BIOGRAPHICAL SKETCH

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NAME: TYAGI, SANJAY

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

POSITION TITLE: 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 Rajasthan, Bikaner, Rajasthan	BS	06/1978	Zoology, Botany, Chemistry
Jawaharlal Nehru University, New Delhi	MS	06/1980	Life Sciences
Jawaharlal Nehru University, New Delhi	MPHIL	06/1982	Theoretical Biology
University of Maryland, College Park, Maryland	PHD	05/1987	Biochemistry

A. Personal Statement

A long-term theme of my research has been the development of nucleic acid analysis and imaging tools, and to utilize them to address important questions in biology. I co-invented molecular beacons in 1996 with Fred Russell Kramer. This technology is used in many laboratories for real-time PCR and has since become a key element in a vibrant diagnostic industry. Millions of molecular beacon-based assays for Tuberculosis (GeneExpert MTB/RIF), HIV (NucliSens EasyQ HIV-1), MRSA (BD GeneOhm MRSA assay), SARS-CoV-2 (Abbott's ID Now) and other diseases are now carried out around the world every year. Subsequently, we adopted molecular beacons for the study of RNA trafficking in live cells. We were able to image the distribution and dynamics of endogenous mRNAs in diverse biological contexts, ranging from Drosophila oocytes to avian fibroblasts. We recognized that for studying the underlying mechanisms of intracellular mRNA transport, it is necessary to have single-molecule sensitivity. To accomplish that, we developed transgenes that contained tandemly repeated sequences as molecular beacon target sites, and alternatively, we utilized as many as 50 hybridization probes for the same mRNA simultaneously (single-molecule FISH) (licensed to Biosearch Technologies and commercially available as Stellaris Probes). With these approaches, individual mRNA molecules become visible as fine fluorescent spots that can be counted and tracked. This approach has rendered *in situ* FISH an exquisitely quantitative and popular technique, even achieving single nucleotide sensitivity for single RNA molecules (Marras *et al* 2019).

We found that mRNAs bound to their entourage of proteins disperse from the gene locus by Brownian motion, and not by active transport, as was previously suspected. We examined RNA-transport granules in hippocampal neurons and showed that they contain just one molecule of mRNA. Our approach also permitted single-molecule analyses of RNA processing events -- by targeting an intron with one set of probes and an exon with another set of differently colored probes, we were able to study the subcellular venues of mRNA splicing.

Another high impact contribution from our laboratory was our studies in which we used our single molecule imaging approach to explore whether the heterogeneity of gene expression previously observed in bacteria and yeast, also manifests itself in higher eukaryotes. We found that mammalian cells exhibit even more acute heterogeneity which likely stems from the genes randomly switching between an off and on state under the influence of chromatin structure. mRNA counting by single-molecule FISH has since become a standard practice in this expanding field. These studies raised two important questions, how do cells prevent such noise from propagating into gene regulatory pathways, and what causes the genes to switch randomly between the on and off states (Fraser *et al* 2021).

Two papers that showcase some recent research in our laboratory:

1. Marras SAE, Bushkin Y, Tyagi S. High-fidelity amplified FISH for the detection and allelic discrimination of single mRNA molecules. Proc Natl Acad Sci U S A. 2019 Jul 9;116(28):13921-13926. PubMed Central PMCID: PMC6628817.

2. Fraser LCR, Dikdan RJ, Dey S, Singh A, Tyagi S. Reduction in gene expression noise by targeted increase in accessibility at gene loci. Proc Natl Acad Sci U S A. 2021 Oct 19;118(42) PubMed Central PMCID: PMC8545487.

NCBI Bibliography: https://www.ncbi.nlm.nih.gov/myncbi/sanjay.tyagi.2/bibliography/public/

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2012 -	Professor, Rutgers University, Newark, NJ
2004 -	Member, Public Health Research Institute, Newark, NJ
2004 - 2012	Associate Professor, University of Medicine and Dentistry of New Jersey, Newark, NJ
2000 - 2004	Associate Member, Public Health Research Institute, New York, NY
1988 - 2000	Senior Research Assocaite, Public Health Research Institute, New York, NY
1987 - 1988	Research Associate, Public Health Research Institute, New York, NY
1982 - 1987	Research and Teaching Assistant, University of Maryland, College Park, MD

Honors

2018	Thomas Alva Edison Award, Research and Development Council of New Jersey
2012	Excellence in Research Award, University of Medicine in Dentistry of New Jersey (Now Rutgers Universty)
2005	Jacob Heskel Gabbay Award in Biotechnology and Medicine, Jacob and Louise Gabbay Foundation; https://www.brandeis.edu/rosenstiel/gabbay-award/past.html

C. Contribution to Science

1. **Molecular Beacons.** Introduction of this technology transformed nucleic acids analysis in research and clinical diagnostics. We were the first to demonstrate real-time monitoring of amplification of specific DNA in sealed tubes. The method eliminated the possibility of carryover contamination in PCR, expanded its dynamic range by three orders of magnitude, and created the possibility of imaging specific mRNAs in living cells. We also introduced the concept of "dark universal" quenchers, which allowed multiplex PCRs for 4-6 different targets simultaneously and are now being used in a large variety of fluorogenic probes. Diagnostic assays using this technology to detect many pathogens are used worldwide.

   a. Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol. 1996 Mar;14(3):303-8. PubMed PMID: 9630890.
   b. Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination. Nat Biotechnol. 1998 Jan;16(1):49-53. PubMed PMID: 9447593.
   c. Bonnet G, Tyagi S, Libchaber A, Kramer FR. Thermodynamic basis of the enhanced specificity of structured DNA probes. Proc Natl Acad Sci U S A. 1999 May 25;96(11):6171-6. PubMed Central PMCID: PMC26854.
   d. Tyagi S, Marras SA, Kramer FR. Wavelength-shifting molecular beacons. Nat Biotechnol. 2000 Nov;18(11):1191-6. PubMed PMID: 11062440.

2. **mRNA Localization.** Localization of mRNAs in particular subcellular zones is important for the phenotype of many differentiated cells. We first demonstrated the detection and tracking of endogenous mRNAs in live oocytes and fibroblasts with 2'-O-methyl molecular beacons. We then analyzed the transport of individual mRNA molecules in the nucleus soon after their synthesis at their gene locus with this approach and showed that intranuclear transport occurs by free diffusion and not by active mechanisms. Earlier cell biologists believed that the nucleus is so dense, and the mRNA protein complexes are so large, that they

would have to be actively transported from the gene locus to the nuclear pores. In neurons certain mRNAs are actively transported as part of “RNA transport granules” to distal reaches of dendrites and contribute to synaptic plasticity. We showed that mRNA transport granules in neurons are not conglomerates of multiple RNAs, as was previously believed, but contain a single mRNA molecule.

a. Bratu DP, Cha BJ, Mhlanga MM, Kramer FR, Tyagi S. Visualizing the distribution and transport of mRNAs in living cells. Proc Natl Acad Sci U S A. 2003 Nov 11;100(23):13308-13. PubMed Central PMCID: PMC263795.
b. Tyagi S, Alsmadi O. Imaging native beta-actin mRNA in motile fibroblasts. Biophys J. 2004 Dec;87(6):4153-62. PubMed Central PMCID: PMC1304924.
c. Vargas DY, Raj A, Marras SA, Kramer FR, Tyagi S. Mechanism of mRNA transport in the nucleus. Proc Natl Acad Sci U S A. 2005 Nov 22;102(47):17008-13. PubMed Central PMCID: PMC1287982.
d. Batish M, van den Bogaard P, Kramer FR, Tyagi S. Neuronal mRNAs travel singly into dendrites. Proc Natl Acad Sci U S A. 2012 Mar 20;109(12):4645-50. PubMed Central PMCID: PMC3311338.

3. **Stochastic mRNA Synthesis.** Using engineered reporter genes with tandemly repeated sequences in their UTR and then employing FISH probes against the repeats, we obtained a census of individual mRNA molecules in single cells. Our analyses showed that mRNA synthesis occurs in bursts that are initiated and end randomly. In combination with the short half-life of mRNAs, this stochastic mRNA synthesis creates great heterogeneity among genetically identical cells. Our 2006 study not only broke new methodological ground for single cell gene expression analyses, it raised questions as to how eukaryotic cells cope with this "noise" in gene expression and what molecular processes give rise to it. Focusing on the first question, we asked how the expression noise in transcription factors affects the noise in the down-stream genes? Our 2012 study identified impediments to propagation of noise and showed that chromatin is simultaneously responsible for creating gene expression noise and for insulating genes from deleterious effects of noise from upstream sources. Focusing on the second question, in 2021 we dissected the molecular mechanism of gene expression noise by targeted decondensation of gene loci.

a. Fraser LCR, Dikdan RJ, Dey S, Singh A, Tyagi S. Reduction in gene expression noise by targeted increase in accessibility at gene loci. Proc Natl Acad Sci U S A. 2021 Oct 19;118(42) PubMed Central PMCID: PMC8545487.
b. Shah K, Tyagi S. Barriers to transmission of transcriptional noise in a c-fos c-jun pathway. Mol Syst Biol. 2013;9:687. PubMed Central PMCID: PMC3792345.
c. Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. PLoS Biol. 2006 Oct;4(10):e309. PubMed Central PMCID: PMC1563489.

4. **Single-molecule FISH.** Improving on an earlier approach by Robert Singer, we developed a powerful method for detection of single mRNA molecules in situ. About 50 small oligonucleotide probes are used to bind to the same mRNA simultaneously which renders each molecule so intensely fluorescent that they can be seen as diffraction limited spots by a fluorescence microscope. We showed that this approach permits sensitive single cell gene expression analysis, not only via microscopy, but also by flow cytometry. The method has opened a new avenue for the study of cellular heterogeneity, RNA localization and processing and is currently used in hundreds of laboratories around the world as prelabeled probes sets have become commercially available (as Stellaris Probes from Biosearch Technologhies).

a. Marras SAE, Bushkin Y, Tyagi S. High-fidelity amplified FISH for the detection and allelic discrimination of single mRNA molecules. Proc Natl Acad Sci U S A. 2019 Jul 9;116(28):13921-13926. PubMed Central PMCID: PMC6628817.
b. Bushkin Y, Radford F, Pine R, Lardizabal A, Mangura BT, Gennaro ML, Tyagi S. Profiling T cell activation using single-molecule fluorescence in situ hybridization and flow cytometry. J Immunol. 2015 Jan 15;194(2):836-41. PubMed Central PMCID: PMC4350577.
c. Raj A, van den Bogaard P, Rifkin SA, van Oudenaarden A, Tyagi S. Imaging individual mRNA molecules using multiple singly labeled probes. Nat Methods. 2008 Oct;5(10):877-9. PubMed Central PMCID: PMC3126653.

5. **Intracellular Venues of mRNA Splicing.** Introns are usually removed co-transcriptionally soon after their synthesis, however, for alternatively spliced exons it is important to defer splicing until all the optional exons are synthesized. Through single molecule transcript visualization, it was shown that during regulated alternative splicing, splicing is uncoupled from transcription and occurs after the release of mRNA from the gene. Through live cell single-molecule imaging we showed that post-transcriptional splicing tends to occur at the periphery of nuclear speckles, sites where splicing factors are clustered.

    a. Vargas DY, Shah K, Batish M, Levandoski M, Sinha S, Marras SA, Schedl P, Tyagi S. Single-molecule imaging of transcriptionally coupled and uncoupled splicing. Cell. 2011 Nov 23;147(5):1054-65. PubMed Central PMCID: PMC3245879.

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

Current Research Support

R01CA227291 Sanjay Tyagi (PI) 02/16/2018 – 01/31/2023
Background free amplified single-molecule FISH for in situ and flow cytometric applications

Major completed Research Support

NIH 1R01AI106036-01 Sanjay Tyagi, Yuri Bushkin, Marila Gennaro (co-PIs) 09/07/2012 – 08/31/2017
Rapid Analysis of Single T Cell Immunity Signatures in Tuberculosis

R01 MH079197-01 Sanjay Tyagi (PI) 07/03/07-05/31/12
Imaging the Transport of Individual mRNA Molecules to the Active Synapses

R03 AI072105-01 Sanjay Tyagi (PI) 07/15/07-06/30/09
High throughput PCR assays for diagnosing tuberculosis

R01 GM070357-01 Sanjay Tyagi (PI) 04/01/04-03/31/08
Visualizing the Movement of mRNAs in Living Cells

R01 ES010536-01 Sanjay Tyagi (PI) 09/30/99-09/29/02
Detecting mRNAs in Living Cells with Molecular Beacons