BIOGRAPHICAL SKETCH

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NAME: Gu, Guoping (Sam)

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

POSITION TITLE: Associate 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)*	Completion Date MM/YYYY	FIELD OF STUDY
Peking University, Beijing, China	B.S.	07/1999	Biotechnology
University of California, Santa Cruz, California, USA	Ph.D.	12/2006	Molecular, Cell, and Developmental Biology
Stanford University, Palo Alto, California, USA	Postdoctoral	07/2012	Molecular Biology and Genomics

A. Personal Statement

My scientific passion is to characterize the underlying mechanism of RNA-mediated chromatin regulation and to explore the novel mechanisms of transgenerational epigenetic inheritance (TEI). My current work focuses on the *C. elegans* nuclear RNAi pathway, an evolutionarily conserved epigenetic silencing pathway that acts against transposons and foreign DNA. The defining features of this pathway are small RNA-guided chromatin modification and transcriptional repression at a target gene. In *C. elegans*, nuclear RNAi is essential for genome stability and promotes germline immortality under heat stress. Nuclear RNAi can be conveniently triggered by feeding worms with dsRNA, which leads to heritable silencing at the target gene for multiple generations. These features, together with powerful genetics and genomic tools and a three-day reproductive cycle, make *C. elegans* a highly tractable system to pioneer understanding of TEI in animals.

My strengths that uniquely qualify me for this R35 application are listed below.

Training and experience. I have more than 20 years of research experience in studying non-coding RNA and chromatin biology in *C. elegans*. My research program was initiated from my postdoctoral work in Dr. Andrew Fire's lab, where I pioneered epigenomic studies and technology development for *C. elegans*. In collaboration with Dr. Scott Kennedy, we demonstrated that (1) an siRNA-mediated heterochromatin response in *C. elegans* occurs in a highly target-specific manner and (2) this response can last for multiple generations. This work is among a series of papers published in 2012 that established the *C. elegans* nuclear RNAi pathway as a leading system to study animal transgenerational epigenetics.

Productivity. I have published 9 corresponding-author papers since arriving at Rutgers in 2012. These works were all supported by my R01, which is my sole major funding source. These nine publications cover the broad topics of small RNA, chromatin, transcription, transgenerational epigenetics, and germline development. Such integrated approach is essential to study a highly complex pathway such as nuclear RNAi.

Expertise. In the past decade, my lab has acquired a diverse set of expertise in high-throughput sequencing methodologies for both RNA and chromatin analyses, protein biochemistry (particularly the histone methylase

enzymology), single-molecule and conventional fluorescence microscopy, and genomic and epigenomic editing. My expertise in RNA and chromatin biology has been sought by colleagues from US and foreign institutes, which resulted in six collaborative papers since 2012. We are currently assisting three collaborative projects using both *C. elegans* and mammalian systems. On the receiving end, I am also grateful that Dr. Simone Sidoli will continue to perform mass spec analysis for histone modifications. Dr. Sidoli is a world expert on this subject and is a coauthor of our 2029 eLife paper. His support letter is included in this application.

<u>*Innovations.*</u> Our published works in the past five years made numerous breakthrough discoveries, for example, a novel histone modification, its writer enzymes, and a new mechanism of siRNA regulation. These conceptual innovations identified several layers of previously unknown complexity in nuclear RNAi. My lab also made numerous technical innovations over the years including a revised RNA-seq protocol that was tailored for nuclear RNAi-targeted RNAs, applying auxin-induced degradation system in nuclear RNAi research, developing a customizable siRNA-generator system. Both our conceptual and technical innovations have significantly advanced our field of research.

<u>*Service.*</u> I served as an *ad hoc* reviewer on the NIH Molecular Genetics (MG) study section in June of 2022. I also served as a peer reviewer for *Science, Nature, PLoS Genetics, Molecular Cell, Genes&Development, Genetics, Development, eLife, PNAS, Nucleic Acid Research, Epigenetics and Chromatin, and Cell Report*.

Please note the I have published all my research products under the name “Sam Guoping Gu”.

In summary, the R35 grant is well suited to my research program, which carries a balanced portfolio of well-defined questions and explorative pursuits of unknowns in the field of non-coding RNA and epigenetics. Supported by our past achievements and expertise, as well as the generous local and broader scientific network, I am confident that my lab is in an excellent position to accomplish both the short and long-term goals proposed in this application.

Research products that highlight my experience and qualifications for this project:

1. Gajic Z, Kaur D, Ni J, Zhu Z, Zhebrun A, Gajic M, Kim M, Hong J, Priyadarshini M, Frøkjær-Jensen C, **Gu S**. Target-dependent suppression of siRNA production modulates the levels of endogenous siRNAs in the *Caenorhabditis elegans* germline. ***Development.*** 2022 Aug 15;149(16). PMID: 35876680; PMCID: PMC9481970
   Funding: NIH NIGMS R01GM111752 to **Gu SG**., KAUST OSR-CRG2019-4016 to C.F.J.

2. Schwartz-Orbach L, Zhang C, Sidoli S, Amin R, Kaur D, Zhebrun A, Ni J, **Gu SG**. *Caenorhabditis elegans* nuclear RNAi factor SET-32 deposits the transgenerational histone modification, H3K23me3**.** ***eLife.*** 2020 Aug 17. PMID: 32804637; PMCID: PMC7431132.
   Funding: NIH NIGMS R01GM111752 to **Gu SG**., Rutgers Busch Biomedical Grant to **Gu SG.**, New Jersey Commission on Cancer Research, DCHS19PPC030 to Schwartz-Orbach L

3. Kalinava N, Ni JZ, Gajic Z, Kim M, Helen Ushakov, and **Gu SG**. *C. elegans* Heterochromatin Factor SET-32 Plays an Essential Role in Transgenerational Establishment of Nuclear RNAi-Mediated Epigenetic Silencing. ***Cell Reports***. 2018 Nov 20;25(8):2273-2284. PMID: 30463021; PMCID: PMC6317888
   Funding: NIH NIGMS R01GM111752 to **Gu SG**.

4. Ni JZ, Kalinava N, Chen E, Huang A, Trinh T, **Gu SG**. A transgenerational role of the germline nuclear RNAi pathway in repressing heat stress-induced transcriptional activation in *C. elegans*. ***Epigenetics Chromatin.*** 2016;9:3. PMID: 26779286; PMCID: PMC4714518.
   Funding: NIH NIGMS R01GM111752 to **Gu SG**., Rutgers Busch Biomedical Grant to **Gu SG.**

B. Positions, Scientific Appointments, and Honors

<u>Positions and Employment</u>
2019-present Associate Professor, Molecular Biology and Biochemistry Department, Rutgers University, NJ

2012-2019 Assistant Professor, Molecular Biology and Biochemistry Department, Rutgers University, NJ
2007-2012 Fellow, Department of Pathology, Stanford University School of Medicine, CA
2006-2007 Fellow, Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA

Other Experience and Professional Memberships

2017-now Member, Genomic Instability and Cancer Genetics Research Program, Rutgers Cancer Institute of New Jersey
2013-now Member, Genetics Society of America
2013-now Member, RNA Society
2013-now Mentor for the School of Arts and Sciences Honors Program

C. Contributions to Science

1. **The establishment and maintenance phases of nuclear RNAi have distinct genetic requirement**
Background: The epigenetically repressed states both have to be *initiated* and then *maintained*. Published work has largely focused on the inheritance or maintenance aspects of this pathway. The transient nature of the establishment phase makes it difficult to delineate the molecular events and genetic requirement. Central findings: We demonstrated that the establishment and maintenance of nuclear RNAi are two distinct processes with different genetic requirements. The rate-limiting step of the establishment phase is a transgenerational, chromatin-based process. The heterochromatin factor SET-32 is critical for silencing establishment. Influence of the work: Our experimental design establishes a powerful approach to address temporal mechanisms in chromatin dynamics. Identifying the bottleneck step provides critical insight into the regulation of this pathway. The phenotype of establishment defect has not been observed for any other genes in the nuclear RNAi pathway and constitutes a novel functional assignment in a conserved player. **This work also prompted us to investigate the enzymological property of SET-32, which led to the discovery of a novel histone modification involved in RNAi (see #3 in this section).** My specific roles: corresponding author.

   Kalinava N, Ni JZ, Gajic Z, Kim M, Helen Ushakov, and **Gu SG**. *C. elegans* Heterochromatin Factor SET-32 Plays an Essential Role in Transgenerational Establishment of Nuclear RNAi-Mediated Epigenetic Silencing. ***Cell Reports***. 2018 Nov 20;25(8):2273-2284. PMID: 30463021; PMCID: PMC6317888
   Funding: NIH NIGMS R01GM111752 to **Gu SG**.

2. **The spatial and temporal dynamics of the nuclear RNAi-targeted RNA transcripts in *C. elegans***
Background: A well-recognized paradox of nuclear RNAi is that the target locus has to be expressed in order to maintain its transcriptional silencing state. This paradox is the key for the transgenerational reinforcement of epigenetic silencing memory. However, the underlying mechanism in *C. elegans* was unknown due to the lack of the fundamental knowledge of (1) when and where the nuclear RNAi silencing occurs and (2) when and where the transcriptional activation occurs in this organism. Central findings: We found that the transcription and silencing cycle of endogenous nuclear RNAi targets is tightly coupled with the early embryogenesis and germline mitotic and meiotic cell cycles. Transcriptional activation and silencing of endogenous targets occur in the same cells type at the same developmental stages in *C. elegans*, suggesting a negative-feedback loop model. RNA transcripts of the endogenous targets accumulate in the nucleus and are largely depleted in the cytoplasm. This striking feature of nuclear localization persists even when the targets are actively transcribed in the nuclear RNAi-defective mutants. Influence of the work: Our study provides the first characterization of the transcriptional and silencing dynamics of endogenous nuclear RNAi targets in an animal model. This was done at the single cell resolution throughout the reproductive cycle of *C. elegans*. Nuclear localization of the endogenous target transcripts suggests that they are associated with RNA processing defect, which may be mechanistically linked to “foreign” and “self” RNA recognition. My specific roles: corresponding author.

Ni JZ, Kalinava N, Galindo-Mendoza S, **Gu SG**. The spatial and temporal dynamics of the nuclear RNAi-targeted RNA transcripts in *Caenorhabditis elegans*. ***Development***. 2018 Oct 22;145(20) PMID: 30254142; PMCID: PMC6215403.
Funding: NIH NIGMS R01GM111752 to **Gu SG**.

3. **The discoveries of H3K23me3 as a novel nuclear RNAi-induced histone modification and SET-32, the first biochemically validated H2K23 histone methyltransferase (HMT) in any organism**
Background: Our 2017 paper (PMID: 28228846) made a surprising finding that the well-known heterochromatin mark H3K9me3 is completely dispensable for the maintenance of nuclear RNAi-mediated silencing. In addition, while three different histone methyltransferases (HMTs) are required for H3K9me3, they have distinct functions in nuclear RNAi. These findings made us wonder: (1) What other histone modifications are involved in this pathway and (2) What are the corresponding enzymes that deposit these marks. Central findings: Nuclear RNAi factor SET-32 deposits H3K23me3 *in vitro* and *in vivo*. Exogenous dsRNA induces transgenerational heritable H3K23me3 at target genes. H3K23me3 marks the endogenous targets of nuclear RNAi in *C. elegans* germline. The functional relationship between the two heterochromatin marks H3K23me3 and H3K9me3 is complex and dependent on the local genomic context. Influence of the work: H3K23me3 is conserved from plants to animals, but its function and regulation are largely elusive. Pubmed search of “H3K23 methylation” found only 22 papers, comparing to 2071 for H3K9 methylation and 1468 for H3K27 methylation (as of December 2022). The identification of the first biochemically validated H3K23 methyltransferase (SET-32) and its essential role in nuclear RNAi from this work marks a breakthrough of this understudied histone modification and therefore significantly advances the histone code theory. **This work also provides the foundational knowledge and experimental framework for the Section I of my R35 proposal.** My specific roles: corresponding author.

Schwartz-Orbach L, Zhang C, Sidoli S, Amin R, Kaur D, Zhebrun A, Ni J, **Gu SG**. *Caenorhabditis elegans* nuclear RNAi factor SET-32 deposits the transgenerational histone modification, H3K23me3**.** ***eLife.*** 2020 Aug 17. PMID: 32804637; PMCID: PMC7431132.
Funding: NIH NIGMS R01GM111752 to **Gu SG**., Rutgers Busch Biomedical Grant to **Gu SG.**, New Jersey Commission on Cancer Research, DCHS19PPC030 to Schwartz-Orbach L

4. **Developing a new transgenerational gene silencing technology in *C. elegans*** Background: In addition to dsRNA, piRNA can also induce robust transgenerational gene silencing in *C. elegans.* A customizable piRNA-induced gene silencing technology was a much-needed expansion of the *C. elegans* toolbox. Central findings: Dr. Christian Frøkjær-Jensen’s lab (corresponding author) and my lab in collaboration developed a transgene-based approach that enables the expression of synthetic piRNAs in *C. elegans* germline. The piRNA-mediated interference (piRNAi) is more efficient than RNAi, can be multiplexed and induce heritable gene silencing that can persist for four to six generations. Influence of the work: Due to its high efficiency, specificity, and scalability, piRNAi has become a powerful genetic tool to study small RNA, transgenerational epigenetics, and germline development of *C. elegans*. **piRNAi will be used as a key method in both Sections of this R35 proposal.** My specific roles: a collaborator, performed all ChIP-seq, small RNA-seq, and mRNA-seq experiments.

Priyadarshini M, Ni JZ, Vargas-Velazquez AM, **Gu SG**, Frøkjær-Jensen C. Reprogramming the piRNA pathway for multiplexed and transgenerational gene silencing in *C. elegans.* ***Nature Methods***. 2022. PMID: 35115715; PMCID: PMC9798472.
Funding: KAUST OSR-CRG2019-4016 to C.F.J., NIH NIGMS R01GM111752 to **Gu SG**., Rutgers Busch Biomedical Grant to **Gu SG.**

5. **Target-dependent suppression of siRNA production distinguishes self from non-self siRNAs in *C. elegans* germline** Background: It is well known that endogenous siRNAs (endo-siRNAs) produced from transposons and other repetitive DNA ensure the silencing of these regions in eukaryotic genomes. However, endo-siRNAs are not limited to these non-self DNA. For various reasons, endo-siRNAs are also produced against self-genes in different organisms including mammals. The self-gene targeting siRNAs can play a different function from the transposon-targeting siRNAs. In some conditions, the self-gene

targeting siRNAs can cause a run-away silencing and therefore need to be repressed. The existence of self-targeting and non-self-targeting endo-siRNAs, as well as their different fate and functions, raises a fundamental question: How do cells distinguish these two populations of endo-siRNAs? Central findings: (1) Germ cells use a sequence-homology based mechanism to distinguish self-targeting and non-self-targeting siRNAs. (2) The production of self-targeting siRNAs is repressed by the mRNAs of the target gene. (3) Normally, non-self-targeting siRNAs are allowed to accumulate in the absence of the target mRNA. But they are sensitive to siRNA suppression when abundant target mRNAs are provided. (4) siRNA suppression requires the P granules. (5) Ectopic siRNAs, when not suppressed, can silence the target gene. Influence of the work: Our findings explain why the abundance of self-targeting siRNAs is low but the non-self-targeting siRNAs high, and further increase the complexity of the relationship between mRNA and siRNAs: mRNA is the target of siRNA for RNAi; mRNA is also the template of siRNA synthesis by RNA-dependent RNA polymerase (i.e., mRNA is necessary for RNAi). On top of that, our work shows that mRNA can suppress homologous siRNAs. **This work also provides the foundational discovery and experimental framework for the Section II of my R35 proposal.** My specific roles: Corresponding author.

Gajic Z, Kaur D, Ni J, Zhu Z, Zhebrun A, Gajic M, Kim M, Hong J, Priyadarshini M, Frøkjær-Jensen C, **Gu S**. Target-dependent suppression of siRNA production modulates the levels of endogenous siRNAs in the *Caenorhabditis elegans* germline. ***Development.*** 2022 Aug 15;149(16). PMID: 35876680; PMCID: PMC9481970
Funding: NIH NIGMS R01GM111752 to **Gu SG**., KAUST OSR-CRG2019-4016 to C.F.J.

A full list of my publications can be found at:
https://www.ncbi.nlm.nih.gov/myncbi/sam.gu.1/bibliography/public/