Das K, Patel SS, Kim H. The dynamic landscape of transcription initiation in yeast mitochondria. Nat Commun. 2020 Aug 27;11(1):4281. PubMed Central PMCID: PMC7452894.
c. Sultana S, Solotchi M, Ramachandran A, Patel SS. Transcriptional fidelities of human mitochondrial POLRMT, yeast mitochondrial Rpo41, and phage T7 single-subunit RNA polymerases. J Biol Chem. 2017 Nov 3;292(44):18145-18160. PubMed Central PMCID: PMC5672038.
d. Ramachandran A, Basu U, Sultana S, Nandakumar D, Patel SS. Human mitochondrial transcription factors TFAM and TFB2M work synergistically in promoter melting during transcription initiation. Nucleic Acids Res. 2017 Jan 25;45(2):861-874. PubMed Central PMCID: PMC5314767.

4. MITOCHONDRIAL DNA REPLICATION I became interested in mitochondrial research (replication and transcription) when Twinkle was discovered by Hans Spelbrink as the human mitochondrial helicase homologous to T7 helicase. I was intrigued that many of the point mutations in Twinkle associated with mitochondrial diseases are the same ones we reported as helicase-deficient from our genetic screen with T7 helicase. We put considerable effort into obtaining soluble and active Twinkle from bacterial expression and succeed in these efforts. We discovered that Twinkle forms hexamers, binds both ssDNA and dsDNA, and has DNA annealing and strand exchange activities. We aim to determine if the DNA annealing activity of Twinkle is involved in mtDNA deletions.

a. Johnson LC, Singh A, Patel SS. The N-terminal domain of human mitochondrial helicase Twinkle has DNA-binding activity crucial for supporting processive DNA synthesis by polymerase γ. J Biol Chem. 2023 Jan;299(1):102797. PubMed Central PMCID: PMC9860392.
b. Sen D, Patel G, Patel SS. Homologous DNA strand exchange activity of the human mitochondrial DNA helicase TWINKLE. Nucleic Acids Res. 2016 May 19;44(9):4200-10. PubMed Central PMCID: PMC4872091.
c. Sen D, Nandakumar D, Tang GQ, Patel SS. Human mitochondrial DNA helicase TWINKLE is both an unwinding and annealing helicase. J Biol Chem. 2012 Apr 27;287(18):14545-56. PubMed Central PMCID: PMC3340288.

5. HCV AND RIG-I HELICASES In parallel with studies of ring-shaped helicases, we have been studying the mechanisms of non-ring-shaped helicases, focusing initially on the one encoded by hepatitis C virus. We elucidated the translocation and unwinding mechanism, showing that HCV helicase forms multimers on nucleic acid that unwind DNA with a high processivity using a novel mechanism that does not require cooperativity in ATPase between the subunits of the multimer. This mechanism is

found now in many helicases, including RecQ, Dda, UvrD family of helicases, and the general principles also apply to motor proteins such as chromatin remodelers. Our interest in HCV led us to study a newly discovered innate immune response receptor called RIG-I, which belongs to the helicase family of proteins but plays a role in detecting viral RNAs in the cytoplasm. RIG-I is an essential protein that recognizes a variety of commonly infecting viruses, including Influenza, Hepatitis C, Dengue, West Nile, Respiratory Syncytial, Reovirus, and Ebola. In collaboration with Joseph Marcotrigiano at Rutgers, we published the first high-resolution structure of RIG-I bound to dsRNA and ATP. We showed that ATPase activity is used in proofreading RNA and assembling signaling-competent oligomers on RNA. This research has applications for developing broad antiviral agents, therapies for autoimmune-related dysfunctions, and adjuvants to mimic the natural early immune response following virus infection.

a. Schweibenz BD, Devarkar SC, Solotchi M, Craig C, Zheng J, Pascal BD, Gokhale S, Xie P, Griffin PR, Patel SS. The intrinsically disordered CARDs-Helicase linker in RIG-I is a molecular gate for RNA proofreading. EMBO J. 2022 May 16;41(10):e109782. PubMed Central PMCID: PMC9108607.
b. Devarkar SC, Schweibenz B, Wang C, Marcotrigiano J, Patel SS. RIG-I Uses an ATPase-Powered Translocation-Throttling Mechanism for Kinetic Proofreading of RNAs and Oligomerization. Mol Cell. 2018 Oct 18;72(2):355-368.e4. PubMed Central PMCID: PMC6434538.
c. Devarkar SC, Wang C, Miller MT, Ramanathan A, Jiang F, Khan AG, Patel SS, Marcotrigiano J. Structural basis for m7G recognition and 2'-O-methyl discrimination in capped RNAs by the innate immune receptor RIG-I. Proc Natl Acad Sci U S A. 2016 Jan 19;113(3):596-601. PubMed Central PMCID: PMC4725518.
d. Jiang F, Ramanathan A, Miller MT, Tang GQ, Gale M Jr, Patel SS, Marcotrigiano J. Structural basis of RNA recognition and activation by innate immune receptor RIG-I. Nature. 2011 Sep 25;479(7373):423-7. PubMed Central PMCID: PMC3430514.

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