BIOGRAPHICAL SKETCH

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NAME: **JEANETTE GOWEN COOK**

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

POSITION TITLE: ASSOCIATE PROFESSOR

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Chapel Hill	B.S. with honors	05/1990	Biology
University of California, Berkeley	Ph.D.	12/1996	Molecular & Cell Biol.
Duke University Medical Center	Postdoc	05/2004	Molecular Genetics

A. Personal Statement.

Research Statement. *My long-term aspiration is to understand how the human cell cycle is organized to maintain genome stability.* One of the *principal goals* in this endeavor is to determine molecular mechanisms of cell cycle entry and exit and to uncover regulatory networks at the transitions into and out of S phase.

As a graduate student I studied the function of the first MAP kinase identified, the yeast Kss1 enzyme. My work contributed several significant findings through identification of novel MAP kinase substrates and uncovering cellular strategies that maintain signaling specificity. (My degree was conferred in 1996, and I continued on that project until 1997.) This early training in signal transduction during a period of the field's explosive growth provides my independent lab with a unique perspective on cell cycle control. For example, we have levied this expertise to explore how MAP kinase signaling in human cells directly impacts cell cycle transitions and cell cycle events, an interface not normally investigated by either the cell cycle or signaling fields. My postdoctoral studies were devoted to the independent development of DNA replication origin licensing studies in the context of mammalian cell cycle control. I discovered new points of licensing regulation involving protein-protein and protein-chromatin interactions as cells exit and re-enter the cell cycle. The portfolio of protocols and reagents I developed to manipulate human licensing proteins were instrumental in the establishment of an independent research program at the University of North Carolina.

My lab is now adept in the analysis of key human DNA replication proteins by protein-protein interactions, ubiquitination, and phosphorylation, manipulating expression of target human proteins by RNAi-mediated knockdown or ectopic expression and inhibition of signaling pathways using pharmacological and genetic approaches. We apply a comprehensive battery of tests that measure cell cycle progression, DNA replication kinetics, protein and RNA abundance, chromatin association, protein interactions, mitotic progression, DNA damage markers, spindle checkpoint activity, live cell single cell analysis, etc. Our proficiency with these techniques plus our knowledge of cell cycle control, cell cycle checkpoints, and signaling pathways allows us to gain mechanistic insight into specific regulatory events relevant for cell cycle control and genome stability.

The multidisciplinary nature of the research in our laboratory provides a rich training environment that includes experience in biochemistry, genetics, molecular biology, computational biology, cell biology, and cancer biology. Moreover we collaborate extensively with many labs at UNC including those led by prominent senior faculty members. Thus, trainees in my lab have meaningful interactions with accomplished faculty who provide additional resources and mentoring. Throughout my time as an independent faculty member, I have made significant contributions to the training of predoctoral students and postdocs from diverse groups both in my own laboratory and in the broader UNC-CH community.

Teaching/Training Statement. I am course co-director for a graduate course in cell biology; postdocs and students in my lab with interest in developing their teaching portfolios have been guest lecturers. I organize a

monthly research meeting of UNC labs studying the cell cycle where trainees present their recent results. I have served on more than 50 PhD thesis committees and have mentored many UNC and summer visiting undergraduates in the lab. Thus, I am confident that trainees in my community receive not only the individual scientific training and experience required to develop as independent and productive investigators but also the broader professional skills required to develop as excellent communicators, instructors, colleagues, and leaders in the scientific community. Cook lab members have been highly successful in obtaining both intramural training grant appointments and extramural fellowships.

I have served as Associate Dean for Graduate Education for the entire UNC biomedical research community since 2013, and in that capacity I am the director of the umbrella first-year graduate program and coordinator of several professional development programs including our NIH-funded Initiative for Maximizing Student Diversity (IMSD), our post-baccalaureate training program (UNC-PREP), and the career development resources for postdocs and graduate students through the Training Initiatives in Biomedical and Biological Sciences and the new ImPACT program funded by an NIH BEST award. In these roles I lead the UNC Office of Graduate Education. The institutional commitment to excellence in graduate education supports the office and its programs that extend from the undergraduate level (our summer programs) to post-doctoral trainees (career exploration). Our efforts have been recognized by the AAMC (e.g. for science outreach in 2014), and by continued funding of our educational programs from NIH and other sources. The combined activities of the Office of Graduate Education are led by me and a group of five full-time PhD-trained Directors plus six full-time staff that continually strive to improve the recruitment, retention, development, and educational and career outcomes for the spectrum of life science graduate students at UNC. These staff supported by NIH grants and institutional resources allow me to spend *at least 50%* of my time with my lab.

B. Positions and Honors

Positions and Employment

1990-1991	**Research Technician**, Cloning and DNA sequence analysis of the dihydropteroate synthase gene of *Escherichia coli*; Burroughs Wellcome Co., Research Triangle Park, NC
1991-1997	**Graduate Student/brief Post-doc**, Activation and function of the *Saccharomyces cerevisiae* MAP kinase, Kss1. Advisor: Professor Jeremy Thorner, Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, UC Berkeley.
1997-2004	**Post-doctoral researcher/Senior Research Associate**, Regulation of DNA replication in mammalian cells, Advisor: Professor Joseph R. Nevins, Department of Genetics/HHMI, Duke University Medical Center.
2004-2011	**Assistant Professor**, Departments of Biochemistry & Biophysics and Pharmacology, School of Medicine, University of North Carolina at Chapel Hill
2011-present	**Associate Professor**, Departments of Biochemistry & Biophysics and Pharmacology, University of North Carolina Medical School, Chapel Hill
2013-present	**Associate Dean for Graduate Education**, School of Medicine, University of North Carolina at Chapel Hill

Selected Honors and Awards

1990	H.V.P. Wilson Award for Excellence in Cellular and Molecular Biology, UNC-CH
1990	Phi Beta Kappa, University of North Carolina, Chapel Hill.
1992-1996	Merck Academic Program Development (ADP) Predoctoral Fellowship.
1997	Elected to Sigma Xi, University of California, Berkeley.
1997-2000	American Cancer Society post-doctoral research fellowship
2003-2008	The National Cancer Institute's Howard Temin Award (K01)
2010	Academy of Educators Teaching Excellence Award (UNC-SOM)
2011	Cold Spring Harbor "Eukaryotic DNA Replication," - invited speaker/session chair
2010-2014	The Jefferson-Pilot Fellowship in Academic Medicine
2012-2017	Induction into the Academy of Educators (UNC-SOM)
2014	Cold Spring Harbor, "The Cell Cycle" – invited speaker/session chair
2015	Gordon Research Conference, "Cell Growth and Proliferation" – invited speaker/session chair
2015	Cold Spring Harbor "Eukaryotic DNA Replication," - invited speaker/session chair

Other Experience and Professional Memberships

Editorial Board- *The Journal of Biological Chemistry*
National Institutes of Health study section service: MGA (2010, 2013), F08 (2012), NCI SEP-2 Omnibus R21-R03 (2014), CSRS (2014, regular member 2015-2021), Fellowships: Oncology (2014), American Cancer Society: Genetic Mechanisms in Cancer (2008, ad hoc), American Heart Association (2009, ad hoc), Mary Kay Foundation (2012), Cancer Research UK (2011, ad hoc). Manuscript review for more than 17 different journals. Member, Sigma Xi, American Association for the Advancement of Science, American Society for Biochemistry and Molecular Biology, American Society for Cell Biology, American Society for Microbiology

C. Contributions to Science

1) Function and regulation of MAPK signaling pathways. My graduate studies sought to identify substrates of the budding yeast kinase, Kss1, the first-discovered messenger-activated protein kinase (MAPK). At the time this work began, much was known about how MAPKs are activated, but less was known about how they transmit information to affect cellular systems. An extensive screen discovered two new transcriptional regulators as substrates of Kss1 and linked their regulation to a developmental switch between typical growth and invasive/pseudohyphal growth. I also made the surprising discovery that the binding of Kss1 to its substrates and activators when the upstream signaling cascade is "off" acts as a critical negative regulator of the signaling pathway. This was the first demonstration that the kinase in its inactive form is biologically important and was later shown (by others) to also be a key feature of mammalian MAPK signaling pathways.

a. **J.G. Cook**, L. Bardwell, S.J. Kron, J. Thorner, Two novel targets of the MAP kinase Kss1 are negative regulators of invasive growth in the yeast Saccharomyces cerevisiae. *Genes Dev* 10, 2831-2848 (1996); PMID 8918885
b. **J.G. Cook**, L. Bardwell, J. Thorner, Inhibitory and activating functions for MAPK Kss1 in the S. cerevisiae filamentous-growth signalling pathway. *Nature* 390, 85-88 (1997); PMID9363895
c. L. Bardwell, **J.G. Cook**, D. Voora, D.M. Baggott, A.R. Martinez, J. Thorner, Repression of yeast Ste12 transcription factor by direct binding of unphosphorylated Kss1 MAPK and its regulation by the Ste7 MEK. *Genes Dev* 12, 2887-2898 (1998); PMCID 317171
d. L. Bardwell, **J.G. Cook**, E.C. Chang, B.R. Cairns, J. Thorner, Signaling in the yeast pheromone response pathway: specific and high-affinity interaction of the mitogen-activated protein (MAP) kinases Kss1 and Fus3 with the upstream MAP kinase kinase Ste7. *Mol Cell Biol* 16, 3637-3650 (1996); PMCID 231359

2) Biological factors that promote DNA replication origin licensing. My postdoctoral studies investigated the regulation of the initial step of DNA replication, "origin licensing" as mammalian cells exit and re-enter the cell cycle. At the time the work began, the signaling pathways triggering cyclin synthesis and cell cycle entry were known (e.g. MAPK signaling), but how this change in cellular state translates to regulation of DNA replication origin licensing through the DNA loading of the MCM helicase complex was unknown. I developed a functional assay for manipulating and monitoring MCM loading *in vivo* and used this assay to explore two mechanisms of MCM loading inhibition in quiescent cells and to identify a novel lysine acetyltransferase that binds MCM. This work continued in my independent position by defining a true cell cycle checkpoint that responds to MCM loading. The existence of this checkpoint had been debated within the field prior to this work, but our demonstration that the checkpoint only functions in normal but not p53-deficient transformed cells helped resolve the issue. A functional genetic screen for synthetic interactions with origin licensing mutations in budding yeast discovered that dimethylation of lysine 4 of histone H4 is required for efficient replication. Since the locations of replication initiation are not determined by DNA sequence, this study revealed a new aspect of chromatin structure that helps define replication origins.

a. **J.G. Cook**, C.H. Park, T.W. Burke, G. Leone, J. DeGregori, A. Engel, J.R. Nevins, Analysis of Cdc6 function in the assembly of mammalian prereplication complexes. *Proc Natl Acad Sci* U S A 99, 1347-1352 (2002); PMID 11805305
b. **J.G. Cook**, D.A. Chasse, J.R. Nevins, The regulated association of Cdt1 with minichromosome maintenance proteins and Cdc6 in mammalian cells. *J Biol Chem* 279, 9625-9633 (2004); PMID 14672932
c. K.R. Nevis, M. Cordeiro-Stone, **J.G. Cook**, Origin licensing and p53 status regulate Cdk2 activity during G(1). *Cell Cycle* 8, 1952-1963 (2009); PMCID 2972510
d. L.F. Rizzardi, E. S. Dorn, B.D. Strahl, **J.G. Cook**, DNA replication origin function is promoted by H3K4 di-methylation in Saccharomyces cerevisiae. Genetics 192, 371-384 (2012); PMCID 3454870

3) *Cellular and genotoxic stress regulation of DNA replication origin licensing.* As key origin licensing factors were identified, understanding their regulation by intracellular and extracellular signals became important. We discovered a novel 492 kDa ubiquitin E3 ligase, Huwe1, that is responsible for the DNA damage-induced degradation of the licensing protein, Cdc6. Huwe1 was contemporaneously shown by others to target critical proliferation and apoptosis proteins such as p53, c-myc, and Mcl-1. We used this information to define a new regulatory pathway that stimulates the degradation of Cdc6 and a second critical licensing protein, Cdt1, when genomic DNA is aberrantly replicated more than once, i.e. "re-replication." We developed a single-molecule assay to quantify re-replication induced by Cdt1 de-regulation using DNA fiber analysis and provided evidence that transformed cells constitutively re-replicate in culture. We further discovered that human Cdt1 is a substrate of two stress-activated messenger activated protein kinases, p38 and JNK. Our thorough investigation of this interaction revealed that Cdt1 phosphorylation by stress MAPKs controls both its stability and function. Stress MAPKs are activated during mitosis, during quiescence or differentiation, and in response to a variety of pathophysiological conditions leading to the conclusion that Cdt1 regulation by stress MAPK signaling is a common phenomenon that restricts origin licensing.

a. J.R. Hall, E. Kow, K.R. Nevis, C.K. Lu, K.S. Luce, Q. Zhong, **J.G. Cook**, Cdc6 stability is regulated by the Huwe1 ubiquitin ligase after DNA damage. Mol Biol Cell 18, 3340-3350 (2007); PMCID PMC1951745
b. J.R. Hall, H.O. Lee, B.D. Bunker, E.S. Dorn, G.C. Rogers, R. J. Duronio, **J.G. Cook**, Cdt1 and Cdc6 are destabilized by rereplication-induced DNA damage. J Biol Chem 283, 25356-25363 (2008); PMCID PMC2533066
c. E.S. Dorn, P.D. Chastain, 2nd, J.R. Hall, **J.G. Cook**, Analysis of re-replication from deregulated origin licensing by DNA fiber spreading. Nucleic Acids Res 37, 60-69 (2009); PMCID PMC2615611
d. S. Chandrasekaran, T.X. Tan, J.R. Hall, **J.G. Cook**, Stress-stimulated mitogen-activated protein kinases control the stability and activity of the Cdt1 DNA replication licensing factor. Molecular and Cellular Biology 31, 4405-4416 (2011); PMCID 3209262

4) Cell cycle organization and dynamics. To achieve in-depth understanding of dynamics and interactions in the eukaryotic cell cycle, we conducted extensive proteomic and genetic screens to uncover new relationships. Our screen for Cdt1-interacting proteins led to a very surprising discovery that Cdt1 is not only required for origin licensing but also required for the stable attachment of chromosomes to the mitotic spindle. A thorough mechanistic study defined the interaction partner of Cdt1 as the loop domain of the Hec1 protein, and (using super-resolution microscopy) we showed that Cdt1 is required for a large conformational change in Hec1 during microtubule attachment. This newly-identified function of Cdt1 is entirely separate from its role in origin licensing and partly explains why deregulation of Cdt1 has such profound effects on genome stability. This study underscored the multi-functional nature of key cell cycle proteins and shed light on the unique nature of Cdt1 in both chromosome replication and segregation. We uncovered a CDK1-dependent mechanism of Cdt1 regulation during late S phase that allows it to accumulate in G2 and then function in kinetochore attachment without inducing re-replication. We further showed that this mechanism controls several other key cell cycle regulators, including the p21 CDK inhibitor and the Set8 lysine methyltransferase. Most recently, we determined a molecular mechanism that determines a stereotypical order of protein degradation at the G1/S transition using a combination of biochemical, molecular genetic, and quantitative live cell imaging approaches. We exploited the new knowledge of the mechanism underlying that order to cause p21 and Cdt1 to be degraded at the same time instead of sequentially. This maniuplation impaired normal S phase progression, suggesting that the order of molecular events at cell cycle transitions ensures genome stability.

a. D. Varma, S. Chandrasekaran, L.J. Sundin, K.T. Reidy, X. Wan, D.A. Chasse, K.R. Nevis, J.G. Deluca, E.D. Salmon, **J.G. Cook**, Recruitment of the human Cdt1 replication licensing protein by the loop domain of Hec1 is required for stable kinetochore-microtubule attachment. *Nature Cell Biology* 14, 593-603 (2012); PMCID 3366049
b. K.R. Lane, Y. Yu, P.E. Lackey, X. Chen, W.F. Marzluff, **J.G. Cook**, Cell cycle-regulated protein abundance changes in synchronously proliferating HeLa cells include regulation of pre-mRNA splicing proteins. *PLoS One* 8, e58456 (2013) PMCID 3592840.
c. Rizzardi, L.F., D. Varma, K.E.Coleman, J.P. Matson, S. Oh, **J.G. Cook**. (2014) CDK1-dependent Inhibition of the E3 Ubiquitin Ligase, $CRL4^{CDT2}$, Ensures Robust Transition from S Phase to Mitosis. *J. Biol. Chem*. 2014 Nov 19. pii: jbc.M114.614701

d. Coleman, K.E. G.D. Grant, R. A. Haggerty, K. Brantley, E. Shibata, B. Workman, A. Dutta, D. Varma, J.E. Purvis, and **J.G Cook** (2015) Sequential replication-coupled destruction at G1/S ensures genome stability. *Genes & Development*, Epub ahead of print. PMID: 26272819.

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