## BIOGRAPHICAL SKETCH

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NAME: Zhang, Zhiguo

eRA COMMONS USER NAME (credential, e.g., agency login): zhang1

POSITION TITLE: Professor, Institute for Cancer Genetics

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 |
|---|---|---|---|
| National University of Defense Technology, China | B.S. | 07/1988 | Applied Chemistry |
| Dalian Institute of Chemical Physics, China | M.S. (Equiv) | 10/1992 | Physical Chemistry |
| University of Utah, Salt Lake City, UT | Ph.D. | 10/1998 | Biochemistry |
| Cold Spring Harbor Lab, Cold Spring Harbor, NY | Postdoctoral | 10/1998-04/2003 | Biochemistry/Genetics |

### A. Personal Statement

I have a broad background in biochemistry, genetics, epigenetics and cancer epigenetics. The first long-term goal of our laboratory is to determine how chromatin states are inherited during S phase of the cell cycle and how this process when going awry, leads to genome instability. Specifically, we focus on elucidating nucleosome assembly pathways are regulated using a variety of systems including yeast, mouse embryonic stem cells and human cells. We have made major contributions to this field as outlined in “Contribution to Science”. Our second long-term goal is to determine how epigenetic changes drive tumorigenesis and drug resistance. My laboratory is one of the first laboratories that discovered how onco-histone mutations, H3K27M (found in diffuse intrinsic midline glioma (DMG)) and H3.3K36M (found in chondroblastoma and head and neck cancer), reprogram cancer epigenomes and drive tumorigenesis. Currently, we aimed at identification and characterization of epigenetic vulnerability of H3K27M cells.

Ongoing and pending projects I would like to highlight include:

1. R35 GM118015 (PI: Zhang, Z) 09/2016-08/2026
NIH/NIGMS
Title: Mechanisms of Epigenetic Inheritance.
Goals: The goal of this grant is to determine mechanisms by which epigenetically determined chromatin states are inherited during mitotic cell divisions in yeast and mammalian cells.
2. R01 NS132344-01 03/2023 -02/2028
NIH/NINDS
Title: Roles of chromatin remodeler CHD2 in diffuse midline glioma with onco-histone mutations
Goals: We will determine molecular mechanisms underlying the dependence of H3.1K27M DIPG cells on CHD2 chromatin remodeler and the function of CHD2 in the regulation of the interactions between neurons and tumor cells.

**Pending:**

3. R01 CA277605-01A1 07/2003-08/2028
Title: **Epigenetic dependence of diffuse midline glioma with H3K27M mutation**
Goals: We will elucidate molecular mechanisms underlying the dependence of H3K27M DIPG cells on Brg1,

the catalytic subunit of mammalian SWI/SNF chromatin remodeling complex and identifying key proteins that work with Brg1 in the regulation of gene expression and cell fitness of DIPG cells.

**Selected reviews**

a. Zhang X and **Zhang Z**. Onco-histone mutations in Diffuse Intrinsic Pontine Glioma (2019). **Trends in Cancer** 5: 799-808. PMCID: PMC698369

b. Serra-Cardona A and **Zhang Z**. DNA-replication coupled nucleosome assembly in the passage of epigenetic information and cell identity (2018). **Trend in Biochemical Science (TIBS)** 43:136-148. PMICD PMC5805396.

**B. Positions, Scientific Appointments, and Honors**

Positions

| | |
|---|---|
| 2023-present | Clyde and Helen Wu Professor of Epigenomics and Molecular Biology |
| 2016-present | Professor of Epigenomics and Molecular Biology in the Department of Pediatrics Genetics and Development, Institute for Cancer Genetics, Columbia University Irving Medical Center |
| 2012-2016 | Professor, Department of Biochemistry and Molecular Biology, Mayo Clinic Rochester, Rochester, MN |
| 2008-2012 | Associate Professor, Department of Biochemistry and Molecular Biology, Mayo Clinic Rochester, Rochester, MN |
| 2003-2008 | Assistant Professor, Department of Biochemistry and Molecular Biology, Mayo Clinic Rochester, Rochester, MN |
| 1988-1989 | Research Technician, Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian, China |

Scientific Appointment and Profession Memberships

| | |
|---|---|
| 2017-present | Member and Vice Chair of International Affairs Committee, Biophysical Society of China |
| 2016-present | Member, New York Academy of Sciences |
| 1998- | Member, American Association for the Advancement of Science |
| 1998-present | Member, American Society for Biochemistry and Molecular Biology |
| 2013, 2019-2020 | Member, Society for NeuroOncology |

Grant review services

| | |
|---|---|
| 2021 | Ad hoc member, NCI PO1 reviewing |
| 2018-2022 | Regular member of the NCSD (now CSF1) study section |
| 2005, 2006, 2009, 2018 | Ad hoc Grant Reviewer for National Science Foundation |
| 2006-2017 | Ad hoc grant reviewer for multiple panels each year at NIGMS and NCI |

Other services

| | |
|---|---|
| 2014-present | Editorial board, Journal of Biological Chemistry |
| 2003-present | Ad hoc reviewer for eLife, Cancer Cell, Science, Mol Cell, Genes & Dev, PNAS, EMBOJ, Nature Communications, NAR, JCB, BBA etc. |

Honors

| | |
|---|---|
| 2009-2014 | Scholar, Leukemia and Lymphoma Society. |
| 1999-2002 | |

| | |
|---|---|
| 1998 | Postdoctoral Fellowship from Damon Runyon – Walter Winchell Cancer Research Foundation. |
| 1988 | James W. Prahl Award for Outstanding Graduate Student, University of Utah School of Medicine. |
| | Outstanding Graduate Award from the University of Defense Technology, China. |

**C. Contributions to Science**

We made the following key discoveries on the regulation of nucleosome assembly pathways, the first step in the inheritance of chromatin structures, and onco-histone mutations.

**1. Discoveries of genes involved in deposition of new H3-H4 (H3.1-H4) following DNA replication.** Following DNA replication, replicating DNA is assembled into nucleosome using both newly synthesized H3-H4 and parental histone H3-H4. We have discovered and comprehensively characterized, using a combination of biochemical, genetic and structural biology approaches, the functions of Rtt101, Rtt106 and Rtt109 in the regulation of nucleosome assembly of new H3-H4 in budding yeast. First, Rtt106 is a histone chaperone for H3-H4 and functions with CAF-1 to deposit new $(H3\text{-}H4)_2$ tetramers during S phase. Second, we discovered that Rtt109 is a histone acetyltransferase that utilizes the Asf1-H3-H4 complex as substrates for H3 lysine 56 acetylation (H3K56ac), a mark on new H3. In collaboration with Dr. Rui-Ming Xu's laboratory, we have determined the structure of Rtt109-Asf1-H3-H4 complex, revealing insight into how Rtt109 utilizes the Asf1-H3-H4 complex as substrates for acetylation of H3K56. Finally, we have demonstrated that the Rtt101-Mms1 E3 ligase ubiquitylates H3K56ac-H4 at H3K122, another mark on H3 we identified, and regulates the handoff of H3-H4 from Asf1 to CAF-1 and Rtt106 for nucleosome assembly. These studies have significantly increased our understanding of nucleosome assembly of newly synthesized H3-H4 during S phase of the cell cycle.

a. Han JH, Zhou H, Horazdovsky B, Zhang KL, Xu RM and **Zhang Z**. Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication (2007). **Science** 315:653-55. (This study was highlighted in Nature review Molecular and Cellular Biology). PMID: 17272723
b. Li Q, Zhou H, Wurtele H, Davies B, Horazdovsky B, Verreault A*, and **Zhang Z**.* Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly (2008). **Cell** 134:24455. (This study was highlighted in Cell). PMCID: PMC2597342.
c. Han J, Zhang H, Zhang H, Wang Z, Zhou H, and **Zhang Z**. A Cul4 E3 ubiquitin ligase regulates histone hand off during nucleosome assembly (2013). **Cell** 155:817-29. PMCID: PMC3994564
d. Zhang, L*., Serra-Cardona*, A., Zhou, H., Wang, M., Yang, N., **Zhang, Z#,** and Xu, R#. Multisite substrate recognition in Asf1-dependent acetylation of histone H3K56 by Rtt109 (2018). **Cell** 174: 818-830. (*Equal contribution; # co-corresponding authors). PMCID: PMC6103222.

**2. Discoveries of two conserved pathways that mediate the transfer of parental H3-H4 onto replicating DNA strands following DNA replication.** How parental H3-H4 tetramers, the primary carriers of epigenetic information, is transferred onto replicating DNA strands for nucleosome reassembly, was largely unknown. Using the eSPAN (**e**nrichment and **S**equencing of **P**rotein **A**ssociated **N**ascent strand) that can discern whether a protein is enriched at leading or lagging strands of DNA replication forks in budding yeast and mammalian cells we developed, we discovered that Dpb3-Dpb4 (POLE3 and POLE4 in mammalian cells), two subunits of leading strand DNA polymerase, Pol$\varepsilon$, function as a H3-H4 chaperone for the transfer of parental H3-H4 onto leading strand of DNA replication forks. On the other hand, the Mcm2-Ctf4-Pol$\alpha$ axis facilitates the transfer of parental H3-H4 onto lagging strands. Furthermore, these two pathways are conserved from yeast to mammalian cells. Finally, mouse ES cells deficient in these two pathways show defects in silencing of endogenous retro-viral elements (ERVs). These studies reveal insight into a pathway that has been puzzling scientists for over four decades.

c. Yu, C., Gan, H., Serra-Cardona, A., Zhang, L., Gan, S., Sharma, S., Johansson, E., Chabes, A., Xu, R.M. and **Zhang, Z**. A mechanism for preventing asymmetric histone segregation onto replicating DNA strands (2018). **Science**, 361,1386-1389. (This study was previewed in Science and recommended by faculty 1000). PMCID: PMC6597248.
d. Gan, H*., Serra-Cardona, A*., Zhou, H., Labib, K., Yu, C*. and **Zhang, Z*.** The Mcm2-Ctf4-Pol$\alpha$ Axis facilitates parental histone H3-H4 transfer to lagging strands (2018). **Mol Cell**, 72: 140-151. PMCID: PMC6193272.

c. Li Z, Hua X, Serra-Cardona A, Xu X, Gan S Zhou Z, Chen C Xu R and **Zhang Z.** DNA polymerase α interacts with H3-H4 and facilitates the transfer of parental histones to lagging strands (2020). **Science Advances**, 6, eabb5820. PMCID:PMC7449674.
d. Serra-Cardona A, Duan S, Yu C and **Zhang Z**. H3K4me3 recognition by the COMPASS complex guides the restoration of this mark following DNA replication (2022). **Science Advances**, 8: eabm6246 PMCID: PMC9075808.

**3. Mechanisms whereby nucleosome assembly of H3.3 is regulated.** In mammalian cells, canonical histones H3.1/H3.2 are assembled into nucleosomes in the DNA replication-coupled process. In contrast, histone H3 variant H3.3, differing from H3.1/H3.2 by four or five amino acids, is assembled into nucleosome through replication-independent nucleosome assembly pathway involving histone chaperones, HIRA and Daxx. Moreover, H3.3 regulates gene transcription, DNA repair and heterochromatin formation. It is believed that the diverse functions of H3.3 are regulated in part by proteins that assemble H3.3 into distinct chromatin domains. Therefore, it is very important to determine the regulation of nucleosome assembly of H3.3. We discovered that H4S47 is phosphorylated by the Pak2 kinase, and this modification and Pak2 promotes nucleosome assembly of H3.3. Moreover, Pak2 regulates cellular senescence and organismal aging. In addition, we also observed HIRA is modified by OGT, which in turn regulates nucleosome assembly of H3.3 during cellular senescence. Unexpectedly, we discovered that single-stranded DNA binding protein RPA, best known for its role in DNA replication and DNA repair, interacts with histone chaperone HIRA and facilitates the deposition of H3.3 at gene regulatory elements. Recently, we discovered pathways that mediate the nucleosome assembly of parental H3.3.

a. Kang B, Pu M, Hu G, Wen W, Dong Z, Zhao K, Stillman, B and **Zhang Z**. Phosphorylation of H4 serine 47 promotes HIRA-mediated nucleosome assembly (2011). **Genes Dev** 25:1359-64. PMCID: PMC3134079.
b. Lee J and **Zhang Z**. O-linked N-acetyglucosamine transferase (OGT) interacts with HIRA complex and regulates nucleosome assembly and cellular senescence (2016). **Proc. Natl. Acad. Sci. (USA)** 113: E3213-E3220. PMCID: PMC4988580.
c. Zhang H, Gan H, Wang Z, Lee J, Zhou H, Ordog T, Wold M, Ljungman M, **Zhang Z.** RPA Interacts with HIRA and Regulates H3.3 Deposition at Gene Regulatory Elements in Mammalian Cells (2017). **Mol Cell** 65:272-284. PMCID: PMC5460635
d. Xu X, Duan S, Hua X, Li Z, He R, and **Zhang Z.** Stable inheritance of H3.3-containing nucleosomes during mitotic cell divisions (2022). **Nature Communications**, 13: 2514.

4**. Coupling leading and lagging strand DNA synthesis under replication stress is a key role for the DNA replication checkpoint pathway.** DNA synthesis proceeds continuously at leading strands and discontinuously at lagging strands, with different proteins involved in leading and lagging strand DNA synthesis. Therefore, strand-specific information will allow us to gain insight into DNA synthesis in an unprecedented way. We developed three strand-specific sequencing methods, BrdU-IP-ssSeq that can measure relative amounts of leading and lagging strand DNA synthesis; ChIP-ssSeq that can reveal whether a protein binds ssDNA and dsDNA in cells; and eSPAN that can measure relative amount of proteins at leading and lagging strands. Using these methods, we discovered that PCNA is unloaded from lagging strands of DNA replication forks under replication stress and Rad53 regulates the PCNA unloading from lagging strands. Second, we found that coupling leading and lagging strand DNA synthesis under replication stress is a novel function of DNA replication checkpoint pathway. Mechanistically, we discovered that Rad53 functions in this pathway by the upregulation of dNTP levels, by attenuating the replication function of Mrc1-Tof1 complex and by unloading PCNA from lagging strands.

a. Yu C, Gan H, Han J, Zhou Z, Farrugia G, Ordog T, and **Zhang Z.** Strand-specific analysis shows distribution of proteins at replication forks and unloading of PCNA from lagging strands when forks stall (2014). **Mol Cell** 56:551-563. (This study is highlighted in Mol Cell and Faculty 1000). PMCID: PMC4362665
b. Gan H, Yu C, Devbhandari S, Sharma S, Han J, Chabes A, Remus D and **Zhang Z**. Checkpoint kinase Rad53 couples leading and lagging strand DNA synthesis under replication stress (2017). **Mol Cell** 68, 446-455. PMCID: PMC5802358.
c. Yu C, Gan H, and **Zhang Z**. Both DNA Polymerases epsilon and Delta Contact Active and Stalled Replication Forks differently (2017). **Mol Cell Biol**: e00190-17. PMCID: PMC5640813.

d. Serra-Cardona A, Yu C, Zhang X, Hua X, Yao Y, Zhou J, Gan H and **Zhang Z.** A mechanism for Rad53 to couple leading and lagging strand DNA synthesis under replication stress in budding yeast (2021). **Proc Natl Acad Sci USA** 118: E2109334118. PMID: 34531325.

**5. Molecular insights into onco-histone mutations and drug resistance.** It was reported that genes encoding H3.3 as well as H3.1 are mutated in high-grade pediatric brain tumors, giant cell tumor of bone and chondroblastoma. There are 13 genes encoding H3.1/H3.2, and two genes encoding H3.3. It was largely unknown how these histone mutations, occurring at one allele of one histone H3 gene, impact tumorigenesis. We discovered that H3.1K27M found in high-grade pediatric brain tumors dominantly reprograms H3K27 methylation, whereas H3.3K36M found in head and neck cancer and chondroblastoma rewires H3K36 methylation. Recently, we discovered that both H3.1K27M and H3.3K27M tumor cells depend on Brg1, the catalytic subunit of mammalian SWI/SNF chromatin remodeling complex. Finally, we discovered a novel MGMT enhancer that regulates MGMT expression and temozolomide resistance.

a. Chan KM, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, Gupta N, Mueller S, James CD, Jenkins R, Sarkaria J, and **Zhang Z.** The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression (2013). **Genes Dev** 27:985-90. PMCID: PMC3656328.
b. Fang D, Gan H, Lee J, Han J, Wang Z, Riester SM, Jin L, Chen J, Zhou H, Wang J, Zhang H, Yang N, Bradley EW, Ho TH, Rubin BP, Bridge JA, Thibodeau SN, Ordog T, Chen Y, van Wijnen AJ, Oliveira AM, Xu R, Westendorf JJ, **Zhang Z** (2016). The histone H3.3K36M mutation reprograms the epigenome of chondroblastomas (2016) **Science**, 352: 1344-1348. PMCID: PMC5460624.
c. Chen X, Zhang M, Gan H, Wang H, Lee JH, Fang D, Kitange GJ, He L, Hu Z, Parney IF, Meyer FB, Giannini C, Sarkaria JN, and **Zhang Z**. A novel enhancer regulates MGMT expression and promotes temozolomide resistance in glioblastoma (2018). **Nature communications** 9:2949. PMCID: PMC6063898. (in Editor's Highlights in Nature Communications).
d. Mo, Y., Duan, S., Zhang, X., Hua, X., Zhou, H., Wei, H., Watanabe, J., McQuillan, N., Su, Z., Gu, W., Wu, C., Vakoc, C. R., Hashizume, R., Chang, K., and **Zhang, Z**. Epigenome programing by H3.3K27M mutation creates a dependence of pediatric glioma on SMARCA4 (2022). **Cancer Discovery**, 12: 2906-29. PMCID: PMC9722525. This study along with an independent study from Drs. Filbin and Qi was spotlighted in Cancer Discovery.

**Complete List of Published Work in MyBibliography:**

https://www.ncbi.nlm.nih.gov/myncbi/zhiguo.zhang.1/bibliography/public/

## BIOGRAPHICAL SKETCH

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NAME: Junqing Kang

eRA COMMONS USER NAME (JUNQING):

POSITION TITLE: Postdoc

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 |
|---|---|---|---|
| Tsinghua University | PHD | 08/2023 | Epigenetics and biochemistry |
| Columbia University | PHD | | Epigenetics and biochemistry |

**A. Personal Statement**

**I studied epigenetics during transcription during my PHD and begin my study on epigenetics during DNA replication using biochemistry. I used to participate in some epigenetic protein or protein complexes' structure determination, including epigenetic protein-histone, protein-DNA, large epigenetic complexes. Currently, I am focus on DNA replication coupled parental histone transfer process, which is the most important step for epigenetic inherence and genome stability in every cell cycle and remains poorly understood.**