BIOGRAPHICAL SKETCH

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NAME: PATEL, SMITA S

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion 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 mechanisms of helicases and polymerases that are 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 who 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, 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, 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 the Rutgers University. I have trained 10 undergraduates, 20 graduate students, and 10 post-docs that are currently working in science related areas in medicine, industry, federal labs, or academia.

B. Positions and Honors

Positions and Employment

1992 - 1996	Assistant Professor, The Ohio State University, Columbus, OH
1996 - 1998	Associate Professor, The Ohio State University, Columbus, OH
1997 - 2002	Member, NIH Biochemistry Study Section
1998 - 2002	Associate Professor, UMDNJ-RWJMS, Piscataway, NJ
2001 -	Co-Organizer, FASEB meeting on Helicases: Structure, Function and Roles in Human Disease
2002 -	Ad hoc Reviewer, NSF, ACS, NIH MGA, Eureka, Pathway to Independence, MSFE, PCMB, Special Emphasis panel,

2002 - 2013	Professor, UMDNJ-RWJMS, Piscataway, NJ
2005 -	Gender Equity Program Mentor, CUNY
2011 -	Editorial Board, JBC
2013 -	Professor, Rutgers-RWJMS, Piscataway, NJ

Other Experience and Professional Memberships

-	Member, ASBMB
2013 -	Member, CERT (Community Emergency Response Team)

Honors

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

C. Contribution to Science

1. MECHANISM AND STRUCTURE OF RING-SHAPED HELICASES We discovered the ring-shaped hexameric structure of T7 helicase and demonstrated that the helicase ring binds DNA in the central channel. These are now recognized as general features of ring-shaped helicases. The structure raised immediate question and the one that intrigued me the most was 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. Our model provided the basis to understand the translocation mechanism of ring-shaped helicases and used as the basis to derive a more precise model from crystal structures of ring-shaped helicases determined more recently. One of our unexpected finding was that the replicative helicase on its own is a poor unwindase that uses a mostly passive mechanism to unwind DNA. This finding led us to investigate the role of DNA polymerase in stimulating the unwinding the replication fork and the coordination between the helicase, polymerase, and primase.

    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 PMID: 7731998; 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 PMID: 9144181; 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 PMID: 17604719; PubMed Central PMCID: PMC2699903.

2. COORDINATION BETWEEN HELICASE, POLYMERASE, AND PRIMASE We discovered the mechanistic basis for the coupling between helicase and polymerase at the fork junction. We showed that an actively

synthesizing T7 DNA polymerase is needed to stimulate the unwinding activity of T7 helicase. When coupled the helicase and polymerase create an efficient combined motor that moves in steps of one-nucleotide to unwind dsDNA and copy the nascent DNA strand. We demonstrated that cooperativity for leading strand DNA synthesis requires close proximity of the helicase and polymerase at the leading strand fork junction and functional coupling requires that the distance between the two enzymes is no more than one nucleotide. Primases synthesize short RNA primers that initiate lagging strand DNA synthesis, and primases are associated with the helicase and DNA polymerase as part of the replisome. We determined the architecture of the replisome and using single molecule FRET demonstrated a priming loop between helicase and primase. Such a loop was also demonstrated in the other prokaryotic replisomes and appears to be a general consequence of active replication.

a. Nandakumar D, Patel SS. Methods to study the coupling between replicative helicase and leading-strand DNA polymerase at the replication fork. Methods. 2016 May 9;PubMed PMID: 27173619.
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 PMID: 26675048; 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;4PubMed PMID: 25970034; 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):1129-38. PubMed PMID: 24630996; PubMed Central PMCID: PMC4010093.

3. MECHANISM OF TRANSCRIPTION BY T7 RNA POLYMERASE Parallel with the studies of T7 helicase and DNA polymerase, my lab has been studying the enzymology of single-subunit RNA polymerases. Our goal was to delineate the biochemical pathway of transcription initiation by quantifying the multi-step process to understand how a single subunit enzyme is able to both 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 aided our research of the related mitochondrial enzymes.

a. Deshpande AP, Patel SS. Interactions of the yeast mitochondrial RNA polymerase with the +1 and +2 promoter bases dictate transcription initiation efficiency. Nucleic Acids Res. 2014 Oct;42(18):11721-32. PubMed PMID: 25249624; PubMed Central PMCID: PMC4191429.
b. Tang GQ, Nandakumar D, Bandwar RP, Lee KS, Roy R, Ha T, Patel SS. Relaxed rotational and scrunching changes in P266L mutant of T7 RNA polymerase reduce short abortive RNAs while delaying transition into elongation. PLoS One. 2014;9(3):e91859. PubMed PMID: 24651161; PubMed Central PMCID: PMC3961267.
c. Kim H, Tang GQ, Patel SS, Ha T. Opening-closing dynamics of the mitochondrial transcription pre-initiation complex. Nucleic Acids Res. 2012 Jan;40(1):371-80. PubMed PMID: 21911357; PubMed Central PMCID: PMC3245942.
d. Tang GQ, Deshpande AP, Patel SS. Transcription factor-dependent DNA bending governs promoter recognition by the mitochondrial RNA polymerase. J Biol Chem. 2011 Nov 4;286(44):38805-13. PubMed PMID: 21911502; PubMed Central PMCID: PMC3207421.

4. MITOCHONDRIAL DNA REPLICATION AND TRANSCRIPTION I became interested in mitochondrial research (replication and transcription), when Twinkle was discovered by Hans Spelbrink as the human mitochondrial helicase homologous to the phage T7 DNA helicase. I was intrigued that many of the point mutations in the Twinkle helicase that were associated with various mitochondrial diseases were the same ones that we had isolated from our genetic screen with the T7 helicase. We put a considerable effort in

obtaining soluble and active Twinkle protein from bacterial expression and succeed in these efforts. We discovered that Twinkle forms hexamers and binds both ssDNA and dsDNA and more interestingly has DNA recombination activities that represents a novel function in the human mitochondria. Replication and transcription are intimately connected in maintaining mitochondrial DNA as transcription is needed for replication. The mitochondrial DNA is transcribed by a single subunit class of RNAPs that 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 bending and melting of the promoter. Using smFRET, we showed that the initiation complex undergoes dynamic opening/bending and closing/unbending transitions that are modulated by initiating ATPs.

a. Sen D, Patel G, Patel SS. Homologous DNA strand exchange activity of the human mitochondrial DNA helicase TWINKLE. Nucleic Acids Res. 2016 Feb 16;PubMed PMID: 26887820.
b. 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 PMID: 22383523; PubMed Central PMCID: PMC3340288.

5. HCV AND RIG-I HELICASES In parallel with the studies of ring-shaped helicases, we have been studying the mechanisms of hepatitis C virus helicase, which is a non-ring shaped helicase. We elucidated the nucleic acid translocation and unwinding mechanism showing that the HCV helicase forms multimers on nucleic acid that unwinds nucleic acids 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 the HCV led us to studies of 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 recognize 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, Chemistry we published the first high resolution structure of RIG-I bound to dsRNA and ATP and more recently showed that it binds to capped dsRNAs as well. 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. Ramanathan A, Devarkar SC, Jiang F, Miller MT, Khan AG, Marcotrigiano J, Patel SS. The autoinhibitory CARD2-Hel2i Interface of RIG-I governs RNA selection. Nucleic Acids Res. 2016 Jan 29;44(2):896-909. PubMed PMID: 26612866; PubMed Central PMCID: PMC4737149.
b. 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 PMID: 26733676; PubMed Central PMCID: PMC4725518.
c. 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 PMID: 21947008; PubMed Central PMCID: PMC3430514.
d. Rajagopal V, Gurjar M, Levin MK, Patel SS. The protease domain increases the translocation stepping efficiency of the hepatitis C virus NS3-4A helicase. J Biol Chem. 2010 Jun 4;285(23):17821-32. PubMed PMID: 20363755; PubMed Central PMCID: PMC2878546.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 GM111959-02
PATEL, SMITA S (PI)
09/15/14-05/31/18
Structural and mechanistic studies of self and non-self recognition by RIG-I
Role: PI

R35 GM118086-01
PATEL, SMITA S (PI)
05/01/16-04/30/21
Mechanistic studies of nucleic acid enzymes involved in DNA replication, transcription, and innate immunity
Role: PI