BIOGRAPHICAL SKETCH

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NAME: Louder, Robert

POSITION TITLE: Postdoctoral Fellow

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE	START DATE MM/YY	END DATE MM/YYYY	FIELD OF STUDY
Coastal Carolina University, Conway, SC	B.S.	08/2008	05/2012	Applied Physics
Coastal Carolina University, Conway, SC	B.S.	08/2008	05/2012	Biochemistry
University of California, Berkeley, CA	Ph.D.	08/2012	12/2017	Biophysics
Johns Hopkins University, Baltimore, MD	Postdoctoral Training	03/2018	Current	Biology/ Biophysics

A. Personal Statement

A common theme throughout my research career thus far has been a desire to develop fundamental principles that govern the complex processes underlying cellular function. Given that the most fundamental level of cellular processes is the atomic-scale interactions between molecules, I view structural biology as the prevailing approach towards a basic understanding of cells. My beginnings in an X-ray crystallography lab as an undergraduate played an important role in developing my passion for characterizing biomolecular structures, opening my eyes to the wealth of information that a structure can provide in terms of understanding how macromolecules function[1]. I then chose to carry out my Ph.D. research in the lab of Eva Nogales in order to learn how to use cryo-electron microscopy (cryo-EM) to characterize the structure and function of large macromolecular assemblies that are vital to cellular function but are too complex to study by other methods. In Eva's lab, I instantly developed a passion for the field of eukaryotic transcription, viewing it as a system that is fundamental to the regulation of cellular outcomes, whereby an immensely complex network of regulatory cues is integrated into a virtually singular output. My thesis work significantly advanced our understanding of the core eukaryotic transcriptional machinery, showing how the general transcription factor TFIID recognizes the promoter regions of genomic DNA and nucleates the assembly of the transcription preinitiation complex[2,3]. Additionally, due to the complexity and lability of transcription complexes, I amassed a wealth of experience in the application of cryo-EM towards the structural characterization challenging targets[4].

While my research in the Nogales lab was mainly focused on investigating the basic mechanisms of the core transcriptional machinery, I eventually developed an interest in understanding how the chromatin environment of genes regulates the process of transcription. Thus, I chose to carry out my postdoctoral research with Carl Wu at Johns Hopkins University, an expert in the field of chromatin biology with a sincere and well-demonstrated devotion towards understanding the relationships between gene expression and chromatin regulation. Here, I have to expanded upon my repertoire of skills by learning from the experts in biochemistry and chromatin available in the Wu lab, while also applying my own expertise in electron microscopy towards the characterization of chromatin structure.

1. McLellan JS, Pancera P, Carrico C, Gorman J, Julien J, Khayat R, **Louder R**, Pejchal R, Sastry M, Dai K, O'Dell S, Patel N, Shahzad-ul-Hussan S, Yang Y, Zhang B, Zhou T, Zhu J, Boyington JC, Chuang G, Diwanji D, Georgiev I, Kwon YD, Lee D, Louder MK, Moquin S, Schmidt SD, Yang Z, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Burton DR, Koff WC, Walker LM, Phogat S, Wyatt R, Orwenyo J, Wang L, Arthos J, Bewley CA, Mascola JR, Nabel GJ, Schief WR, Ward AB, Wilson IA, and

Kwong PD. (2011). Structure of HIV-1 gp120 V1V2 domain with broadly neutralizing antibody PG9. *Nature*, **480**:336-43. PMCID: PMC3406929.

2. **Louder RK**, He Y, López-Blanco JR, Fang J, Chacón P, Nogales E. (2016). Structure of promoter-bound TFIID and insight into human PIC assembly. *Nature*, **531**:604-09. PMCID: PMC4856295.
3. Patel AB[†], **Louder RK**[†], Fang J, Nogales E. (2018). Architecture of human TFIID and mechanism of promoter binding. *Science*, **362**: eaau8872. PMCID: PMC6446905. [†]Contributed equally.
4. Nogales E, **Louder RK**, He Y. (2016). Cryo-EM in the study of challenging systems: the eukaryotic transcription initiation process. *Curr Opin Struct Biol.*, **40**:120-127. PMCID: PMC5161697.

B. Positions and Honors

Positions

2008 - 2012	Summer Research Intern, National Institute of Allergy and Infectious Diseases, NIH
2012 - 2017	Graduate Student under Eva Nogales, Biophysics Graduate Group, UC Berkeley, CA
2018 -	Postdoctoral Fellow under Carl Wu, Dept. of Biology, Johns Hopkins University, Baltimore, MD
2020 -	EM advisor to the Integrated Imaging Center, Johns Hopkins University, Baltimore, MD

Honors

2008-2012	Presidential Merit and Dean's Excellence Scholarships, Coastal Carolina University
2012	Merit Award, National Institute of Allergy and Infectious Diseases, NIH
2012	Student of the Year Award, Department of Physics, Coastal Carolina University
2013 - 2015	Molecular Biophysics Training Program, National Institute for General Medical Sciences
2016	Young Investigator Life Sciences Award, Thermo Fisher Scientific Materials & Structural Analysis Division
2016 - 2017	Graduate Student Fellowship Award, California Cancer Research Coordinating Committee
2019 - 2022	Ruth L. Kirschstein National Research Service Award (NIH F32 Individual Fellowship)

C. Contributions to Science

1. <u>Structural basis of HIV-1 immune recognition (undergraduate research)</u>

The development of an effective HIV-1 vaccine is a paramount objective in global health research. The primary target for neutralizing antibodies against HIV-1 is the trimeric envelope glycoprotein spike (Env), thus a structural understanding of the mechanisms of antibody recognition of conserved "sites of vulnerability" on HIV Env is of critical importance in designing immunogens that elicit effective HIV neutralization responses in humans. Such is the goal of the Structural Biology Section of the Vaccine Research Center at the NIH, where I spent my summers as an undergraduate, assimilating the tenets of structural biology while working along-side top-contributors to the HIV-1 vaccine development effort. I solved multiple crystal structures of broadly neutralizing antibodies derived from HIV-infected patients who were resistant to the virus. By comparing these with other structures of similar antibodies in complex with their epitope on HIV Env, we uncovered a structural motif in the complementary-determining regions of this class of antibodies that explained their broadly neutralizing activity. A separate class of non-neutralizing antibodies recognizes another site of vulnerability on HIV Env that is only accessible upon binding to the cell-surface receptor CD4. By solving the crystal structure of an antibody in this class in complex HIV Env and CD4, I contributed to our understanding of how this class induces antibody-dependent cellular cytotoxicity that can result in immune protection without direct neutralization of the virus.

a. McLellan JS, Pancera P, Carrico C, Gorman J, Julien J, Khayat R, **Louder R**, Pejchal R, Sastry M, Dai K, O'Dell S, Patel N, Shahzad-ul-Hussan S, Yang Y, Zhang B, Zhou T, Zhu J, Boyington JC, Chuang G, Diwanji D, Georgiev I, Kwon YD, Lee D, Louder MK, Moquin S, Schmidt SD, Yang Z, Bonsignori M, Crump JA, Kapiga SH, Sam NE, Haynes BF, Burton DR, Koff WC, Walker LM, Phogat S, Wyatt R, Orwenyo J, Wang L, Arthos J, Bewley CA, Mascola JR, Nabel GJ, Schief WR, Ward AB, Wilson IA, and

Kwong PD. (2011). Structure of HIV-1 gp120 V1V2 domain with broadly neutralizing antibody PG9. *Nature*, **480**:336-43. PMCID: PMC3406929

b. Acharya P, Tolbert WD, Gohain N, Wu X, Yu L, Liu T, Huang CC, Kwon YD, **Louder RK**, Luongo TS, McLellan JS, Pancera M, Yang Y, Zhang B, Flinko R, Foulke JS Jr, Sajadi MM, Kamin-Lewis R, Robinson JE, Martin L, Kwong PD, Guan Y, DeVico AL, Lewis GK, and Pazgier M. (2014). Structural definition of an antibody-dependent cellular cytotoxicity response implicated in reduced risk for HIV-1 infection. *J Virol.*, **88**:12895-906. PMCID: PMC4248932

2. Structural and functional characterization of the eukaryotic transcription machinery

The general transcription factor TFIID is a critical component of the transcription preinitiation complex (PIC) that is responsible for recognizing the promoter regions of genes and nucleating assembly of the PIC on promoter DNA. My graduate work focused on using cryo-electron microscopy (cryo-EM) to study the structure of TFIID and its interaction with promoter DNA. Our cryo-EM structures of promoter-bound TFIID revealed the structural basis of TFIID's recognition of conserved promoter sequence motifs, showing how the TAF1 and TAF2 subunits of TFIID make extensive contacts spanning over 35 bp of the core promoter using highly conserved regions rich in positively-charged residues. These structures have also elucidated the mechanism by which the TFIID complex precisely loads TBP onto the TATA region of promoter DNA, such that it can be properly positioned to enable accurate loading of RNA Pol II. By analyzing these structures in the context of previous structural and biochemical data, we have generated a model that explains the functional importance of TFIID's large conformational rearrangement for accurate and regulated loading of TBP onto promoter DNA. In recent, unpublished, work, we have solved cryo-EM structures of free TFIID at high resolution, resulting in a complete, atomic level description of the architecture of the full 19-polypeptide TFIID complex. These new structures have led to a wealth of insight into the function of the complex, including potential mechanisms for TFIID recruitment by activators and promoter-proximal nucleosomes.

a. **Louder RK**, He Y, López-Blanco JR, Fang J, Chacón P, Nogales E. (2016). Structure of promoter-bound TFIID and insight into human PIC assembly. *Nature*, **531**:604-09. PMCID: PMC4856295

b. Nogales E, Fang J, **Louder RK**. (2016). Structural dynamics and DNA interaction of human TFIID. *Transcription,* **8**:55-60. PMCID: PMC5279711

c. Nogales E, **Louder RK**, He Y. (2016). Structural Insights into the Eukaryotic Transcription Initiation Machinery. *Annu Rev Biophys.*, **46**:59-83. PMCID: PMC5161697

d. Nogales E, Patel AB, **Louder RK**. (2017). Towards a Mechanistic Understanding of Core Promoter Recognition from Cryo-EM Studies of Human TFIID. *Curr Opin Struct Biol.*, **47**:60-66. PMCID: PMC5723225

e. Patel AB[†], **Louder RK**[†], Fang J, Nogales E. (2018). Architecture of human TFIID and mechanism of promoter binding. *Science*, **362**: eaau8872. PMCID: PMC6446905. [†]Contributed equally.

3. Advancing the use of cryo-EM for structural characterization of challenging targets

Recent technological advances in the field of cryo-EM have ushered in a new era in which high-resolution structural determination of large macromolecular assemblies can be achieved using relatively standard procedures. However, despite these advances, many targets remain challenging to solve by cryo-EM, for reasons including scarcity of the sample, poor stability of the complexes, and most often, the intrinsic flexibility of biological molecules. Eukaryotic transcription complexes suffer from all of these shortcomings, and thus, throughout my graduate work, I was required to address and overcome these challenges in order to successfully characterize the structure and function of the eukaryotic transcription machinery. This led to multiple improvements in the cryo-EM workflow that I have been able to share with other cryo-EM researchers through publications, personal communications, and conference presentations. These improvements include 1) biochemical optimization, such as the optimization of buffer conditions for maintaining the integrity of labile complexes or optimization of chemical crosslinking strategies, 2) optimization of cryo-EM sample preparation such as through the chemical modification of EM substrates, and 3) novel image processing workflows that can handle heterogenous sets of single particle images and maximize map quality in more stable regions of a complex through focused refinement schemes. In addition to my own work, the work of several other projects in

the lab have benefited from these trials, as noted in the acknowledgement sections of the papers cited below (c and d), for which I am not an author.

a. **Louder RK**, He Y, López-Blanco JR, Fang J, Chacón P, Nogales E. (2016). Structure of promoter-bound TFIID and insight into human PIC assembly. *Nature*, **531**:604-09. PMCID: PMC4856295
b. Nogales E, **Louder RK**, He Y. (2016). Cryo-EM in the study of challenging systems: the eukaryotic transcription initiation process. *Curr Opin Struct Biol.*, **40**:120-127. PMCID: PMC5161697
c. Greber BJ, Ngyuen, THD, Fang J, Afonine PV, Adams PD, Nogales E. (2017). The cryo-electron microscopy structure of human transcription factor IIH. *Nature*, **549**: 414-17. PMCID: PMC5844561
d. Poepsel S, Kasinath V, Nogales E. (2018). Cryo-EM structures of PRC2 simultaneously engaged with two functionally distinct nucleosomes. *Nat Struct Mol Biol.*, **25**: 154-62. PMCID: PMC5805599

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BIOGRAPHICAL SKETCH

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NAME: Wu, Carl

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
St. Mary's College of California	B.S. summa cum laude	05/1974	Biology-Chemistry
Harvard University	A.M.	03/1977	Biology
Harvard University	Ph.D.	06/1979	Biology

A. Personal Statement

I am interested in the structure and function of chromatin—the complex of genomic DNA, histones, nonhistone proteins and nuclear RNAs—which orchestrates the highly selective expression of eukaryotic genomes for normal growth, development, and homeostasis, and whose impairment is causally linked to many diseases, especially cancer. The importance of chromatin in gene regulation and disease was not evident when I entered the field over three decades ago. By developing novel biochemical methods for chromatin analysis and applying them to model genes and organisms to elucidate general principles relevant to human biology, my group made key contributions that significantly advanced and expanded the field to its present prominence in contemporary biological research. These advances include development of an indirect end-labeling technique to map the first DNase hypersensitive sites in metazoan genomes (Wu, 1980), the first chromatin footprint of transcription factor binding, leading to purification of one of the first sequence-specific transcription activators (Wu 1984; Wu et al. 1987), biochemical reconstitution of DNase hypersensitivity and discovery of the first cell-free, ATP-dependent chromatin remodeling activity (Tsukiyama et al. 1994), and identification of SWR1, a novel ATP-dependent histone exchange enzyme catalyzing variant histone dynamics at regulatory elements (Mizuguchi et al. 2004).

In addition to my long laboratory record of technical innovation applied to important biological problems, I have been privileged to mentor a number of outstanding post-doctoral trainees, who have since assumed leading positions in the chromatin field. For 15 years, I also served as head of a small department at NCI focused on chromosome biology, recruiting new talent to the NIH intramural research program. After 3 decades at NCI, I returned to the laboratory by moving with a few staff to the Transcription Imaging Consortium, Janelia Research Campus, HHMI, where several years were invested in learning single-molecule fluorescence microscopy while continuing biochemical work on histone variants. Late in 2016, I established a new laboratory at Johns Hopkins University with space and instrumentation to combine biochemical and single-molecule imaging approaches (super-resolution fluorescence and cryo-electron microscopy) with the power of yeast genetics to the study of chromatin structure and transcription control. While retaining intellectual links to my former institutions, I am excited to direct an academic laboratory with new graduate and undergraduate students, integrating them into our overall research effort.

1. Wu, C. The 5' ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature 286: 854-860 (1980).
2. Wu, C., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. and Ueda, H. Purification and properties of Drosophila heat shock activator protein. Science 238: 1247-1253 (1987).

3. Tsukiyama, T., Becker, P.B., and Wu, C. ATP-dependent nucleosome disruption at a heat shock promoter mediated by binding of GAGA transcription factor. Nature 367: 525-532 (1994).
4. Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303: 343-348 (2004).

B. Positions and Honors

Positions

1974 – 1979	Graduate student under Sarah Elgin, Harvard University
1979 – 1982	Post-doctoral fellow under Walter Gilbert, Harvard University
1982 – 1989	Principal Investigator, Laboratory of Biochemistry, National Cancer Institute, USA
1989 – 1996	Chief, Laboratory of Biochemistry, National Cancer Institute, USA
1996 – 2006	Chief, Laboratory of Molecular Cell Biology, National Cancer Institute, USA
2006 – 2011	Chief, Laboratory of Biochemistry & Molecular Biology, National Cancer Institute, USA
2007 – 2012	NIH Distinguished Investigator, National Cancer Institute, USA
2012 – 2016	Senior Fellow & Lab Head Janelia Research Campus, Howard Hughes Medical Institute, USA
2016 – present	Bloomberg Distinguished Professor Dept. of Biology, Johns Hopkins University Dept. of Molecular Biology & Genetics, Johns Hopkins School of Medicine

Honors

1979	Election to Harvard Society of Fellows (Junior Fellow 1979-82)
1987	Outstanding Young Scientist Award, Maryland Academy of Sciences
1992	ASBMB Young Investigator Award
1996	Appointment to Senior Biomedical Research Service, National Institutes of Health
1996	Election to Chair, Gordon Conference on Chromatin Structure & Function
1998	Election to American Academy of Arts and Sciences
2005	Recognition of 3 publications in a Nature Milestone in Gene Expression over 50 years
2006	Election to US National Academy of Sciences
2006	Election to Academia Sinica
2007	Appointment as NIH Distinguished Investigator
2007	Election to European Molecular Biology Organization (Foreign Associate)
2010	Election to the US National Academy of Medicine (formerly Institute of Medicine)

Keynote & Distinguished Lectures (from 2012)

2012	Keynote Lecture, Cold Spring Harbor Conference on Epigenetics and Chromatin, New York
2013	NIH Director's Wednesday Afternoon Lecture, National Institutes of Health
2013	Keynote Lecture, Physical and Biological Sciences Research Conference, University of California Santa Cruz
2014	Distinguished Lecture, Center for Cancer Epigenetics, M.D. Anderson Cancer Center, Texas
2014	Keynote Lecture, Cold Spring Harbor Asia Conference on Epigenetics, Chromatin & Transcription
2015	Keynote Lecture, Penn State Summer Symposium in Molecular Biology, "Chromatin and Epigenetic Regulation of Transcription"
2015	Marie Curie Seminar, Institute Curie, France
2015	Keynote Lecture, EMBO Conference Nuclear Structure and Dynamics, France
2016	Distinguished Lecture, Dept. of Biological Sciences, U. Maryland Baltimore County
2016	Keynote Lecture, Dept. of Biochemistry & Biophysics Annual Retreat, U. of Rochester
2017	EMBO Keynote Lecture, Barcelona Conference on Epigenetics & Cancer

C. Contribution to science

1. Discovery and mapping of DNase hypersensitive sites in metazoan chromatin.

The discovery of the nucleosome as the fundamental unit of chromatin organization in 1973-74 raised the key issue of nucleosome function in gene expression. As a graduate student, I was inspired by the findings of Weintraub and Groudine (1976), who provided the first evidence that nucleosomes within the (globin) transcription unit had altered, DNase I-sensitive structures. The limitations of that study, which relied on solution hybridization of highly digested chromatin products, prompted a new approach to measure initial cleavage kinetics of large chromatin domains by combining with Southern blotting with gentle (first-hit) DNase I digestion. This enabled the discovery of local, DNase 'hypersensitive' sites of ~100 bp occurring every 5-10 kb for sampled Drosophila loci. As an independent post-doctoral fellow, I adapted the Maxam-Gilbert principle of mapping chemical cleavages for DNA sequencing to develop the indirect end-labeling technique, and revealed the first genomic positions of DNase hypersensitive sites at upstream promoter sequences, beyond gene bodies of Drosophila heat shock and rat preproinsulin genes. These findings introduced the concept of an open chromatin architecture at gene promoters, since extended to transcription enhancers and replicated in hundreds of laboratories world-wide. DNase hypersensitive sites can be considered one of the first 'epigenetic' signatures of functional chromatin, and new developments based on deep sequencing technologies attest to its utility and importance as a functional genomic marker for human chromatin in normal cell development and pathogenesis.

a. Wu, C., Bingham, P.M., Livak, K.J., Holmgren, R. and Elgin, S.C.R. The chromatin structure of specific genes (I). Cell 16: 797-806 (1979).
b. Wu, C., Wong, Y.C., and Elgin, S.C.R. The chromatin structure of specific genes (II). Cell 16: 807-814 (1979).
c. Wu, C. The 5' ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature 286: 854-860 (1980).
d. Wu, C. and Gilbert, W. Tissue-specific exposure of chromatin structure at the 5' terminus of the rat preproinsulin II gene. Proc. Natl. Acad. Sci. USA 78: 1577-1580 (1981).

2. Discovery of the first transcription factor footprints and purification of Heat Shock Transcription Factor HSF1.

The discovery and molecular characterization of prokaryotic gene repressors in the 1970's raised the question whether similar transcription factors existed to control gene activity in eukaryotic organisms. With the ultimate goal of understanding the molecular basis of DNase hypersensitivity, I began work as a P.I. in the NCI intramural program by developing an exonuclease protection technique to identify transcription factor-DNA interactions within a DNase hypersensitive site. This led to detection of the first constitutive and inducible transcription factor footprints on Drosophila chromatin. The chromatin footprinting assay enabled identification of the heat shock transcription activator (HSF1) in cell-free extracts, and its purification, cloning and biochemical characterization as a heat and cell stress-inducible, sequence-specific DNA binding protein. These findings enabled collaborative genetic identification of Drosophila HSF1 mutants, proving that HSF1 is the master regulator of the metazoan cell stress response, and the cloning of and patent award for the human HSF1 cDNA. Our molecular and genetic reagents facilitated advances in the regulation of cell stress chaperones by the conserved HSF family, and in the modulatory function of stress chaperones on protein mis-folding in neurodegenerative diseases. HSF1 has also become the subject of renewed medical interest as a powerful regulator of core cellular activities maintaining the growth of human metastatic cancers.

a. Wu, C. Two protein-binding sites in chromatin are implicated in the activation of heat shock genes. Nature 309: 229-234 (1984).
b. Wu, C. Activating protein factor binds in vitro to upstream control sequences in heat shock gene chromatin. Nature 311: 81-84 (1984).
c. Wu, C., Wilson, S., Walker, B., Dawid, I., Paisley, T., Zimarino, V. and Ueda, H. Purification and properties of Drosophila heat shock activator protein. Science 238: 1247-1253 (1987).
d. Jedlicka, P., Mortin, M.A. and Wu, C. Multiple Functions of Drosophila Heat Shock Transcription Factor in vivo. EMBO J. 16: 2452-2462 (1997).

3. Discovery and biochemical characterization of ATP-dependent chromatin remodeling enzymes

To elucidate the molecular basis of DNase hypersensitive chromatin, we developed a robust, cell-free extract from Drosophila embryos that efficiently assembles nucleosome arrays on DNA plasmids. Investigation of constitutive GAGA transcription factor binding to reconstituted chromatin led to the discovery of a cooperating ATP-dependent activity that repositions nucleosomes on heat shock promoters, creating a constitutive DNase hypersensitive site for stress-inducible HSF1 binding. Purification of a four-component protein complex called Nucleosome Remodeling Factor (NURF) by classical multi-step chromatography identified the catalytic ATPase ISWI, a member of the SWI/SNF family implicated in transcriptional regulation, and the largest subunit NURF301, corresponding to *Enhancer of Bithorax*, a mutant previously isolated by Laureate geneticist Ed Lewis but lost from his collection. Our findings, together with parallel studies by others on SWI/SNF, introduced a major new direction to the chromatin field, emphasizing the importance of ATP-dependent enzymes cooperating with sequence-specific transcription factors throughout metazoan development for nucleosome remodeling. Three publications from our laboratory have been cited as a Nature Milestone in Gene Regulation over the past 50 years. ATP-dependent chromatin remodeling along with post-translational histone modifications are key eukaryotic additions to the bacterial paradigm of sequence-gene regulators, are clearly implicated in many cancers, and provide important pathways for drug development.

a. Tsukiyama, T., Becker, P.B., and Wu, C. ATP-dependent nucleosome disruption at a heat shock promoter mediated by binding of GAGA transcription factor. Nature 367: 525-532 (1994).
b. Tsukiyama, T., and Wu, C. Purification and properties of an ATP-dependent Nucleosome Remodeling Factor. Cell 83:1011-1020 (1995).
c. Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., Wu, C. ATP-Dependent Histone Octamer Sliding Mediated by the Chromatin Remodeling Complex NURF. Cell 97: 833-842 (1999).
d. Badenhorst, P., Voas, M., Rebay, I., and Wu, C. Biological functions of the ISWI chromatin remodeling complex NURF. Genes Dev. 16: 3186-3198 (2002).

4. Discovery and characterization of ATP-dependent histone H2A.Z variant exchange

Nonallelic, conserved minor variants of the canonical H2A histone such as H2A.Z had been known for decades. Genetic studies indicated that histone H2A.Z was functionally important, but the mechanisms of H2A.Z assembly into chromatin were obscure. Bioinformatics and systematic purification of potentially novel ISWI-related chromatin remodeling enzymes from budding yeast led to our discovery of the INO80 subfamily of ATP-dependent chromatin remodelers, and to the first connection between chromatin remodeling and the histone variant H2A.Z. Histone H2A.Z is specifically incorporated on dynamic nucleosomes flanking promoters and enhancers genome-wide by SWR1, a conserved 14-component remodeler related to INO80. SWR1 uses the energy of ATP hydrolysis - not to slide nucleosomes - but to evict histone H2A, replacing it with H2A.Z, contributing to a genome-wide signature of permissive chromatin for transcription. The mechanism of histone H2A.Z replacement by SWR1, the precise functions of the H2A.Z nucleosome in facilitating proper transcription, and the reversal of the H2A.Z nucleosome to its canonical state are problems at the leading edge of chromatin dynamics and transcription. H2A.Z overexpression is increasingly associated with disease, including metastatic breast carcinoma, colorectal cancer and neurological disorders, so understanding the mechanism and functions of SWR1 and H2A.Z will contribute to approaches for pharmacological intervention.

a. Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303: 343-348 (2004).
b. Wu, W.H., Alami, S., Luk, E., Wu, C.H., Sen, S., Mizuguchi, G., Wei, D., and Wu, C. Swc2 is a widely conserved H2AZ binding module essential for ATP-dependent histone exchange. Nat. Struct. Mol. Biol. 12: 1064-71(2005).
c. Luk E, Ranjan A, Fitzgerald PC, Mizuguchi G, Huang Y, Wei D, Wu C. Stepwise Histone Replacement by SWR1 Requires Dual Activation with Histone H2A.Z and Canonical Nucleosome. Cell 143(5):725-36 (2010).
d. Ranjan A, Mizuguchi G, Fitzgerald PC, Wei D, Wang F, Huang Y, Luk E, Woodcock CL, Wu C. Nucleosome-free Region Dominates Histone Acetylation in Targeting SWR1 to Promoters for H2A.Z Replacement. Cell 154:1232-45 (2013). PMCID:PMC3815578

5. Discovery of a novel histone chaperone for centromere-specific variant nucleosome assembly

Our findings of histone H2A.Z incorporation by the SWR1 complex raised the question of the deposition mechanism for another prominent histone variant, CENP-A (CenH3), which is localized specifically to centromeres and plays a key role in kinetochore assembly for the segregation of daughter chromosomes in cell division. The cell biology of CENP-A had been analyzed extensively, but surprisingly little was known regarding the assembly mechanism of the CENP-A nucleosome. We undertook a biochemical approach to identify factors physically interacting with CENP-A (Cse4 in budding yeast), and purified Scm3, a conserved Cse4-specific histone chaperone with AT-rich DNA-binding properties. Scm3 localizes to yeast centromeres in vivo, undergoes dynamic exchange with a free chaperone pool in the nucleus, and appears to insure against catastrophic loss of chromatin-kinetochore interactions in the yeast cell cycle. Collaborative structural studies elucidated the physical basis for Scm3 chaperone binding to Cse4 histone, and informed studies of the human CENP-A chaperone, HJURP. Our findings have contributed to the fundamental architecture and function of the kinetochore, and the pathology of CENP-A associated with chromosome aneuploidy and human cancers.

a. Mizuguchi, G., Xiao, H., Wisniewski, J., Smith, M.M. and Wu, C. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere specific nucleosomes. Cell 129: 1153-1164 (2007).
b. Zhou Z, Feng H, Zhou BR, Ghirlando R, Hu K, Zwolak A, Jenkins L, Xiao H, Tjandra N, Wu C, Bai, Y. Structural basis for recognition of centromere specific histone H3 variant by chaperone Scm3. Nature 472:234-7 (2011). PMCID:PMC3077455
c. Xiao H, Mizuguchi G, Wisniewski J, Huang Y, Wei D, Wu C. Nonhistone Scm3 Binds to AT-Rich DNA to Organize Atypical Centromeric Nucleosome of Budding Yeast. Mol Cell. 43:369-80 (2011). PMID: 21816344
d. Wisniewski J, Hajj B, Chen J, Mizuguchi G, Xiao H, Wei D, Dahan M, Wu C. Imaging the fate of histone Cse4 reveals *de novo* replacement in S phase and subsequent stable residence at centromeres. eLIFE May 20 (2014). PMCID:PMC4067749

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D. Research Support

Ongoing Research Support

Johns Hopkins University Bloomberg Distinguished Professorship start-up funds. 7/1/2016 – present.
Project: Biochemistry and cell biology of chromatin and transcription. The goal of this project is to elucidate the mechanisms by which transcription factors and chromatin architecture regulate transcription in eukaryotic cells.

R01GM125831, Wu (PI) 1/1/2018 – 12/31/2021
NIH, NIGMS
Title: Mechanism of histone H2A.Z exchange catalyzed by SWR1 chromatin remodeler.
The goals of this project are to use biochemical approaches to elucidate the mechanism of ATP-dependent histone H2A.Z exchange by the yeast SWR1 chromatin remodeling complex, develop an *in vitro* single-molecule imaging platform to elucidate histone exchange, and determine structures for SWR1 and its components.

Completed Research Support

HHMI Janelia Research Campus, Wu (Lab Head) 3/1/2012 – 6/30/2016
Project: Biochemistry and cell biology of histone and transcription factor dynamics, with the goal of using biochemistry and new single-molecule imaging techniques to elucidate chromatin and transcription mechanisms.