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

I have dedicated my career to study chromatin, beginning with the structure determination of the nucleosome (Luger et al., 1997). We are now interested in the structure and mechanism of large macromolecular assemblies involved in chromosome organization. We are working towards a quantitative, mechanistic, and structural description of nucleosome assembly/disassembly and are investigating the structure and function of histone chaperones (Liu et al., 2020), chromatin remodeling factors, and histone variants. We are also interested in the evolutionary origin of the nucleosome and study histone-based chromatin in non-eukaryotic organisms (e.g. Liu et al, 2021). My lab uses a wide palette of approaches, such as x-ray crystallography, cryo-EM, analytical ultracentrifugation, fluorescence-based affinity assays, hydrogen-deuterium exchange coupled to mass spectrometry, atomic force microscopy, and life-cell microscopy. In the past 10 years, I have also led a research program addressing the structure, enzymology and in vivo role of poly-ADP-ribose)-polymerases. I have been particularly focused on the interactions of PARP1 and PARP2 with chromatin (Rudolph et al., 2021a), and its mechanism of activation.

My research is highly collaborative, both with colleagues at CU Boulder and all over the world. I have a long-standing track record and investment in mentoring undergraduates, graduate students and postdocs. I am a training faculty member in the NIH-funded Biophysics Training Program and the Signaling and Cellular Recognition Training Program, as well as an HHMI Gilliam Mentor. Rigor, reproducibility, ethics and safety are important elements of training in my lab. Overall, 22 graduate students have obtained their Ph.D. from my lab, and I have trained ~ 25 postdocs. I also mentor many junior female faculty throughout the US. I have chaired departmental recruitment committees, and I am active in numerous international and national advisory activities to funding agencies and academic research enterprises. Throughout my career, I have served the NIH in many functions (e.g., standing and ad hoc member of study sections, NIH intramural research site visits, Protein Structure Initiative SAB, Center Grant reviews, and the NAGMS council).

Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). "Crystal structure of the nucleosome core particle at 2.8 A resolution." Nature **389**(6648): 251-260.

Liu, Y., K. Zhou, N. Zhang, H. Wei, Y. Z. Tan, Z. Zhang, B. Carragher, C. S. Potter, S. D'Arcy and K. Luger (2020). "FACT caught in the act of manipulating the nucleosome." Nature **577**(7790): 426-431.

Liu, Y., Bisio, H., Toner, C.M., Jeudy, S., Philippe, N., Zhou, K., Bowerman, S., White, A., Edwards, G., Abergel, C.*, et al.* (2021). Virus-encoded histone doublets are essential and form nucleosome-like structures. Cell *184*, 4237-4250 e4219.

Rudolph, J., U. M. Muthurajan, M. Palacio, J. Mahadevan, G. Roberts, A. Erbse, P. N. Dyer and K. Luger (2021). "The BRCT Domain of PARP1 Binds Intact DNA and Mediates Intrastrand Transfer." Mol.Cell (24), 4994-5006 e4995.

B. Positions

1990-1994 Postdoctoral Fellow, Dept. of Molecular Biology and Biophysics, 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
2015-2019 Affiliate Professor, Dept. of Biochemistry and Molecular Biology, Colorado State University
2015-present Professor, Dept. of Chemistry and Biochemistry, University of Colorado, Boulder
2015-present Jennie-Smoly-Caruthers Endowed Chair of Biochemistry, University of Colorado, Boulder
2023-present Distinguished Professor, 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
2006 Meeting organizer: Keystone meeting: Regulation of eukaryotic transcription, 2006
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
2010-2015 IRSF, member of the Scientific Review Board
2010-2013 National Advisory General Medical Sciences Council
2011-2012 NIGMS Protein Structure Initiative Advisory Committee, Chair
2011 Meeting organizer: Keystone meeting: Histone code: Fact or Fiction, 2011
2011 Nucleic acids GRC: vice chair
2012 4DCellFate project SAB member
2012 Biophysical Society National Lecturer
2013 Nucleic acids GRC: vice chair
2014-present 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
2017-present Chair, Departmental graduate student recruitment committee
2018 Associate Member, EMBO
2018 Member, National Academy of Science
2018-present Member of the Standing Committee on Research Misconduct, CU Boulder
2018-present Member of the PNAS editorial board
2019-present Member of the European Research Council (ERC) consolidator grant review committee

2020-present: NIHGM MIRA R35 review panel.
2020-present Member of the 'Expertengremium', Excellence initiavive DFG and German Government
2021 Rusnak Named Lectureship, Mayo Clinic
2021 The Jerry A. Weisbach Memorial Lecture; Rockefeller University
2021 Hans Tuppy Lecture, Austrian Academy of Science, University of Vienna
2021 21st Irving L. Schwartz Lectureship, Icahn School of Medicine, Mt. Sinai
2022 Steven T. Rosen Lecture, Northwestern University
2023 Distinguished Professor, University of Colorado, Boulder

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 the hundreds of labs that analyze 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. 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).

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.

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.

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.

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). We show that CENP-N promotes centromere-specific higher order structure (Zhou et al., 2022)

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.

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.

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.

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*.

Zhou, K., Gebala, M., Woods, D., Sundararajan, K., Edwards, G., Krzizike, D., . . . Luger, K. (2022). CENP-N promotes the compaction of centromeric chromatin. *Nat Struct Mol Biol*, *29*(4), 403-413.

Histone-based chromatin in non-eukaryotic organisms

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.

We have determined the structure of archaeal chromatin to understand the origins of the eukaryotic nucleosome (Mattiroli et al., 2017a). We continue our investigations into archaeal chromatin (Bowerman et al., 2021) and have expanded into histone-based chromatin of giant viruses, using a combination of structural biology, biochemistry, and cell biology (Liu et al., 2021). Our investigations of these 'proto-eukaryotic nucleosomes' 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).

Mattiroli, F., Bhattacharyya, S., Dyer, P.N.,…Luger, K. (2017a). Structure of histone-based chromatin in Archaea. Science *357*, 609-612.

Bowerman, S., Wereszczynski, J., and Luger, K. (2021). Archaeal chromatin 'slinkies' are inherently dynamic complexes with deflected DNA wrapping pathways. Elife *10*.

Liu, Y., Bisio, H., Toner, C.M., Jeudy, S., Philippe, N., Zhou, K., Bowerman, S., White, A., Edwards, G., Abergel, C*., et al.* (2021). Virus-encoded histone doublets are essential and form nucleosome-like structures. Cell *184*, 4237-4250 e4219.

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.

Nucleosome assembly and exchange 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 et al., 2013). In 2020, we determined the cryo-EM structure of the ubiquitous histone chaperone FACT in the process of assembling the nucleosome (Liu et al., 2020). This structure provides unprecedented mechanistic insight into how this chaperone both stabilizes and destabilizes chromatin during the passage of the DNA or RNA polymerase. Most recently, we have explored the structure and biochemistry of the ATP-dependent chromatin remodeler SMARCAD1 (Markert et al., 2021).

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*.

D'Arcy, S., K. W. Martin, T. Panchenko, X. Chen, S. Bergeron, L. A. Stargell, B. E. Black and K. Luger (2013). Chaperone Nap1 Shields Histone Surfaces Used in a Nucleosome and Can Put H2A-H2B in an Unconventional Tetrameric Form." Mol Cell **51**(5): 662-677.

Liu, Y., Zhou, K., Zhang, N., Wei, H., Tan, Y.Z., Zhang, Z., Carragher, B., Potter, C.S., D'Arcy, S., and Luger, K. (2020). FACT caught in the act of manipulating the nucleosome. Nature *577*, 426-431.

Markert, J., Zhou, K., and Luger, K. (2021). SMARCAD1 is an ATP-dependent histone octamer exchange factor with de novo nucleosome assembly activity. Sci Adv *7*, eabk2380.

Poly [ADP-ribose] polymerase (PARP-1)

The abundant nuclear enzyme PARP-1 is a key player in the recognition of DNA damage and initiates the DNA damage response, but is also abundantly bound to chromatin where it acts as a chromatin architectural protein. In research supported by this grant from the NCI, we have quantified the movements of DNA-dependent PARPs in the cell and in vitro (Mahadevan et al., 2019; Rudolph et al., 2018), and demonstrated how its enzymatic properties are changed upon interaction with the newly discovered accessory protein histone parylation factor (HPF1) (Rudolph et al., 2021b). We have shown that PARP1 and PARP2 are NOT activated by RNA (resolving a controversial issue in the literature). We have recenty determined the cryoEM structure of PARP2 as it bridges two nucleosomes, as well as identifed and a previously undescribed DNA binding domain of PARP1 that is only required for binding to intact DNA rather tan to DNA damage. We have determined its cryo-EM structure bound to nucleosomes and show that it is required for PARP1 movement by the 'monkey bar mechanism' (Rudolph et al., 2021a).

Mahadevan, J., Jha, A., Rudolph, J., Bowerman, S., Narducci, D., Hansen, A. S., & Luger, K. (2022). Dynamics of endogenous PARP1 and PARP2 during DNA damage revealed by live-cell single-molecule imaging. *iScience*, *in press*, 2022.2003.2012.484081.

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

Rudolph, J., Roberts, G., Muthurajan, U. M., & Luger, K. (2021b). HPF1 and nucleosomes mediate a dramatic switch in activity of PARP1 from polymerase to hydrolase. *Elife*, *10*..

Rudolph, J., Muthurajan, U.M., Palacio, M., Mahadevan, J., Roberts, G., Erbse, A., Dyer, P.N., and Luger, K. (2021a). The BRCT Domain of PARP1 Binds Intact DNA and Mediates Intrastrand Transfer. Mol Cell *81(24), 4994-5006 e4995.*.

Full online list of publications: https://www.ncbi.nlm.nih.gov/pubmed/?term=luger+k+not+cow

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

Ongoing Research Support:

R01CA218255 (Luger) 8/01/2017 – 7/31/2027 2.4 calendar

NCI/NIH
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.

1U24GM139174 (Hoenger) 9/8/2020 – 8/31/2024
NIH Office of the Director (no funds awarded to the PI or her lab)
Title: CU BOULDER CENTER FOR CRYO-ET (CCET)
Role: CoPI
High-end electron microscopy and cryo-electron tomography are highly specialized and expensive technologies. To pool expertise and high-end equipment, the creation of hub-like service centers, open to the scientific community, has been propagated since several years. Here we propose to form a service unit that is integrated in a wider network, acting together for the benefit of the EM community in a similar fashion as synchrotrons are for X-ray protein crystallography projects.

Howard Hughes Medical Institute 9/16/2005 – 12/31/2022 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.