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RNA Society Awards Committee

Dear Awards Committee members,

Re: The RNA Society Outstanding Career Researcher Award (Member Number 6016)

My name is Narasimhan Sudarsan and I am currently a Research Specialist at the Howard Hughes Medical Institute in Professor Ronald Breaker's laboratory at Yale University. I enclose materials in application for the RNA Society Outstanding Career Researcher Award.

I am grateful for your consideration of my application.

Yours sincerely,
Narasimhan Sudarsan

Summary of Research Accomplishments (Member 6016)

The expertise in RNA biochemistry that I acquired upon coming to Yale, combined with my extensive previous experience in microbial genetics, yielded me a unique perspective for furthering the Breaker lab's efforts in discovering new classes of non-coding RNAs, notably riboswitches, and validating these structures. In this position, I have solved the function of this new class of metabolite-sensing RNAs, through leading individual and team projects, as well as making significant technical and intellectual contributions in other efforts led by other members of the lab.

Over the years, our lab has identified many classes of conserved RNA structures through bioinformatic approaches. These structures are a novel group of putative riboswitches and ribozymes and include several RNAs that possibly function to perform complex biochemistry. In my role as a research scientist, I have worked on individually developed projects towards solving these novel RNA motifs, as well as mentored graduate students by troubleshooting technical issues, providing advice regarding experimental design and guiding them to the successful completion of their projects.

Our earliest study on riboswitches was on the first natural aptamer involved in gene control to be discovered was the adenosylcobalamin-sensing RNA that regulates B12 transport in *E. coli*. For this study, I led the genetic experiments needed to show the regulation in response to cobalamin, with various mutations on the RNA, to have a structure model in complement with the biochemical demonstration of directing binding shown by the graduate student on the project. This was especially vital as we did not have a phylogeny derived structure from bioinformation at the time.

These initial discoveries helped me in future studies throughout the course of my career. Some of the most significant research studies include following. I completed the initial discovery and validation of metabolite-sensing RNAs in eukaryotes, by identifying these elements bioinformatically in plant and fungal genomes and subsequently verifying the RNA-thiamine pyrophosphate interaction. Thus, far, this is first and only riboswitch class to be discovered in eukaryotes. I led the project that resulted in the first examples of tandem riboswitch architectures, where I had predicted that certain mRNAs should harbor tandem sensing domains for different metabolites and would perform complex genetic control. Based on my predictions, bioinformatics was employed to find the sam-adocobalamin tandem. This work led to new studies on more complex riboswitch architectures, including one of my studies on the first natural example of an allosteric group I intron ribozyme controlled by c-di-GMP. My work on the identification of c-di-GMP riboswitch solved the mechanism of gene control by which c-di-GMP regulates, which had been unknown for almost two decades. This work helped identify additional class of second messenger sensing riboswitches for c-AMP-GMP and c-di-AMP. Furthermore, I have also worked on the discovery of new RNA sensors and identification of their cognate ligands, which has revealed new biology. For example, when we identified the fluoride-sensing RNA, we had understood that biology has a well-developed fluoride toxicity mitigation system and the associated proteins are conserved in all three domains of life.

Narasimhan Sudarsan, Ph.D.

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Education

Ph.D., Biotechnology
Madurai Kamaraj University, Madurai, India
Dr. Vaithilingam Sekar, Advisor
Thesis: Molecular cloning of ICP gene *cryIA(c)* from *Bacillus thuringiensis* in to a phyllosphere colonizing *Bacillus megaterium*

M.Sc., Biotechnology
Madurai Kamaraj University, Madurai, India
Dr. R. Jayaraman, Advisor
Thesis: Intergenic suppression of the *fitA76*(ts) mutation by a rifampicin resistant *rpoB* allele in *Escherichia coli*

Research & Professional Experience

Howard Hughes Medical Institute Research Specialist 2007 - Present
Yale University, Department of Molecular, Cellular, and Developmental Biology
Dr. Ronald Breaker, HHMI Investigator

- Study of long non coding RNA (*ole/oap)* in *B. halodurans* sporulation
- Identification and study of c-AMP-GMP riboswitches in bacteria
- Genetics of c-di-AMP riboswitch in *B. subtilis*
- Genetics of the *crcB*/fluoride regulon, and fluoride riboswitch in bacteria
- Identification and characterization of c-di-GMP riboswitches in eubacteria
- c-di-GMP and RNA biology in *Clostridium difficile*
- Mentoring doctoral students and advising postdoctoral associates in their projects

Associate Research Scientist 2004-2007
Yale University, Department of Molecular, Cellular and Developmental Biology
Dr. Ronald Breaker, Mentor

- Focus: Identification of new riboswitches and other regulatory RNA elements
- Led a team that demonstrated that riboswitches could be a novel class of antimicrobial drug target. This project helped launch a start up biotech company (BioRelix) for developing new class of antibiotics
- Identified and studied riboswitches with tandem architectures that perform complex gene control functions
- Characterized riboswitch-targeting mechanism for antibacterial thiamin and lysine analogs
- Mentored doctoral and undergraduate students toward successful completion of riboswitch projects
- Advised on vector design, growth conditions, and microbial genetics of various bacteria

Post-doctoral Research Associate 2000-2003
Yale University, Department of Molecular, Cellular, and Developmental Biology
Dr. Ronald Breaker, Mentor

- Focus: *In vitro* selection of allosteric ribozymes and characterizing novel RNA elements
- Designed experiments to validate the detection of biological samples by immobilized RNA switches (*in vitro* selected allosteric ribozymes)
- Involved in the first project that confirmed the existence of riboswitches and identified the first amino acid sensing riboswitch (lysine riboswitch)

Post-doctoral Fellow 1998-1999
University of Nebraska, Lincoln, School of Biological Sciences
Dr. Kenneth Nickerson, Advisor

- Focus: Construction of cDNA library from the lepidopteran insect *Manduca sexta* and to clone the bacterial toxin specific receptor.
- Proposed and examined the role of the ClpP protease in detergent resistance in bacteria

Golda-Meir Fellow 1996-1998
Hebrew University of Jerusalem, Hadassah Medical School
Dr. Hanna Engelberg-Kulka, Advisor
Studied toxin-antitoxin module in *E. coli*

Doctoral studies 1989-1996
Madurai Kamaraj University, School of Biotechnology
Dr. Vaithilingam Sekar, Advisor

- Focus: Develop tools for pest management in cotton using bacterial toxins
- Isolated a phyllosphere colonizing *Bacillus megaterium* that could colonize malvaceous plants including cotton
- Cloned the *cryIA(c)* gene from *Bacillus thuringiensis var kurstaki* in to the phyllosphere colonizing *Bacillus megaterium* and studied the efficacy of control of Heliothis army worms in cotton plants
- Developed techniques for transformation and tracking of the isolated bacterium in the environment

Publications

1. White, N, Sadeeshkumar, H, Sun, A, **Sudarsan, N**, Breaker, RR. (2021) Lithium-sensing riboswitch classes regulate expression of bacterial cation transporter genes. (Submitted)

2. White, N, Sadeeshkumar, H, Sun, A, **Sudarsan, N**, Breaker, RR. (2021) A bacterial riboswitch class for Na+ regulates genes for ion transport, osmoregulation and ATP production. (Submitted)

3. Sherlock, ME, **Sudarsan, N**, Breaker, RR. (2018) Riboswitches for the alarmone ppGpp expand the collection of RNA-based signaling systems. **Proc Natl Acad Sci USA.** Jun 5;115(23):6052-6057.

4. Sherlock, ME, **Sudarsan, N**, Stav, S, Breaker, RR. (2018) Tandem riboswitches form a natural Boolean logic gate to control purine metabolism in bacteria. **Elife.** Mar 5; 7: e33908.

5. Nelson, JW, **Sudarsan, N,** Phillips, GE, Stav, S, Lunse, CE, McCown, PJ, Breaker, RR. (2015) Control of bacterial exoelectrogenesis by c-AMP-GMP. **Proc Natl Acad Sci USA.** Apr 28;112(17):5389-94. Equal contribution with the first author.

6. Nelson, JW, **Sudarsan, N**, Furukawa, K, Weinberg, Z, Wang, JX, Breaker, RR. (2013) Riboswitches in Eubacteria sense the second messenger c-di-AMP. **Nat Chem Biol**. **9(12): 834-39.**

7. Furukawa, K, Gu, H, **Sudarsan**, **N**, Hayakawa, Y, Hyodo, M and Breaker, RR. (2012) Identification of ligand analogues that control c-di-GMP riboswitches. **ACS Chem Biol. 7(8): 1436-43.**

8. Baker, JL, **Sudarsan, N**, Weinberg, Z, Roth, A, Stockbridge, RB ,and Breaker RR.(2012) Widespread genetic switches and toxicity resistance proteins for fluoride. **Science 335:** 233-35 Equal contribution with the first author**.**

9. Chen, AG, **Sudarsan, N** and Breaker, RR. (2011) Mechanism for gene control by a natural allosteric group I ribozyme. **RNA 17 (11)** 1967-72**.**

10. Meyer, MM, Hammond, MC, Salinas, Y, Roth, A, **Sudarsan, N** and Breaker, RR. (2011) Challenges of ligand identification for riboswitch candidates, **RNA Biol 8(1) 5-10**

11. Lee, ER, Baker, JL, Weinberg, Z, **Sudarsan, N** and Breaker RR. (2010) An allosteric self-splicing ribozyme triggered by a Bacterial second messenger. **Science 329:** 845-48

12. Lee, ER, **Sudarsan, N** and Breaker, RR. (2010) Riboswitches that sense c-di-GMP. In AJ Wolfe and KL Visick (ed.), The second messenger cyclic diguanylate, **ASM Press**, Washington, DC. pp. 215- 229. **Book chapter**

13. **Sudarsan, N,** Lee, ER, Weinberg, Z, Moy, RH, Kim, JN, Link, KH and Breaker, RR. (2008) Riboswitches in eubacteria sense the second messenger cyclic di-GMP. **Science 321:** 411-413

14. Weinberg, Z, Barrick, J, Yao, Z, Roth, A, Kim, J, Gore, J, Wang, J, Lee, E, Block, K, **Sudarsan, N,** Neph, S, Tompa, M, Ruzzo, W and Breaker RR (2007) Identification of 22 candidate structured RNAs in bacteria using the CMfinder comparative genomics pipeline. **Nucleic Acids Res**. 35: 4809-4819

15. Cheah, MT, Wachter, A, **Sudarsan, N** and Breaker, RR. (2007) Control of alternative RNA splicing and gene expression by eukaryotic riboswitches. ***Nature* 447:** 497-500

16. Blount, KF, Wang, X, Lim, J, **Sudarsan, N**, and Breaker, RR.(2007) Antibacterial compounds that target lysine riboswitches. ***Nat Chem Biol***. **3:** 44-49

17. **Sudarsan, N,** Hammond, MC, Block, K, Welz, R, Barrick, JE, Roth, A and Breaker, RR. (2006) Tandem Riboswitch architectures exhibit complex gene control functions. ***Science* 314:** 300-304

18. **Sudarsan, N,** Cohen-Chalamish, S, Nakamura, S, Emilsson, GM and Breaker, RR. (2005) Thiamine pyrophosphate riboswitches are targets for the antimicrobial compound pyrithiamine. ***Chem Biol* 12**: 1325-1335 (***Featured article of the issue***)

19. Barrick, JE, **Sudarsan, N,** Weinberg, Z, Ruzzo, W and Breaker, RR. (2005) 6S RNA is a widespread regulator of eubacterial RNA polymerase that resembles an open promoter. ***RNA* 11**:774-784

20. Barrick, JE, Corbino, KA, Winkler, WC, Nahvi, A, Mandal, M, Collins, J, Lee, M, Roth, A **Sudarsan, N,** Jona, I, Wickiser, JK and Breaker, RR. (2004) New RNA motifs suggest an expanded scope for riboswitches in bacterial genetic control. ***Proc Natl Acad Sci USA* 101**:6421-6426

21. **Sudarsan, N**, Wickiser, JK, Nakamura, S, Ebert, MS and Breaker, RR. (2003) An mRNA structure in bacteria that controls gene expression by binding lysine. ***Genes Dev* 17**:2688-2697

22. Winkler, WC, Nahvi, A, **Sudarsan, N**, Barrick, JE and Breaker, RR. (2003) An mRNA structure that controls gene expression by binding S-Adenosylmethionine. ***Nat Struct Biol* 10**:701-707

23. Breaker, RR, Emilsson, GM, Lazarev, D, Nakamura, S, Puskarz, I, Roth, A and **Sudarsan, N**. (2003) A common speed limit for RNA-cleaving ribozymes and deoxyribozymes. ***RNA* 9**:949-957

24. **Sudarsan, N**, Barrick, JE and Breaker, RR. (2003) Metabolite-binding RNA domains are present in the genes of eukaryotes. ***RNA*** **9**:644-647(**First information on the presence of riboswitches in higher organisms**)

25. Nahvi, A, **Sudarsan, N**, Ebert, MS, Zou, X, Brown, KL and Breaker, RR (2002) Genetic control by a metabolite-binding mRNA. ***Chem Biol*** **9**:1043-1049

26. Rajagopal, S, **Sudarsan, N** and Nickerson, KW. (2002) Sodium dodecyl sulfate hypersensitivity of *clpP* and *clpB* Mutants of *Escherichia coli.* ***Appl Environ Microbiol*** **68**:4117-4121

27. Seetharaman, S, Zivarts, M, **Sudarsan, N** and Breaker, RR (2001) Immobilized RNA Switches for the analysis of complex chemical and biological mixtures. ***Nat Biotechnol*** **19**:336-341 (***Cover Story***)

28. Engelberg-kulka, H, Reches, M, **Narasimhan, S**, Schoulaker-Schwartz, R, Klemes, y, Aizenman, E and Glaser, G. (1998) *rexB* of bacteriophage lambda is an anti-cell death gene. ***Proc Natl Acad Sci USA*** 95:15481-15486 (First name and last name got reversed by mistake. Hence Narasimhan S is Sudarsan N)

29. **Sudarsan, N**, Suma, NR, Vennison, SJ and Sekar, V. (1994) Survival of a strain of *Bacillus megaterium* carrying a lepidopteran-specific gene of *Bacillus thuringiensis* in the phyllosphere of various economically important plants. ***Plant Soil*** **167**:321-324

Patents

- Breaker, RR, Nahvi, A, **Sudarsan, N**, Ebert, MS, Winkler, WC, Barrick, JE and Wickiser, JK (2002) Riboswitches, Methods for their use, and compositions for use with riboswitches. 03781294.8-2101-US0329589
- Breaker, R, Blount, K, Wang, X, Lim, J, and **Sudarsan, N**. (2006) Antibacterial lysine analogs that target lysine riboswitches. Provisional Application.

Memberships and Honors

- **Invited Speaker:** DIMACS Conference on Effects of Genome Structure and Sequence on the Generation of Variation and Evolution. Rutgers University. New Brunswick NJ August **2011.** Metabolite-dependent alteration of RNA folding, splicing and function
- **Invited Speaker:** FASEB meeting on Mechanisms and regulation of Prokaryotic transcription conducted at Saxtons River Vermont. June **2009**. Transcription regulation by c-di-GMP sensing riboswitches.
- **Invited Speaker:** Microbiology Symposium: United States Military Academy West Point NY Nov **2008**: Genetic Regulation by Riboswitches.
- **Invited Speaker:** Genetic regulation by riboswitches. National Center for biological Sciences. Bangalore India Sep **2007**
- **Invited Speaker**: FASEB meeting on Post-transcriptional Control of Gene Expression: Effectors of mRNA Decay Conducted in Tuscon Arizona. July **2004**. Role of Riboswitches in Post-Transcriptional control of Gene Expression
- **Federation of American Societies for Experimental Biology (FASEB) Award:** Best poster award based on Scientific Merit at the meeting on Post-transcriptional Control of Gene Expression: Effectors of mRNA Decay conducted in Tuscon Arizona July 6-11 **2002.**
- **Golda-Meir Postdoctoral Fellowship Award: From the Golda Meir Foundation,** (**1996**) One of the 17 recipients of the international award in that year

Conference Presentations

- An mRNA structure that regulates gene expression by sensing L-lysine inside the Cell
  Sudarsan, N, Wickiser, JK, Ebert, MS and Breaker, RR
  Gordon Research Conference meeting on Nucleic Acids held at Newport RI, June 2003

- Riboswitches as new targets for antimicrobial drug development
  Sudarsan,**N**, Barrick, JE, Cohen-Chalamish, S, Ebert. MS, Mandal, M
  Nahvi, A, Wickiser, JK, Winkler, WC, Emilsson, GM and Breaker, RR
  Gordon Research Conference on "Chemical & Biological Terrorism Defense" held at Buellton CA, March 23-28, 2003

- Genetic control by a metabolite-binding RNA
  Nahvi, A, Sudarsan, N, Ebert, MS, Zou, X, Brown, KL and Breaker, RR
  FASEB Summer Research on Post-Transcriptional Control of Gene Expression: Effectors of mRNA Decay held at Tuscon AZ July 2002

- Metabolite-sensing riboswitches control fundamental biochemical pathways in bacteria
  Nahvi, A, Winkler, WC, **Sudarsan, N**, Barrick, JE and Breaker, RR
  Annual meeting of the RNA society held at Vienna, Austria July 2003

- Oligonucleotide sensitive hammerhead ribozymes as logic gates
  Wickiser, JK, Cohen-Chalamish, S, Sudarsan, N, Sawichi, B, Ward, DC and Breaker, RR
  International Meeting on DNA based Computer held at Hokkaido University, Japan, June 2002

Teaching Experience

- Trained undergraduate and graduate students in the Breaker laboratory at Yale University in RNA biochemistry and microbial genetics.