BIOGRAPHICAL SKETCH

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NAME: Luger, Karolin, Ph.D.

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

POSITION TITLE: Dept. of Chemistry and Biochemistry, and Jennie-Smoly-Caruthers Endowed Chair of Chemistry and Biochemistry, University of Colorado; Investigator, Howard Hughes Medical Institute

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
University of Innsbruck, Austria	B.S.	05/1983	Biology / Microbiology
University of Innsbruck, Austria	M.S.	05/1986	Biology / Biochemistry
University of Basel (Biocenter), Switzerland	Ph.D.	02/1989	Biochemistry/ Biophysics
Swiss Federal Institute of Technology, Switzerland	Postdoc	12/1994	Structural Biology

A. Personal Statement

My lab studies the structure and function of large macromolecular assemblies involved in chromosome organization. We have years of experience in studying the structural biology of nucleosomes and chromatin and associated proteins. We are working towards a quantitative, mechanistic, and structural description of nucleosome assembly and disassembly and are investigating the role of histone chaperones in these processes. With our move to CU Boulder, we are taking full advantage of the existing Cryo-EM facility, and we are hoping to branch out into cryo-ET, in particular for our investigations of archaeal and viral chromatin. Recently, we were awarded the funds to acquire our own Titan Krios with a K3 camera, an amazing addition to our existing microscopes. The instrument will be installed early in January 2020.

In addition to cryo-EM, we employ x-ray crystallography, analytical ultracentrifugation, fluorescence-based affinity assays, hydrogen-deuterium exchange coupled to mass spectrometry, small angle x-ray scattering, atomic force microscopy. My research is highly collaborative. For example, I have been a PI on a Program Project Grant and other multi-PI grants. I have a long-standing track record in mentoring undergraduates, graduate students and postdocs. Throughout my career, I have served in numerous advisory functions to the NIGMS (e.g., regular and ad hoc member of study sections, site visits, Center Grant reviews, and NAGMS council).

B. Positions and Honors

1990-1994 Postdoctoral Fellow, Dept. of Molecular Biology and Biophysics, Swiss Federal Institute of Technology (ETH), Zürich, Switzerland

1994-1999 Research Assistant Professor (‘Oberassistent’), ETH Zürich, Switzerland

1999-2003 Assistant Professor, Dept. of Biochemistry and Molecular Biology, Colorado State University

2003-2007 Associate Professor, Dept. of Biochemistry and Molecular Biology, Colorado State University

2004-2007 Monfort Professor, Dept. of Biochemistry and Molecular Biology, Colorado State University

2005-present Adjoint Professor, Dept. of Biochemistry and Molecular Genetics, Colorado School of Medicine
2005-present Investigator, Howard Hughes Medical Institute
2007-2015 University Distinguished Professor, Colorado State University
2015-present Affiliate Professor, Dept. of Biochemistry and Molecular Biology, Colorado State University
2015-present Professor, Dept. of Chemistry and Biochemistry, University of Colorado
2015-present Jennie-Smoly-Caruthers Endowed Chair of Chemistry and Biochemistry, University of Colorado

***Honors / Professional Service*:**

1999 Searle Scholar Award
2000 Basil O'Conner Starter Scholar Award
2002 Reviewer, NASA Biomaterials study section
2003 Ad hoc reviewer, NIH BBCB study section
2003 Reviewer, NIH training grants study section
2004 Monfort Professor Award
2005-2009 NIH MSFC study section, regular member
2005-2014 Keystone Symposia, member of the Scientific Advisory Board
2007-present University of Colorado Cancer Center member
2007 Vorarlberg, Austria, State Science Prize
2008-present Journal of Biological Sciences, Editorial Board
2009-2013 EU Marie Curie Nucleosome 4D Network, SAB member and Visiting Scientist
2010 NIH, College of CSR Reviewers
2010-present IRSF, member of the Scientific Review Board
2010-2013 National Advisory General Medical Sciences Council
2011-2012 NIGMS Protein Structure Initiative Advisory Committee, Chair
2012 4DCellFate project SAB member
2013 Biophysical Society National Lecturer
2014 Biophysical Society Fellow
2014-present Journal of Molecular Biology, Editorial Board
2015 Jack E. Cermak Undergraduate Advising Award
2015 Martha L. Ludwig Lectureship in Structural Biology, University of Michigan
2016 HHMI Faculty Scholars Mentoring Board
2016, 2018: Co-organizer and organizer of the Chromatin Gordon Research Conference
2017 Octoberfest Award, LMU Munich
2017 Murray Honors Visiting Scholar, Colorado State University
2017 Member, American Academy of Arts & Sciences
2018 Associate Member, EMBO
2018 Member, National Academy of Science
2020 Hans Tuppy Lecture, Austrian Academy of Science, University of Vienna

C. Contributions to Science

Nucleosome structure, stability, and dynamics

In 1997, the central role of chromatin and nucleosomes in the regulation of transcription, replication, and DNA repair had fully emerged. As a postdoctoral researcher in the lab of Dr. T. Richmond, I was the lead author on a manuscript determining the structure of the nucleosome core particle, the fundamentally repeating unit of chromatin (Luger et al., 1997). Completion of this project entailed the development of methods to prepare recombinant nucleosomes that are now used in every lab that analyzes nucleosomes in vitro. Upon

establishing my own lab, we determined the first structure of a nucleosome in complex with another protein, a peptide from the LANA Protein of Kaposi Sarcoma Herpes Virus (Barbera et al., 2006), and identified the acidic patch on the surface of the nucleosome as a key determinant of nucleosome-nucleosome interactions.

Nucleosomes are multi-component assemblies that undergo a number of structural transitions, as shown in a collaborative study with two labs specializing in single molecule studies (Sheinin et al., 2013). These studies revealed a multitude of finely tuned interactions that hold together the nucleosome, and show evidence for a new dynamic state in which the interface between H2A-H2B dimer and $(H3\text{-}H4)_2$ is exposed. As such, the thermodynamic properties of the nucleosome are difficult to define. We have developed a novel approach to measure nucleosome stability that allows us to thermodynamically define the various steps in nucleosome assembly / disassembly (Andrews et al., 2010), and are in the process of further refining this. We have collaborated with Lewis Kay to demonstrate nucleosome dynamics by NMR (Kitevski-LeBlanc et al., 2018).

Structure of nucleosomes with post-translational modifications and histone variants

When I started my own lab in 1999, the prevailing hypothesis was that epigenetic modifications of chromatin, such as acetylation or methylation, but also incorporation of histone variants, would structurally alter nucleosome to provide a mechanism for regulating access to DNA. We expanded our studies on nucleosome structure to include nucleosomes containing histone variants (Suto et al., 2000); and histones with post-translational modifications (Lu et al., 2008). Our studies revealed that these structures were very similar to that of unmodified nucleosomes. We made the important conclusion that crystallization stabilizes nucleosomes, thereby obscuring any changes in their dynamic properties, and this led us to develop assays to investigate nucleosome stability, dynamics, and the mechanisms of nucleosome assembly. In some cases, post-translational modifications lead to changes in chromatin higher order structure (Lu et al., 2008). We have also characterized the interaction of nucleosomes with the ubiquitous linker histone H1 (White et al., 2016). Most recently, using single-particle cryo-EM combined with in-cell assays, we have determined how the centromeric protein CENP-N decodes and stabilizes the centromeric nucleosome (Pentakota et al., 2017).

The origins of the eukaryotic nucleosome

The massive expansion of the eukaryotic genome was arguably made possible by the early adoption of histones to form nucleosomes as the fundamental organizational unit of chromatin. Because eukaryotic histones are highly conserved, these events must have happened early in eukaryogenesis. We contribute to the debate of karyogenesis by exploring the evolutionary roots of the modern eukaryotic nucleosome and its assembly machinery in either archaea or giant viruses, both of which contain histone-like proteins.
Most recently, we have investigated the structure of archaeal chromatin to understand the origins of the eukaryotic nucleosome (Mattiroli et al., 2017a). We continue our investigations into archaeal chromatin and expand into histone-based chromatin of giant viruses, using a combination of structural biology, biochemistry, and cell biology. Our investigations of these 'proto-eukaryotic nucleosomes' will provide insight into the adaptations to the many challenges of organizing large eukaryotic genomes. Tools that were developed in my lab previously will be essential to stabilize these likely metastable nucleoprotein assemblies to make them suitable for cryo-EM (Edayathumangalam et al., 2005; Edayathumangalam et al., 2004).

Nucleosome assembly factors

A major effort in the lab is to investigate the mechanism by which nucleosomes are assembled and disassembled in the process of transcription and replication. We have elucidated an intriguing mechanism for the major replication-dependent histone chaperone CAF-1 (Mattiroli et al., 2017b, and investigated how the histone chaperone NAP1 interacts with histones {D'Arcy, 2013 #3639). Most recently, we determined the cryo-EM structure of the ubiquitous histone chaperone FACT in the process of assembling the nucleosome (Liu et al., 2019). This structure provides unprecedented mechanistic insight into how this chaperone both stabilizes and destabilizes chromatin during the passage of the DNA or RNA polymerase. We have delved into the mechanism by which centromeric nucleosomes are assembled by the histone chaperones CAL1 and Scm3 (yeast; (Dechassa et al., 2014)).

Poly [ADP-ribose] polymerase

Our studies on the interaction of the abundant nuclear enzyme Poly [ADP-ribose] polymerase 1 (PARP-1) have revealed that auto-modification resulting in the attachment of long poly-ADP-ribose chains to itself alters its interactions with chromatin, and confers on PARP-1 the activity of a powerful histone chaperone (Muthurajan et al., 2014) and a transcriptional activator. PARP-1 is a key player in the recognition of DNA damage and

initiates DNA damage response; it is also a regulator of transcription. We are investigating the movements of all DNA-dependent PARPs in the cell (Mahadevan et al., 2019) and in vitro (Rudolph et al., 2018).

Full online list of publications:
https://www.ncbi.nlm.nih.gov/sites/myncbi/karolin.luger.1/bibliography/40851519/public/?sortby=pubDate&sdirection=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support:

R01CA218255 (Luger)	8/01/2017 – 7/31/2022	2.4 calendar
NCI/NIH	$294,189 annual direct	

Title: Structure and Mechanism of Chromatin-Bound PARP1
Role: PI
We propose to study the structure and activity of PARP1 bound to chromatin DNA. Our quantitative, structural, and mechanistic approaches provide fundamental insight into this abundant multi-faceted enzyme, and will aid in the development of the next generation of PARP inhibitors.

Howard Hughes Medical Institute	9/16/2005 – 12/31/2021 $880,000 annual direct	HHMI does not use effort reporting

Dr. Luger's salary and benefits, and those of one postdoc, three graduate students, a lab manager and admin assistant are paid by HHMI, *exclusive of Dr. Luger's salary and benefits.* HHMI provides funds for operations, which includes travel, supplies, equipment maintenance and equipment under $15,000. All HHMI budgets are determined annually.

Completed Research Support:

GM067777 (Luger)	5/1/2003 – 8/31/2017
NIGMS/NIH	

Title: Functional connections between histone variants and histone chaperones
Goal: The machinery that transcribes and replicates the information encoded in the human genome is prevented access due to the packaging of all genomic DNA with an equal amount of protein to form chromatin. Here we study two functionally related activities that regulate DNA accessibility and thus vital cellular processes through the modulation of chromatin structure. We are using quantitative and structural approaches to gain insight into how structural transitions within chromatin allow vital biological processes to occur.

Andrews, A.J., Chen, X., Zevin, A., Stargell, L.A., and Luger, K. (2010). The Histone Chaperone Nap1 Promotes Nucleosome Assembly by Eliminating Nonnucleosomal Histone DNA Interactions. Mol Cell *37*, 834-842.

Barbera, A.J., Chodaparambil, J.V., Kelley-Clarke, B., Joukov, V., Walter, J.C., Luger, K., and Kaye, K.M. (2006). The nucleosomal surface as a docking station for Kaposi's sarcoma herpesvirus LANA. Science *5762*, 856-861.

Dechassa, M.L., Wyns, K., and Luger, K. (2014). Scm3 deposits a (Cse4-H4)2 tetramer onto DNA through a Cse4-H4 dimer intermediate. Nucleic Acids Res *42*, 5532-5542.

Edayathumangalam, R.S., Weyermann, P., Dervan, P.B., Gottesfeld, J.M., and Luger, K. (2005). Nucleosomes in Solution Exist as a Mixture of Twist-defect States. J Mol Biol *345*, 103-114.

Edayathumangalam, R.S., Weyermann, P., Gottesfeld, J.M., Dervan, P.B., and Luger, K. (2004). Molecular recognition of the nucleosomal "supergroove". Proc Natl Acad Sci U S A *101*, 6864-6869.

Kitevski-LeBlanc, J.L., Yuwen, T., Dyer, P.N., Rudolph, J., Luger, K., and Kay, L.E. (2018). Investigating the Dynamics of Destabilized Nucleosomes Using Methyl-TROSY NMR. J Am Chem Soc *140*, 4774-4777.

Liu, Y., Zhou, K., Zhang, N., Wei, H., Tan, Y.Z., Zhang, Z., Carragher, B., Potter, C.S., D'Arcy, S., and Luger, K. (2019). FACT caught in the act of manipulating the nucleosome Nature *in press*.

Lu, X., Simon, M.D., Chodaparambil, J.V., Hansen, J.C., Shokat, K.M., and Luger, K. (2008). The effect of H3K79 dimethylation and H4K20 trimethylation on nucleosome and chromatin structure. Nature Structural & Molecular Biology *15*, 1122-1124.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature *389*, 251-260.

Mahadevan, J., Rudolph, J., Jha, A., Tay, J.W., Dragavon, J., Grumstrup, E.M., and Luger, K. (2019). Q-FADD: A Mechanistic Approach for Modeling the Accumulation of Proteins at Sites of DNA Damage. Biophys J *116*, 2224-2233.

Mattiroli, F., Bhattacharyya, S., Dyer, P.N., White, A.E., Sandman, K., Burkhart, B.W., Byrne, K.R., Lee, T., Ahn, N.G., Santangelo, T.J.*, et al.* (2017a). Structure of histone-based chromatin in Archaea. Science *357*, 609-612.

Mattiroli, F., Gu, Y., Yadav, T., Balsbaugh, J.L., Harris, M.R., Findlay, E.S., Liu, Y., Radebaugh, C.A., Stargell, L.A., Ahn, N.G.*, et al.* (2017b). DNA-mediated association of two histone-bound complexes of yeast Chromatin Assembly Factor-1 (CAF-1) drives tetrasome assembly in the wake of DNA replication. Elife *6*.

Muthurajan, U.M., Hepler, M.R., Hieb, A.R., Clark, N.J., Kramer, M., Yao, T., and Luger, K. (2014). Automodification switches PARP-1 function from chromatin architectural protein to histone chaperone. Proc Natl Acad Sci U S A *111*, 12752-12757.

Pentakota, S., Zhou, K., Smith, C., Maffini, S., Petrovic, A., Morgan, G.P., Weir, J.R., Vetter, I.R., Musacchio, A., and Luger, K. (2017). Decoding the centromeric nucleosome through CENP-N. elife *6:e33442*.

Rudolph, J., Mahadevan, J., Dyer, P., and Luger, K. (2018). Poly(ADP-ribose) polymerase 1 searches DNA via a 'Monkey Bar' mechanism. Elife *7*.

Sheinin, M.Y., Li, M., Soltani, M., Luger, K., and Wang, M.D. (2013). Torque modulates nucleosome stability and facilitates H2A/H2B dimer loss. Nat Commun *4*, 2579.

Suto, R.K., Clarkson, M.J., Tremethick, D.J., and Luger, K. (2000). Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. Nat Struct Biol *7*, 1121-1124.

White, A.E., Hieb, A.R., and Luger, K. (2016). A quantitative investigation of linker histone interactions with nucleosomes and chromatin. Sci Rep *6*, 19122.