**Pankaj Srivastava**

Research Associate

Dept of Microbiology and Immunology, School of Medicine, UNC-Chapel Hill

Email: srivaspa@unc.edu

**EDUCATION**

PhD in Biotechnology (Molecular Parasitology):

Infectious Disease Research Laboratory, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. 2012

Master’s in Biotechnology (2004):

VBS Purvanchal University, Jaunpur, UP, India.

 Bachelor of Science (2002): Zoology, Botany and Chemistry Lucknow Christian College, Lucknow University, UP, India

**Research Statement**

I have an excellent background in biochemistry, molecular and cell biology. I completed my M.Sc and PhD in Biotechnology followed by postdoctoral fellowship (2011 to 2016) in the area of DNA Replication in the laboratory of Prof. Deepak Bastia at the dept. of Biochemistry and Molecular Biology at Medical University of South Carolina (MUSC), Charleston, SC. During this period, I have been thoroughly trained in number of cutting-edge technologies. One of my main projects was on DDK kinases that I successfully led in lab and ended up with a highly relevant discovery by solving the mechanism of DNA replication termination. Precisely, we have discovered the novel role of cell cycle kinase DDK (CDC7 and DBF4) in controlling replication termination and published in PNAS, 2016. Before this work, DDK’s main involvement was known in controlling replication initiation. We solved the mechanism of replication termination controlled by DDK kinase and concluded that phosphorylation of both fork protection complex protein Tof1 (a protein also responsible for circadian rhythm) and MCM helicase (active form-CMG) is necessary for the programmed fork arrest.

In June 2016, I joined a renal biochemistry lab at MUSC on Staff Scientist position that provides me with the opportunity to develop translational projects. I was engaged in research on kidney podocyte biology. Diseases of the renal glomerulus that result in the nephrotic syndrome are important causes of morbidity and mortality, especially in the aging population such as the Veterans. Injury to the kidney glomerulus is often characterized by heavy proteinuria associated with the loss of kidney filtration barrier commonly referred to as slit diaphragm. Significant progress has been made to discover the proteins localized at the slit diaphragm, yet little is known about the mechanism of actions of these proteins and how it contributes to the actual kidney filtration. My research aim was to understand the function and regulation of the proteins that are the critical components of this slit diaphragm and how they assemble to form a functional glomerular filtration barrier. The focus of my research was to define the intracellular signaling pathways that are activated in response to the activation of slit diaphragm proteins Nephrin and Neph1. For this purpose, I used a combination of biochemical, molecular biology and cell biology approaches to define the functional regions in these proteins and identify their novel interacting partners. We have discovered Hepatocyte Growth Factor as a novel activator (Ligand molecule) of Neph1 and Nephrin membrane proteins.

 After this, in January 2020, I moved to Univ. of Colorado, Denver as a Research Instructor in the laboratory of Prof. Rui Zhao. Here the focus of my project was to investigate the mechanisms of Coupling between RNA polymerase II driven mRNA transcription and splicing mediated by Small Nuclear Ribonucleoproteins. Several reports speculate that transcription machinery interacts with spliceosome (a complex with more than 100 proteins), however its role in coupling with splicing has not been investigated thoroughly. We were trying to mechanistically dissect this coupling using biochemical and structural biology approaches. Briefly, I discovered that RNA-PolII interacts with early spliceosome proteins complex for successful completion of cotranscriptional splicing in eukaryotes.

 In Sept. 2021, I joined UNC as a postdoctoral research associate where the focus of my work was to develop a peptide-based therapy for obesity. I tried to optimize the technically challenging expression and purification of a liver hormone LEAP2 (peptide) using maltose binding fusion protein system as MBP fusion protein tends to be more soluble in bacteria. During the short period of my stay in this lab, I have been able to generate relatively pure peptide. However, after final purification by TEV protease cleavage, it has the tendency to precipitate.

 In July 2022, I joined Cameron lab where the focus of my work is to test the antiviral therapeutic potential of novel nucleotide base analogues using in vitro biochemical and molecular biology approaches against SARS-CoV2. First, we tested if they are substrate for the RNA dependent RNA Polymerase (RdRP) by demonstrating their incorporation and chain termination/delay ability using oligo-RNA as a substrate, and next asked if the incorporated analogues exhibit any resistance to 3’-5’ Exoribonuclease (ExoN) cleavage. We also found that active RNA polymerase bound newly extended RNA template is resistant to 3’-5’ exoribonuclease cleavage. Another objective I am working on is the mechanism of ExoN activity by comparing biochemically and structurally with and without heterotrimeric complex with Nsp16. Recently we have demonstrated that co-expressing Nsp10, 14 and 16, they interact and form a heterotrimeric complex of Nsp14-Nsp10-Nsp16. In-vitro study demonstrating the relevance of the complex will be of high interest.

 My Ph.D. thesis is entitled: “Indian Visceral Leishmaniasis- Studies on Molecular Approaches to Characterization of Clinical Isolates and Diagnosis”. My thesis advisor is Prof. Shyam Sundar, and most of the work was done in collaboration with Prof. Jean Claude Dujardin’s group, Head, dept. of Molecular Parasitology, Univ. of Antwerp, Belgium. I published 8 papers in the field of diagnosis and genetic heterogeneity of Leishmania parasite.

**A selection of my contributions to science are presented below:**

2006-2011: Biomarkers to diagnose Leishmaniases and genetic polymorphism in L. donovani: Developed a PCR based diagnostic assay to detect Leishmania directly from 200 µl of human blood and from asymptomatic individuals. The assay was validated on a very large sample size including healthy controls from endemic and nonendemic areas.

Identified co infection of Leptomonas sp in the Visceral Leishmaniasis patients.

Using serological and molecular based tools, we discovered the markers of recent and past infections in asymptomatic individuals.

Using a combination of genetic markers, we reported the presence of significant genetic polymorphism in Indian Leishmania donovani clinical isolates.

For the first time, reported a unique proteomic and molecular signature in an Amphotericin B resistant Kala-azar patient from India.

2012-2015: Phosphorylation of CMG helicase and Tof1 is required for programmed fork arrest: This research pertains to the DNA replication termination process of DNA. Termination of replication is essential to cell processes, and when it is prevented, it allows formation of an R loop, which leads to genome instability. In addition, termination is needed in cell processes such as differentiation and genetic imprinting. We showed that DDK (cell cycle kinases), a combination of CDC7 and DBF4 proteins, is necessary for fork arrest, and therefore for normal termination of DNA replication. We further determined that the phosphorylation of both TOF1 (essential for normal fork arrest) protein and CMG helicase is crucial for their interaction and is required for programmed fork arrest. These findings addressed the mechanism of control of fork arrest along with the novel function of DDK. The main function of cell cycle kinases CDC7 AND DBF4 (DDK) seems to be in initiation of DNA replication by neutralizing and inhibitory domain located at the N-terminus of MCM4 and recruitment of CDC45 and the GINS sub complex to form an active CMG complex that has vigorous helicase activity. DDK also participates in S phase checkpoint response and in chromosome segregation during meiosis. We have provided the first evidence supporting the conclusion that DDK is also involved in termination of DNA replication at the Ter sites of rDNA and probably at other nonhistone protein dependent barriers. The evidence tends to support the model that DDK exercises this control through the formation of active MCM2-7 helicase which in turn interacts with Tof1 and Csm3 that are known to stabilize stalled forks at replication termini. Further, we have demonstrated that phosphorylation of Tof1 is crucial for the interaction with proteins of replication machinery as revealed by in vitro protein-protein interaction data.

Based on our study, a follow up study by UK group solved the Cryo-EM structure of the fork protection complex interacting with CMG at replisome demonstrating the interaction of the 2 systems and relevance of phosphorylation of Tof1 and CMG. Our study is of very high importance for the general audience as for the first time we have solved the mechanism of eukaryotic replication termination. This work may also have excellent implications for cancer treatment, as hindrance of DNA replication often slows tumorigenesis.

2016-2019: HGF-induced activation of NEPHRIN and NEPH1, a novel mechanism that participates in podocyte recovery from injury: Phosphorylation (activation) and dephosphorylation (deactivation) of the slit diaphragm proteins NEPHRIN and NEPH1 are critical for maintaining the podocyte actin cytoskeleton and, therefore, proper glomerular filtration. However, the mechanisms underlying these events remain largely unknown. Here we show that NEPHRIN and NEPH1 are novel receptor proteins for HGF and can be phosphorylated independently of the MET receptor in a ligand-dependent fashion through engagement of their extracellular domains by HGF. Further, we demonstrate SHP-2 phosphatase-dependent dephosphorylation of these proteins. To establish HGF as a ligand, purified baculovirus-expressed NEPHRIN and NEPH1 recombinant proteins were constructed and used in surface plasma resonance binding experiments. We report high-affinity interactions of NEPHRIN and NEPH1 with HGF, though NEPHRIN binding was 20-fold higher than NEPH1. Additionally, using molecular modeling we constructed peptides that were used to map specific HGF-binding regions in the extracellular domains of NEPHRIN and NEPH1. Finally, using an in vitro model of cultured podocytes and an ex vivo model of Drosophila nephrocytes, and chemically induced injury models, we demonstrate that HGF-induced phosphorylation of NEPHRIN and NEPH1 is centrally involved in podocyte repair. Importantly, the clinical relevance of our study was demonstrated by the plasma analysis of patients with FSGS, which showed significantly elevated levels of HGF consistent with the role of HGF as a component of injury or repair mechanisms. Thus, this is the first study demonstrating a receptor-based function for NEPHRIN and NEPH1. This has important biological and clinical implications for the repair of injured podocytes and the maintenance of podocyte integrity.

2016-2019: Diagnosing recurrent FSGS using a novel cell-based assay: Majority of glomerular diseases suffer from the lack of accurate and timely diagnosis, which is key to developing a successful treatment strategy for these diseases and prevent their progression to ESRD (end stage renal disease). This study demonstrates a novel concept that was used to develop a noninvasive, accurate and economical diagnostic assay with easy commercial adaptability in detecting rFSGS (recurrent focal and segmental glomerulosclerosis), where the glomerular disease FSGS recurs immediately following renal transplant. Importantly, the concepts demonstrated in this study are widely applicable in designing similar diagnostic/prognostic assays for other glomerular diseases requiring renal biopsy as a gold standard for diagnosis. Since FSGS targets podocyte damage and death, our unique approach involved mRNA profiling (RNA-Seq) of cultured podocytes treated with rFSGS patient plasma to reveal upregulated genes involved in cell damage. For concept validation, three upregulated candidate rFSGS responsive genes IL1β, BMF (Bcl2 Modifying Factor), and IGFBP3 were selected, their promoter regions were cloned into a luciferase-based reporter vector and transfected into podocytes to generate stable podocyte cell lines. Strikingly, when exposed to plasma from rFSGS patients, these cell lines showed increased reporter activity; in contrast, no reporter activity was noted with control plasma from other glomerular disease (non-rFSGS and membranous nephropathy) patients. The area under the receiver operating characteristics curves (AUCs) estimated for models discriminating between rFSGS and all other nephropathies and between rFSGS and non-recurrent FSGS ranged from 0.93 to 0.96, respectively. Additionally, the estimated sensitivity was greater than 86% and specificities were greater than 82% for all genes.

**RESEARCH EXPERIENCE**

* **July 2022 to till date** Research Associate, Cameron and Arnold Lab, Dept of Microbiology and Immunology, UNC Chapel Hill.
* **Sept 2021 to May 2022** Postdoctoral Research Associate, Liu Lab, Div. of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, UNC Chapel Hill. Project: Biotechnological applications of LEAP2 peptide.
* **Jan 2020 to Sept 2021** Research Instructor, Zhao Lab, Dept of Biochemistry and Molecular Genetics, Univ of Colorado, Anschutz Medical Campus, Denver-CO on the project entitled “Coupling between Transcription and Splicing mediated by Small Nuclear Ribonucleoproteins”.
* **June 2016 to December 2019** Staff Scientist, Nihalani Lab, Laboratory of Renal Biochemistry, division of Nephrology, Medical University of South Carolina, SC.
* **Sept. 2011 to May 2016** Postdoctoral Fellow, Bastia Lab, Laboratory of Yeast DNA Replication, dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, SC.
* **2006-2011** Ph.D. thesis research entitled “Indian Visceral Leishmaniasis - Studies on Molecular Approaches to Characterization of Clinical Isolates and Diagnosis”.
* **Jan–April 2004** M.Sc. thesis on the topic “Y chromosome polymorphism in Rajput and Brahmin population groups of Himachal Pradesh” National Centre of Applied Human Genetics, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.
* **May, 2003** Summer trainee at National JALMA (Japanese Leprosy Mission for Asia) Institute of Leprosy and other Mycobacterial Disease, Agra, UP, India.

**Publications In Peer Reviewed Journals:**

1. Chinthapatla, R., Sotoudegan, M., Srivastava, P., Anderson, T., Moustafa, I.M., Passow, K.T., Kennelly, S.A., Moorthy, R., Dulin, D., Feng, J., Harki, D., Kirchdoerfer, R., Cameron, C.E., Arnold, J,J., (2023). Interfering with nucleotide excision by the coronavirus 3’-to-5’ exoribonuclease. Nucleic Acid Research, 51, 315-336.

2. Solanki, A., Arif, E., Srivastava, P., Furcht, C.M., Rahman, B., Wen, P., Singh, A., Holzman, L.B., Fitzgibbon, W.R., Budisavljevic, M.N., Lobo, G.P., Kwon, S.H., Han, Z., Lazzara, M.J., Lipschutz, J.H., Nihalani, D., (2021) Phosphorylation of slit diaphragm proteins NEPHRIN and NEPH1 upon binding of HGF promotes podocyte repair. J of Biological Chemistry, 297, 101079

3. Solanki, A., Srivastava, P., Rahman, B., Lipschutz, J.H., Nihalani, D., Arif, E., (2020) The Use of High-Throughput Transcriptomics to Identify Pathways with Therapeutic Significance in Podocytes. International J of Molecular Sciences, 21, 274.

4. Srivastava⃰, P., Solanki⃰, A., Arif, E., Kwon, S., Wolf, B., Janech, M., Budisavljevic, M., Nihalani, D., (2019) Development of a novel cell-based assay to diagnose recurrent Focal and Segmental Glomerulosclerosis. Kidney International, 95, 708-716.

5. Nihalani, D., Solanki, A., Arif, E., Srivastava, P., Rahman, B., Zuo, X., Dang, Y., Alsudan, H., Ghoshroy, S., Sampson, M., Lipschutz, J., (2019) Disruption of the exocyst induces podocyte loss and dysfunction. J of Biological Chemistry, 294, 10104-10119.

6. Arif, E., Solanki, A., Srivastava, P., Tash, B., Holzman, L., Janech, M., Martin, R., Knölker, H-J., Fitzgibbon, W., Deng, P., Budisavljevic, M., Syn, W., Wang, C., Kwon, S., Nihalani, N., (2019) The motor protein Myo1c regulates transforming growth factor-β-signaling and fibrosis in podocytes. Kidney International, 96, 139-158.

7. Arif, E., Solanki, A., Srivastava, P., Rahman, B., Megyesi, J., Janech, M., Kwon, S., Collier, J., Schnellmann, R., Nihalani, D., (2019). Mitochondrial biogenesis induced by the β2-adrenergic receptor agonist formoterol accelerates podocyte recovery from injury. Kidney International, 96, 656-673.

8. Solanki, A., Widmeier, E., Arif, E., Daga, A., Helmstadter, M., Srivastava, P., Kwon, S., Shril, S., Bergmann, C., Huber, T., Hildebrandt, F., Nihalani, D., (2019) Mutations in KIRREL1 a slit diaphragm component cause steroid-resistant nephrotic syndrome. Kidney International, 96, 883-889.

9. Sagar, A., Arif, E., Solanki, A., Srivastava, P., Kwon, S., Ashish, Nihalani, D., (2017) Targeting Neph1 and ZO-1 protein-protein interaction in podocytes prevents podocyte injury and preserves glomerular filtration function. Nature-Scientific Reports, 7, 12047.

10. Bastia, D., Srivastava⃰, P., Zaman⃰, S., Chaudhary⃰, M., Mohanty⃰, B.K., Bacal, J., Langston, L., Pasero, P., O’Donnell, M. (2016) Phosphorylation of CMG helicase and Tof1 is required for programmed fork arrest. PNAS, 113, E3639–E3648.

11. Zaman, S., Chaudhary, M., Jiang, J., Srivastava, P., Mohanty, B., Danielson, C., Humphrey, S., Jazwinski, S., Bastia, D. (2016). Mechanism of regulation of intrachromatid recombination and long-range chromosome interactions In Saccharomyces cerevisiae. Molecular and Cellular Biology, 36,1451-63.

12. Srivastava, P., Gidwani, K., Picado, A., Auwera, G.V.D., Tiwary, P., Ostyn, B., Dujardin, J.C., Boelaert, M., Sundar, S., (2013) Molecular and serological markers of Leishmania donovani infection in healthy individuals from endemic areas of Bihar, India. Tropical Medicine and International Health, 18, 548-54.

13. Srivastava, P., Singh, T., Sundar, S. (2011) Genetic heterogeneity in Leishmania donovani clinical isolates from India. Journal of Clinical Microbiology, 49, 3687-90.

14. Srivastava, P., Mehrotra, S., Tiwari, P., Chakravarty, J., Sundar, S., (2011) Diagnosis of Indian Visceral Leishmaniasis by Nucleic Acid Detection using PCR, Plos One, 6, e19304.

15. Srivastava, P., Prajapati, V.K., Rai, M., Sundar, S., (2011) Unusual Case of Resistance to Amphotericin B in Visceral Leishmaniasis in a Region in India Where Leishmaniases is not Endemic. Journal of Clinical Microbiology, 49, 3088-91.

16. Mohan, S., Srivastava, P., Maheshwari, S.N., Sundar, S., Prakash, R., (2011) Nano Structured Nickel Oxide based DNA Biosensor for Detection of Visceral Leishmaniasis Analyst, 136, 2845-51.

17. Srivastava, P., Prajapati, V., Auwera, GVD., Dujardin, JC., Sundar, S., (2010) Detection of Leptomonas sp. parasites in clinical isolates of Kala-azar patients from India. Infection, Genetics and Evolution, 10, 1145-50.

18. Picado, A., Singh, S, Rijal, S., Sundar, S., Ostyn, B., Chappuis, F., Uranw, S, Gidwani, K., Khanal, B., Rai, M., Paudel, I., Lal, M., Das, P., Kumar, R., Srivastava, P., Dujardin, J.C., Vanlerberghe, V., Andersen, E.W., Davies, C.R., Boelaert, M., (2010) Long lasting insecticidal nets for the prevention of Leishmania donovani infection in India and Nepal: paired cluster randomised trial British Medical Journal, 341, c6760.

**Review Article:**

1) Srivastava, P., Dayama, A., Mehrotra, S., Sundar, S., (2011) Diagnosis of Visceral Leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene: 105, 1-6.

**Abstracts:**

1. Srivastava, P., Dujardin, J.C., Boelart, M., Singh, S.P., Sundar, S., (2010). To study the magnitude of asymptomatic human carriers of Leishmania donovani and the effect of insecticide treated nets (LNs) in an intervention trail in Bihar, India. Fourth US-India Joint Research Training Program Workshop on Intracellular Pathogens, Goa, India (Poster).

2. Srivastava, P., Mehrotra, S., Prajapati, V.K., Sundar, S., (2009). Studies on genetic heterogeneity among isolates from Indian kala-azar patients by PCR-RFLP and PCR-direct sequencing. 4th WorldLeish Congress on Leishmaniasis, Lucknow, India, (Poster).

3. Manandhar, K.D., Kumar, B., Srivastava, P., Gidwani, K., Maurya, R.S., Rai, M., Sundar, S., (2009) Study of antigenic leishmanial protein and its potentiality as diagnostic marker by immunoblot assay. 4th WorldLeish Congress on Leishmaniasis, Lucknow, India (Poster).

4. Mehrotra, S., Prajapati, V.K., Babu, Y., Maurya, R.S., Srivastava, P., Vaish, M., Rai, M., Sundar, S., (2009) Comparative evaluation of primers for diagnosis and prognosis of visceral leishmaniasis. 4th WorldLeish Congress on Leishmaniasis, Lucknow, India (Poster).

5. Srivastava, P., Prajapati, V.K., Mehrotra, S., Sundar, S., (2008) Studies on genetic heterogeneity and diagnostic PCR in Indian visceral leishmaniasis. Training course on “Molecular Biology of Leishmania”, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy (Poster).

6. Srivastava, P., Arif, E., Solanki, A., Kwon, K., Janech, M.G., Nihalani, D., (2017) Diagnosing Recurrent FSGS Using a Novel Cell-Based Assay. American Society of Nephrology conference, San Diego, CA (Talk)

7. Solanki, A., Nihalani, D., Arif, E., Srivastava, P., Lipschutz, J.H., Zuo, X., Su, Y., Dang, Y., Al sudani, H., Ghoshroy, S., (2017) Role of Exocyst Complex in Podocytes. American Society of Nephrology conference, San Diego, CA (Poster).

**NCBI Gen-Bank Submissions:** Accession No-FJ226475, HQ159842 and GU143558

**US PATENT APPLICATION (2020):**

D. Nihalani, **Pankaj Srivastava** et al. (Publication number: 20200109434). **Title:** Development of anovel cell-based assay to diagnose recurrent Focal and Segmental Glomerulosclerosis.

**Number of citations of all papers:** 1200+

**Invited Talks & Presentations**

1. Oral presentation in Nephrology Young Investigators' Forum (Southern Society for Clinical Investigation) 2017 meeting held in New Orleans (Feb, 2017). **Title**: A Novel Role for the Phosphatase SHP2 in Regulating Glomerular Filtration Function.
2. Oral presentation in American Society of Nephrology conference. Nov 2017. **Title**: Diagnosing recurrent FSGS using a novel cell-based assay.
3. Presented my research work in Research and Methods seminar series, dept. of Biochemistry and Molecular Biology, Medical University of South Carolina, SC, USA on 24th March 2015. **Title:** Phosphorylation of CMG helicase and Tof1 is required for programmed fork arrest.

**Experstise:** Molecular Biology, Protein and nucleic acid biochemistry, Cell Biology