BIOGRAPHICAL SKETCH

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NAME: Ravinder Singh

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

POSITION TITLE: Associate Professor, Molecular, Cellular and Development Biology

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
H.A.U., Hisar, India	B.Sc. (Hons.)	1983	Genetics and Plant Breeding
I.A.R.I., New Delhi, India	M. Sc.	1985	Seed Science and Technology
I.A.R.I., New Delhi, India	M. Sc.	1987	Molecular Biology
Baylor College of Medicine, Houston, TX	Ph.D.	1990	Mol.Biol./ Pharmacology
HHMI/UMass Medical Center, Worcester, MA	postdoc	1997	Splicing Regulation

A. Personal Statement

I have extensive experience in RNA biology and have made seminal contributions to this field. My graduate and post-doctoral work focused on how RNA-binding proteins regulate pre-mRNA splicing, which controls sex determination and dosage compensation in Drosophila. As an independent investigator, I've shown unexpected functions and mechanisms for three splicing regulators: U2AF, SXL, and PTB. We have used Drosophila genetics, computational biology, and high throughput approaches to study gene regulation. We are also doing experiments to study Down Syndrome and to screen drugs against ovarian tumors in Drosophila.

1. Singh, R., J. Valcarcel and M.R.Green (1995) Distinct binding specificities and functions of higher eukaryotic polypyrimidine-tract binding proteins. **Science** 268:1173-1176.
2. Robida, M. D., Sridharan, V., Morgan, S. Rao, T., and Singh, R. (2010) Drosophila Polypyrimidine Tract-binding protein (PTB) is necessary for spermatid individualization. **Proc. Natl. Acad. Sci., USA**, 107:12570-12575.
3. Heimiller, J., Sridharan, V., Huntley, J., Wesley, C., and Singh, R. (2014) Drosophila Polypyrimidine Tract binding protein regulates dorso-ventral patterning genes in embryos. **PLoS One** , 9(7): e98585. doi:10.1371/journal.pone.0098585.
4. Singh R. (2015). Bioinformatics Analysis to identify RNA-protein Interactions in Oogenesis. In Bratu D McNeil GP, editors. New York: Humana Press.
5. Sridharan, V., Heimiller, J., Robida, M.D, and R. Singh* **(**2016) High throughput sequencing identifies misregulated genes in the *Drosophila* Polypyrimidine Tract-binding protein (*hephaestus*) mutant defective in spermatogenesis. **PLoS ONE**, 11(3):e0150768. doi: 10.1371/journal.pone.0150768.
6. Singh, R. (2018) A Novel Saturation Mutagenesis Approach: Single Step Characterization of Regulatory Protein Binding Sites in RNA Using Phosphorothioates. **J. Vis. Exp.** (138), e57816, doi:10.3791/57816.
7. Singh, R. (2019) Exploring Sequence Space to Identify Binding Sites for Regulatory RNA-Binding Proteins. J. Vis. Exp. (150), e59635, doi:10.3791/59635.

8. Banerjee, H. and Singh, R. (2021) Genomic and cDNA selection-amplification identifies transcriptome-wide binding sites for the Drosophila protein sex-lethal. PLoS ONE 16(5): e0250592. https://doi.org/10.1371/journal. pone.0250592.
9. Wang, R., Mancini, E., and Singh, R. (2023) Machine Learning approach distinguishes conventional (U2AF-sensitive) and non-conventional (U2AF-insensitive) introns in the fission yeast. (manuscript in preparation).
10. Rogalska, M., Mancini, E., Chambers, J. T., Singh, R., Valcarcel, J.* (2023). We have provided our part of the high throughput data analysis to our collaborator* for a manuscript under preparation.

B. Positions and Honors

Positions and Employment:

1988-90 Ph.D. (with Dr. Ram Reddy), Department of Pharmacology, Baylor College of Medicine, Houston, TX.
1990-97 Post-doctoral Fellow (with Dr. Michael R. Green), Howard Hughes Medical Institute, UMass Medical Center, Worcester, MA.
1997-2003 Assistant Professor, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder
2004- Associate Professor, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder

Honors:

1978-83 Indian Council of Agricultural Research Scholarship
1985-87 Indian Agricultural Research Institute Fellowship
1983-85 Indian Agricultural Research Institute Fellowship
1994-97 Leukemia Society of America Special Fellowship
1991-94 Leukemia Society of America Fellowship
1999-01 Basil O'Connor Research Award, March of Dimes

Awards/Recognition

1983 Second Position in University, B. Sc. (Honors)
1987 Gold Medal in M.Sc. Molecular Biology
1990 The Mead Johnson Award, Annual National Student Research Forum
1989 Busch Award for the Best Graduate Student
1990 Baylor College of Medicine Graduate Symposium Award
1999 Junior Faculty Development Award, University of Colorado, Boulder
2003, '10 Jane and Charlie Butcher Foundation Award
2004, '05 Keck Foundation Award
2007 Marinus Smith Teaching Award
2007 National Academies Education Fellow in the Life Sciences
2016-18 Chair, University of Colorado Faculty Council

C. Contribution to Science

1. The cap structure of the U6 snRNA, an essential spliceosomal snRNA critical for joining pre-mRNA exons, had been a mystery for over a decade. I discovered the novel U6 RNA cap structure and found that the mechanism of capping of the U6 snRNA is fundamentally different from the capping of mRNAs (7-methyl guanosine) and other snRNAs (2,2,7-trimethyl guanosine). mRNA cap formation is coupled to transcription by RNA polymerase II and is independent of the mRNA sequence. In contrast, the capping of U6 snRNA is post-transcriptional, is not coupled to transcription by RNA polymerase III, which transcribes U6, and requires a stem-loop followed by an AUAUAC motif as a capping signal. Finally, this cap confers stability, and is present in numerous other cellular transcripts.

a. Singh, R. and R. Reddy (1989) γ-Monomethyl phosphate: A cap structure in spliceosomal U6 small nuclear RNA. **Proc. Natl. Acad. Sci. USA** 86:8280-8283.
b. Singh, R., S. Gupta and R. Reddy (1990) Capping of mammalian U6 small nuclear RNA in vitro is directed by a conserved stem-loop and AUAUAC sequence: Conversion of a non-capped RNA into a capped RNA. **Mol. Cell. Biol**. 10:939-946.
c. Gupta, S., R.K. Busch, R. Singh and R. Reddy (1990) Characterization of U6 snRNA cap-specific antibodies: Identification of γ-monomethyl GTP cap structure in 7SK and several other human small RNAs**. J. Biol. Chem**. 265:19137-19142.
d. Gupta, S., R. Singh and R. Reddy (1990) Capping of U6 small nuclear RNA in vitro can be uncoupled from transcription. **J. Biol. Chem**. 265:9491-9495.

2. My colleagues and I discovered unexpected properties and functions of RNA-binding proteins. First, I showed that the pyrimidine-tract binding proteins, SXL, PTB, and U2AF^{65}, have distinct RNA-binding specificities and defined the binding site for the master sex-switch protein SXL. I then showed that the mammalian polypyrimidine-tract binding protein PTB is a splicing repressor rather than a splicing factor, as was previously thought. We discovered that the *Drosophila* SXL protein and the human PTB protein repress 3' splice sites on different pre-mRNA using a common molecular mechanism – competition with the general splicing factor U2AF^{65}. For these studies we developed the first *in vitro* alternative splicing assay for SXL and showed how the activation domain (RS domain) of U2AF contacts the branch site and recruits U2 snRNP to the branch point to form the commitment complex.

a. Singh, R., J. Valcarcel and M.R.Green (1995) Distinct binding specificities and functions of higher eukaryotic polypyrimidine-tract binding proteins. **Science** 268:1173-1176.
b. Singh, R. and M.R. Green (1993) Sequence-specific binding of tRNA by glyceraldehyde-3-phosphate dehydrogenase. **Science** 259:365-368.
c. Valcarcel, J., R. Singh, P.D. Zamore and M.R. Green (1993) The protein sex-lethal antagonizes the splicing factor U2AF to regulate alternative splicing of *transformer* pre-mRNA. **Nature** 362:171-5.
d. Valcarcel, J., R. Gaur, R. Singh and M.R. Green (1996) Interaction of U2AF^{65} RS region with pre-mRNA branch point and promotion of base pairing with U2 snRNA. **Science** 273:1706-1709.

3. The RNA Recognition Motif (RRM) is a common RNA-binding domain. Most RRM proteins contain multiple RRMs. How multiple RRMs contribute to RNA recognition, however, had been unknown. My laboratory's detailed analysis of pyrimidine-tract recognition by multiple RRMs, using site-specific laser crosslinking and domain mapping, led to a model by which two RRMs in both U2AF^{65} and SXL recognize the pyrimidine tract using multiple modes of binding, resulting in an ensemble of complexes. This likely involves binding in multiple registers for each RRM. Our findings explained several past observations, and provided a basis for the increased binding affinity for longer pyrimidine tracts, and thus increased strength for associated 3' splice sites. DNA or RNA recognition by an ensemble of complexes was unprecedented. Another unexpected finding from this series of biochemical analysis on RNA recognition was that the RRM3 domain of U2AF is dispensable for splicing *in vitro* and that U2AF and SXL bind to the same RNA site differently.

a. Singh, R., Banerjee, H., and Green, M. R. (2000) Differential Recognition of the Polypyrimidine-tract by the General Splicing Factor U2AF^{65} and the Splicing Repressor Sex-lethal. **RNA,** 6:901-911.
b. Banerjee, H., Rahn, A., Davis, W. and Singh, R. (2003) Sex-lethal and U2 small nuclear ribonucleoprotein auxiliary factor (U2AF^{65}) recognize polypyrimidine-tract using multiple modes of binding. **RNA,** 9:88-99.
c. Banerjee, H. Rahn, A., Gawande, B., Guth, S., Valcarcel, J. and Singh, R. (2004) The conserved RNA Recognition Motif 3 of U2 snRNA Auxiliary Factor (U2AF^{65}) is essential *in vivo* but dispensable for activity *in vitro.* **RNA** 10: 240-253.

4. The polypyrimidine-tract binding protein (PTB) is ubiquitously expressed and has been extensively studied in vertebrates. We discovered unexpected roles for PTB and SXL in the male and the female germline in Drosophila. We found that the dm*PTB* transcript functions in the male germline and is regulated by the somatic sex-determination pathway. We provided genetic evidence that directly linked dm*PTB* function to male fertility by analyzing a *P*-element insertion (*heph*^{2}) in the gene encoding dm*PTB*. Excision of the *P*-

element rescued dm*PTB* expression, gonad morphology, and male fertility. This germline-specific function of dm*PTB* in *Drosophila* males was surprising because of the established view that the vertebrate PTB is ubiquitously expressed. In somatic cells, the master sex-switch protein SXL plays a pivotal role in sexual differentiation and dosage compensation. The role of SXL in splicing and translation regulation in somatic cells is well understood. While it was known that SXL also plays an important role in the female germline, what it does there had been a mystery. We found that SXL regulates *enhancer of rudimentary* in the female germline by a novel mechanism, poly(A) site switching. While SXL regulates splicing of other known targets (*Sxl, tra,* and *msl2*) in somatic cells, this is the first example in which SXL mediates gene regulation via poly(A) switching. The poly(A) site switching is important because it results in production of a longer mRNA, which is subject to translational repression. Thus, identification of a germline-specific target for SXL provides an important handle to understanding the role of SXL in the female germline. Our subsequent computational analysis of the *Drosophila* genome identified five additional targets that are potential candidates for such regulation, suggesting that poly(A) switching may be a common mechanism for SXL regulation.

a. Robida, M. D., and Singh, R. (2003) *Drosophila* polypyrimidine-tract binding protein (PTB) functions specifically in the male germline. **EMBO J.** 22:2924-2933**.**
b. Gawande, B., M. D. Robida, A. Rahn, and R. Singh (2006) *Drosophila* protein Sex-lethal dependent polyadenylation switching in the female germline. **EMBO J.** 25:1263-1272.
c. Robida MD, Rahn A, Singh R (2007) Genome-wide identification of alternatively spliced mRNA targets of specific RNA-binding proteins. **PLoS ONE** 2(6): e520.
d. Shepherd, A.K., Singh, R., and Wesley, C.S. (2009). Notch mRNA expression in Drosophila embryos is negatively regulated at the level of mRNA 3' processing. **PLoS One** *4*, e8063.
e. Hartmann, B, Castelo, R., Miñana, B., Peden, E., Blanchette, M., Rio, D. C., Singh, R., and Valcárcel, J. (2011) Distinct programs of sex-specific alternative splicing regulation in Drosophila melanogaster. **RNA**, 17:453-468.

5. We discovered unexpected diversity in U2AF and Py-tract requirements for 3' splice site recognition. We also identified novel introns whose splicing *in vivo* is unaffected when the function of the essential splicing factor U2AF is conditionally inactivated. These U2AF-insensitive introns may differ in how they assemble pre-spliceosomes. We also identified unconventional pyrimidine-tracts located upstream of the branchsite and showed a conditional role of U2AF. To computationally search for pyrimidine-rich sequences (defined by base composition rather than a defined sequence) that are relevant for RNA processing, we developed a unique search algorithm - Fast-FIND.

a. Sridharan, V. and R. Singh (2007) A Conditional Role of U2AF in Splicing of Introns with Unconventional Polypyrimidine Tracts**. Molecular and Cellular Biology,** 27, 7334-7344**.**
b. Sridharan, V., Joseph Heimiller and R. Singh (2011) Genomic mRNA profiling reveals compensatory mechanisms for the requirement of the essential splicing factor U2AF. **Molecular and Cellular Biology**, 31:652-661.
c. Hamady, M., E. Peden, R. Knight, and R. Singh (2006) Fast-FIND: A novel computational strategy to analyzing combinatorial motifs. **BMC Bioinformatics** 7:1-10.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1z16ckhaHlw5w/bibliography/48173003/public/?sort=date&direction=ascending

D. Research Support:

University of Colorado Research Support/Stipend 2016 — 2017
University of Colorado Research Support/Stipend 2017 — 2018

Submitted

National Institutes of Health

R21

Date submitted: October, 2022 (Declined, learned about the status in Spring 2023; planning to revise and resubmit in the future)

Novel use of the dosage compensation machinery to induce Chromosome 21 silencing in Tc1 mouse cells

Completed Research Support

University Bridge Fund 2011 — 2013

Role: PI

Jane and Charlie Butcher Foundation Award 06/01/2010—10/30/2012

"Bigger Bang for the Next-Generation-Sequencing Buck: Clear and Present Need"

Role: PI

RSG-04-177-01-DDC 07/01/04-6/30/09

American Cancer Society "Female Germline-specific Regulation of *e(r)"*

Role: PI

GM58576 National Institutes of Health 08/01/99-7/31/04

RNA-Protein Interactions in Pre-mRNA Splicing Regulation

Role: PI

Other past support:

Basil O'Connor Starter Scholar Research Award, MARCH OF DIMES,
The Keck Foundation,
Jane and Charlie Butcher Foundation Award
The Colorado RNA Center

Role: PI