## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Weifeng Gu

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

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 Health Science Center, China | M.D. | 07/1997 | Basic Medical Sciences |
| Peking University Health Science Center, China | M.S. | 07/2000 | Immunology |
| University of Rochester, NY | Ph.D. | 10/2005 | Biochemistry |
| University of Massachusetts Medical School, MA | Postdoctoral | 12/2013 | RNAi and Small RNAs |

### A. Personal Statement

The PI has been maintaining a solid record in RNA biology research with several achievements including: **1)** Identified the first enzyme which adds nucleotides in the 3' to 5' instead of 5' to 3' direction as described in the textbook; **2)** Made significant contributions to discovering the roles of $m^1A$ modifications on RNA CAG repeats in neurodegenerative diseases, as detailed below; **3)** Identified 22G-RNAs which regulate almost all genes in the germline cells of *C. elegans*; **4)** Dissected the biogenesis pathway and functions of piRNAs or 21U-RNAs; **5)** Dissected the biogenesis pathway and functions of 26G-RNAs; **6)** Discovered that influenza A virus prefers snatching caps from ncRNAs, especially U1 and U2 snRNAs, to generate viral mRNA; and **7)** Discovered the non-canonical cap-snatching which functions to diversify viral proteome. All these were recognized as breakthroughs in the PI's fields and were all published in top-tier biomedical journals. The PI has obtained broad expertise in RNA biology and genetics as well as advanced bioinformatics and programming skills. These skills have allowed the PI to collaborate with many scientists worldwide and established the PI as an expert in cloning and analyzing RNAs via high-throughput sequencing. Currently the PI focuses on three types of nuclear RNA modifications: 1) ppp-RNA dephosphorylation by PIR-1 related nuclear polyphosphatases including PIR-1 and PIR-2; 2) $m^5C$ modifications on piRNAs or 21U-RNAs; and the differential usage of $m^7G$ and $m^{2,2,7}G$ caps by influenza A virus. These studies will generate new tools for regulating gene expression and treating disease. In summary, the PI demonstrated a proven record of original and innovative research, and has the facility, expertise and motivation to successfully perform this project.

For promoting next-generation researchers especially those under-represented and underserved, the PI has recruited 40 undergraduates including half with minority backgrounds, one female graduate student, and two female postdocs. In addition, the PI recruited 8 high school students including 5 female students. The PI actively participated in scientific outreaching, including presentations and talks at local elementary and high schools which are minority-serving. The PI served as a judge 8 times in California Science & Engineering Fair and once in Riverside Science Fair. At UCR, the PI served as Admission Chair for the GGB graduate program for five years. In addition, the PI served as the Chair of the CMDB graduate program. The PI served in three UCR Senate committees and one committee at the state level.

The PI also actively participated in the activities of the scientific communities, serving as poster judges twice in the international RNA and *C. elegans* meetings and fostering young investigators.

(*** first authorship**).

**a.** **Gu, W.***, Lee, H., Chaves, D., Youngman, E.M., Pazour, G.J., Conte, D.J., Mello, C.C. (2012). CapSeq and CIP-TAP map Pol II start sites and reveal capped-small RNAs as *C. elegans* piRNA precursors. ***Cell***, 151,1488–1500. PMCID: PMC3581324.

**b.** Chaves D.A., Dai H., Li L., Moresco J.J., Oh M.E., Conte D. Jr., Yates J.R. 3rd, Mello C.C., **Gu W**. (2021). The RNA phosphatase PIR-1 regulates endogenous small RNA pathways in C. elegans. ***Mol Cell,*** 81(3):546-557.e5. PMID: 33378643. **Grant No.: R01GM124349.**

**c.** Li L., Dai H., Nguyen A.P., Hai R., **Gu W**. (2020). Influenza A virus utilizes noncanonical cap-snatching to diversify its mRNA/ncRNA. ***RNA***, 26(9):1170-1183. PMID: 32444459

**d.** **Sun, Y., Dai, H,** Dai, X., Yin, J., Cui, Y., Liu, X., Gonzales, G., Yuan, J., Tang, F., Wang, N., Perlegos, A.E., Bonini, N.M., Yang, X.W., **Gu, W**., Wang Y. (2023). $m^1A$ in CAG repeat RNA binds to TDP-43 and induces neurodegeneration. ***Nature*** 623:580-587. **Grant No.: R01GM124349. Dr. Yinsheng Wang and the PI's postdocs shared the first authorship**.

## B. Positions, Scientific Appointments, and Honors

### Positions and Employment

2021- Associate Professor, Dept. of Molecular, Cell & Systems Biology, Univ. of California, Riverside
2014-2020 Assistant Professor, Dept. of Molecular, Cell & Systems Biology, Univ. of California, Riverside

### Scientific Appointments and Professional Memberships

**Grant Review**: 2014 NSF MCB grant; 2017 NSF Career grant; 2018 NSERC Discovery grant (Canada); 2020 ISF grant (Israel); 2022 UCR Delfino grant; 2022 NIH MG Study Section; 2023 NIH VDT Study Section

**Ad hoc Manuscript Reviewer**: For RNA, Nucleic Acid Research, PLoS Genetics, Frontier Immunology, Nature Scientific Reports, etc, with >40 manuscripts since 2014

**Guest Editor:** 2021 PLoS Pathogen

**Associate Editor:** Frontiers in Immunology

**Membership:** RNA Society; Genetics Society of America

**Meeting Organization & Service:** 2019 SoCal RNA Symposium; 2015 International *C. elegans* workshop; poster judge in 2023 RNA meeting; poster judge in 2019 International *C. elegans* meeting

**Scientific Judge:** 2015-2018 and 2020-2023 (CSSF and later renamed as CSEF) in Microbiology, Mammalian Biology, etc; 2015 RIMS Inland Science and Engineering Fair

### Honors

1999 Kwang-Hua Scholarship, Kwang-Hua Education Foundation, Beijing, China
2004 Elon Huntington Hooker Fellowship, Univ. of Rochester, Rochester, NY
2009 Walter S. Bloor Award, University of Rochester, Rochester, NY
2016 Regents Faculty Fellowship, UC Riverside

## C. Contributions to Science

The below contributions are listed according to the publication time. **A Complete List of Publications in MyBibliography** is available from *https://www.ncbi.nlm.nih.gov/myncbi/weifeng.gu.1/bibliography/public/*

**1)** Previous studies indicated that $tRNA^{His}$ has a 5' Guanosine (G) which is added posttranscriptionally in *S. cerevisiae*. However, the functions of this G and the responsible enzyme were unknown. During my Ph.D. training, The PI identified Thg1p, which adds a Guanosine to $tRNA^{His}$ in 3' to 5' direction. This finding was highly innovative since Thg1p is the first enzyme which extends RNA in a reverse direction, as compared with

any RNA/DNA polymerase in the textbook. In addition, The PI developed the first RNA $m^5C$ mapping technique, the bisulfite sequencing.

**a.** **Gu, W.***, Jackman, J., Lohan, A.J., Grayhack, E.J., Phyzicky, E.M. (2003). $tRNA^{His}$ maturation: an essential yeast protein catalyzes addition of a guanine nucleotide to the 5' end of $tRNA^{His}$. ***Gene & Development***, 17(23), 2889-2901. PMCID: PMC289149.

**b.** **Gu W.***, Hurto R.L., Hopper A.K., Grayhack E.J., and Phizicky E.M. (2005). Depletion of *Saccharomyces cerevisiae* tRNA guanylyltransferase Thg1p leads to uncharged tRNA with additional $m^5C$. ***Molecular & Cellular Biology*** 25, 8191-8201. PMCID: PMC1234336

**2)** During the postdoctoral training, the PI was the first to systematically characterize the biogenesis and functions of 22G-siRNAs, especially in WAGO (worm specific Argonautes)-dependent pathway. The PI found that 22G siRNAs belong to two distinct small RNA pathways, based on the Argonaute proteins they bind: **1)** Argonaute WAGOs which target aberrant transcripts and also thousands of annotated protein coding genes, and **2)** Argonaute CSR-1 which targets thousands of functional genes in germline cells. Basically 22G-siRNAs target all worm genes expressed in the germline cells. This is the first work to demonstrate that RNAi can work in a genome-wide manner to regulate the expression of all genes in animals, thereby significantly broadening our understanding of RNAi. Later on the PI demonstrated that 22G-siRNAs also serve as effectors downstream other small RNA pathways, such as 26G siRNAs and 21Us. This 22G work basically laid the groundwork for many RNAi-related regulations in *C. elegans*.

**a.** **Gu, W.***, Shirayama, M.*, Conte, D.Jr.*, Vasale, J.J., Batista, P.J., Claycomb, J.M., Moresco, J.J., Youngman, E.M., Keys, J., Stoltz, M.J., Chen, C.C., Chaves, D., Duan, S., Kasschau, K.D., Fahlgren, N., Yates III, J.R., Mitani, S., Carrington, J.C., Mello, C.C. (2009). Distinct argonaute-mediated 22G-RNA pathways direct genome surveillance in the *C. elegans* germline. ***Molecular Cell***, 36(2), 231-244. PMCID: PMC2776052.

**b.** Claycomb, J.M.*, Batista, P.J.*, Pang, K., **Gu, W.**, Vasale, J.J., Van Wolfswinkel, J.C., Chaves, D., Shirayama, M., Mitani, S., Ketting, R.F., Conte, D.Jr., Mello, C.C. 2009. The Argonaute CSR-1 and its 22G-RNA cofactors are required for holocentric chromosome segregation. ***Cell***, 139(1), 123-134. PMCID: PMC2766185.

The PI collaborated with Dr. Hengchi Lee to study the function and mechanism of 21Us, and found that type 1 21Us may target many germline transcripts. Later on, the PI contributed to Dr. Shirayama Masaki's work, which together with other work indicated that type 1 21Us can initiate epigenetic memory of non-self RNAs. One intriguing question left is how 21Us are generated in *C. elegans*. The PI found that 21Us are made from capped small RNAs (csRNA). This work represented the first clear picture of how piRNAs are generated in any organism. And this was also the first work demonstrating that csRNAs generated during Pol II transcription initiation can serve as functional ncRNAs, as confirmed later in other organisms. In addition, the PI identified type 2 21Us. Unlike type 1 21Us, which are only expressed within two huge clusters on chromosome IV, type 2 21Us are expressed from all Pol II promoters such as those of mRNAs, and may be involved in gene regulation. These innovative findings significantly increased our understanding of how piRNAs function in animals, and were all published in top-tier biomedical journals.

**c.** Lee, H.*, **Gu, W.***, Shirayama, M., Youngman, E.M., Conte, D.Jr., Mello, C.C. (2012). *C. elegans* piRNAs mediate the genome-wide surveillance of germline transcripts. ***Cell***, 150(1), 78-87. PMCID: PMC3410639.

**d.** **Gu, W.***, Lee, H.*, Chaves, D., Youngman, E.M., Pazour, G.J., Conte, D.J., Mello, C.C. (2012). CapSeq and CIP-TAP map Pol II start sites and reveal capped-small RNAs as *C. elegans* piRNA precursors. ***Cell***, 151,1488–1500. PMCID: PMC3581324.

**3)** Influenza virus snatches RNA caps from host RNAs and then add the caps to viral RNAs, a process called cap snatching. In the past 30 years, all the studies have focused on host pre-mRNAs as the source of viral mRNA caps. The PI found that influenza virus A preferentially snatches the caps plus the following 11-13 nucleotides from non-coding RNAs (ncRNAs) and csRNAs, and then attach them to the 5' end of viral mRNAs. Together with David Bartel's lab, the PI found that U1 and U2 snRNAs contribute the most caps snatched by influenza virus A. In addition, the PI also demonstrated that ncRNAs contributed more than 50% caps snatched

by the virus, and csRNAs are snatched at much higher rate compared to that of mRNAs/pre-mRNAs. In all, this work basically changes the textbook view about the mechanism of cap-snatching and leads to a new direction in studying the roles of ncRNAs including csRNAs in cap snatching. Moreover, the PI recently discovered non-canonical IAV cap-snatching, which generates truncated mRNAs and ncRNAs using the internal initiation sites on viral template RNAs, diversifying the viral proteome. Non-canonical IAV cap snatching also prefers caps derived from cellular ncRNAs. The PI also found that a small fraction of viral template RNAs does not bear a 5' triphosphate group as the rest, but instead bears caps snatched from cellular mRNAs. This mechanism further diversifies the viral RNA transcriptome and may generate more viral toxicity.

**a.** **Gu, W.***, Gallagher, G.R., Dai, W., Liu, P., Li, R., Trombly, M.I., ammon, D.B., Mello, C.C., Wang, J.P., Finber, R.W. (2015). Influenza A virus preferentially snatches non-coding RNA caps. ***RNA*** 12, 2067-2075. PMID: 26428694***.*** **The PI was also the sole corresponding author*.***

**b.** Li L., Dai H., Nguyen A.P., Hai R., **Gu W**. (2020). Influenza A virus utilizes noncanonical cap-snatching to diversify its mRNA/ncRNA. ***RNA***, 26(9):1170-1183. PMID: 32444459.

**4)** In *C. elegans*, 26G-RNAs play important roles in regulating ~2,000 genes involved in spermatogenesis and embryogenesis. Previously the PI and others together defined two types of 26G-RNAs, one binding the Argonaute ERGO-1 in the embryos, and the other binding Argonaute ALG-3/4 in the male germline cells. The PI recently dissected the biogenesis pathway of 26G-RNAs and demonstrated how RNA polyphosphatase PIR-1 functions together with Dicer in this pathway. This is a novel mechanism for small RNA biogenesis since small RNAs generated in this manner are not completely phased as other Dicer products including endogenous siRNAs (22G-RNAs), and this 'semi-phased' mode generates much more diversities of small RNAs than the phased manner. Based on the biochemical activity of PIR-1, the PI also developed a convenient and sensitive method to clone ppp-RNAs using the PIR-1 treatment followed by high-throughput sequencing.

**a.** Chaves D.A., Dai H., Li L., Moresco J.J., Oh M.E., Conte D. Jr., Yates J.R. 3rd, Mello C.C., **Gu W**. (2021). The RNA phosphatase PIR-1 regulates endogenous small RNA pathways in C. elegans. ***Mol Cell,*** 81(3):546-557.e5. PMID: 33378643. **Grant No.: 5R01GM124349.**

**b.** Li L., Dai H., Nguyen A.P., **Gu W**. (2020). A convenient strategy to clone small RNA and mRNA for high-throughput sequencing. ***RNA***, 26(2):218-227. PMID: 31754076. **Grant No.: R01GM124349.**

**c.** Vasale, J.J.*, **Gu, W.***, Thivierge, C., Batista, P.J., Claycomb, J.M., Duchaine, T.F., Mello, C.C., Conte, D.Jr. (2010). Sequential rounds of RNA-dependent RNA transcription drive endogenous small-RNA biogenesis in the ERGO-1/Argonaute pathway. ***Proc Natl Acad Sci U S A***. Vol., 107(8), 3582-3587. PMCID: PMC2840456.

**d.** Conine, C.C.*, Moresco, J.J., **Gu, W.**, Shirayama, M., Conte, D.J., Yates III, J.R., Mello, C.C. (2013). Argonautes promote male fertility and provide a paternal memory of germline gene expression in *C. elegans*. ***Cell***, 155, 1532–1544. PMCID: PMC3924572.

**5)** Microsatellite DNA repeats, especially short nucleotide repeats, are strongly associated with several neurodegenerative diseases including Huntington's disease, spinocerebellar ataxia(SCA), amyotrophic lateral sclerosis (ALS). For example, the CAG repeat number is associated with the onset/progression and severity of Huntington's disease and SCA. TDP-43 is usually miss-localized from the nucleus to the cytoplasm and aggregated in the degenerated brain regions, thus serving as clinical hallmarks for these neurological diseases. Dr. Yingsheng Wang, the PI and others collaborated to investigate a key question left unanswered in the field, i.e., what initiates/causes TDP-43 mislocalization/aggregation. The key discovery is that the 'A' nucleotides in CAG repeat containing mRNAs are modified as $m^1A$, generating $Cm^1AG$. This modified trinucleotide repeat recruits TDP-43 to the cytoplasm, leading to TDP-43 mislocalization and aggregation. Then such aggregation causes neurological damage, as demonstrated using animal disease models. The PI's postdoc carried out the *in vivo* animal work using *C. elegans* with associated disease models. This research provides a significant insight into the etiology of several CAG-associated diseases and may lead to a new therapeutic strategy. **Dr. Yinsheng Wang and the PI's postdocs shared the first authorship**.

**a.** **Sun, Y., Dai, H,** Dai, X., Yin, J., Cui, Y., Liu, X., Gonzales, G., Yuan, J., Tang, F., Wang, N., Perlegos, A.E., Bonini, N.M., Yang, X.W., **Gu, W**., Wang Y. (2023). $m^1A$ in CAG repeat RNA binds to TDP-43 and induces neurodegeneration. ***Nature*** 623:580-587. **Grant No.: R01GM124349.**