

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./DUE DATE NSF 22-586 07/26/2023		<input type="checkbox"/> Special Exception to Deadline Date Policy		FOR NSF USE ONLY NSF PROPOSAL NUMBER 2339605	
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TITLE OF PROPOSED PROJECT CAREER: Deciphering strand-specific mechanisms of chromatin replication using a yeast model				SHOW LETTER OF INTENT ID IF APPLICABLE	
REQUESTED AMOUNT \$ 831,908	PROPOSED DURATION (1-60 MONTHS) 60 months	REQUESTED STARTING DATE 02/01/2024		SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE	
THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW					
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PI/PD FAX NUMBER					
NAMES(TYPED)	High Degree	Yr of Degree	Telephone Number	Email Address	
Chuanhe Yu	PhD	2009	507-437-9639	yu000479@umn.edu	
CO-PI/PD					

CERTIFICATION PAGE**Certification for Authorized Organizational Representative(or Equivalent)**

By electronically signing and submitting this proposal, the Authorized Organizational Representative(AOR) is:(1)certifying that statements made here in are true and complete to the best of the individual's knowledge; and(2)agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this proposal. Further, the proposer is hereby providing certifications regarding conflict of interest, flood hazard insurance, responsible and ethical conduct of research, organizational support, and safe and inclusive working environments for off-campus or off-site research, as set forth in the NSF Proposal & Award Policies & Procedures Guide(PAPPG).Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense(U.S.Code,Title 18,Section §1001).

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Certification Regarding Responsible and Ethical Conduct of Research (RECR)

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By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that, in accordance with the NSF Proposal & Award Policies and Procedures Guide, Chapter IX.B., the institution has a plan in place to provide appropriate training and oversight in the responsible and ethical conduct of research to undergraduate students, graduate students, postdoctoral researchers, faculty, and other senior personnel who will be supported by NSF to conduct research. As required by Section 7009 of the America Creating Opportunities to Meaningfully Promote Excellence in Technology, Education, and Science (COMPETES) Act (42 USC 1862o–1), as amended, the training addresses mentor training and mentorship. The AOR shall require that the language of this certification be included in any award documents for all subawards at all tiers.

Certification Regarding Organizational Support

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is certifying that there is organizational support for the proposal as required by Section 526 of the America COMPETES Reauthorization Act of 2010. This support extends to the portion of the proposal developed to satisfy the Broader Impacts Review Criterion as well as the Intellectual Merit Review Criterion, and any additional review criteria specified in the solicitation. Organizational support will be made available, as described in the proposal, in order to address the broader impacts and intellectual merit activities to be undertaken.

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By electronically signing the certification pages, the Authorized Organizational Representative is certifying that the organization will be or is in compliance with all aspects of the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern.

Certification Requirement Specified in the William M.(Mac)Thornberry National Defense Authorization Act for Fiscal Year 2021, Section 223(a)(1) (42 USC 6605(a)(1))

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that each individual employed by the organization and identified on the proposal as senior personnel has been made aware of the certification requirements identified in the William M. (Mac) Thornberry National Defense Authorization Act for Fiscal Year 2021, Section 223(a)(1) (42 USC 6605(a)(1)).

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AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME Patrick Donnell	Electronic Signature	Jul 26 2023 10:49 AM
TELEPHONE NUMBER 612-301-1411	EMAIL ADDRESS donne096@umn.edu	FAX NUMBER

Overview

Eukaryotic chromatin, which consists of DNA and associated proteins such as histones, transcription factors, and chromatin regulators, plays a crucial role in epigenetic stability and gene expression. For this reason, chromatin regulation and replication are crucial in cell differentiation and development. Despite extensive biochemical and molecular research on DNA replication, the mechanisms underlying chromatin replication at each DNA strand (leading and lagging) remain unclear, as do the role of chromatin regulators. It is critical to address this knowledge gap because understanding the mechanisms of asymmetric chromatin replication, including the deposition of histones and other epigenetic marks, will provide fundamental insights into cell differentiation in higher organisms. Our inability to identify the mechanisms underlying chromatin replication primarily stems from technical challenges in distinguishing between the continuous leading strand of DNA and the discontinuous lagging strand. Fortunately, the study of chromatin replication has recently gained momentum from novel in vitro and in vivo models, as well as cutting-edge technologies such as in vivo DNA labeling techniques and next-generation sequencing. Building upon these advances, the Principal Investigator (PI) has developed innovative methodologies using a budding yeast model system to map major DNA replication fork components on two DNA replication strands and investigate the mechanisms by which parental histones are recycled during DNA replication. In the proposed work, these methods will allow us to achieve the following specific objectives: (1) investigate the mechanism by which leading and lagging strands are coupled during chromatin replication, (2) study the biochemical and functional strand preferences of chaperones for newly produced H3-H4 histones (Caf1, Asf1, Rtt106), and (3) examine the DNA strand preferences of transcription factors during the DNA replication process. Throughout, STEM graduate and undergraduate students will lead the yeast mutation screens, genetic studies, and sequencing and bioinformatic analyses essential for the success of this project. In addition, as part of the Integrated Educational Plan, the PI will develop an epigenomics summer training course for undergraduates and a yeast genetic training program for high school students, with a particular focus on recruiting students from underrepresented groups to stimulate their interest in science and future STEM careers. The proposed research and educational activities will establish a solid foundation for understanding the mechanisms of chromatin replication and retaining high-achieving students in STEM.

Intellectual Merit

This proposal will make significant intellectual contributions to the DNA/chromatin replication research community by developing a novel method called Genomic Enrichment of ssDNA and Sequencing (GESS-seq). This method holds great potential for advancing the field of DNA replication and DNA repair research because it allows researchers to characterize the replication fork structure without the need for sophisticated procedures. Using a combination of powerful genetic approaches and our established methodologies, we will also provide novel information about the strand-specific roles of newly discovered histone chaperones. Finally, we will produce novel data on the binding patterns of chromatin transcription factors on the leading and lagging strands of replicating DNA, including determining whether they bind concurrently or sequentially on the two strands. In short, by employing innovative genetic, genomic, and molecular biology approaches, we will significantly advance the research community's understanding of how asymmetric chromatin replication is regulated, particularly regarding differences between the leading and lagging DNA strands. This line of inquiry may shed light on the mechanisms underlying asymmetric cell division processes, such as those that occur during stem cell development.

Broader Impacts

This project will yield substantial benefits for both the research community and society at large. The new scientific knowledge and tools we create will further two of NSF's Strategic Objectives: advancing the frontiers of research and enhancing research capability. A key aspect of this work is creating research experiences and learning opportunities that will enhance the confidence and STEM learning of students from underrepresented groups who are pursuing degrees and careers in biological fields. By training 5 graduate students, 50 undergraduates, and 50 high school students in the areas of microbial biology, genetics, genomics, and bioinformatics, with a priority on recruiting members of underrepresented groups, this project will also further two additional NSF Strategic Objectives: ensuring accessibility and inclusivity and unleashing STEM talent for America.

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1. OVERVIEW & CAREER MOTIVATION

Since the discovery of the DNA double helix, geneticists have dedicated substantial effort to understanding DNA replication mechanisms. Initial discoveries, such as the identification of DNA polymerase and the isolation of DNA replication Okazaki fragments, were made in bacteria. However, eukaryotic cells, with their histones and complex chromatin structures, possess a more intricate chromatin replication system. My research interests lie in understanding the mechanisms of eukaryotic chromatin replication. These interests were driven by my PhD training in transposable element activity and postdoctoral training in yeast mating type switch, both phenomena associated with chromatin replication. My long-term goal is to unravel the molecular mechanisms underlying chromatin replication and their profound impact on genome integrity, as chromatin replication underpins fundamental biological processes, ensures genetic stability and inheritance, and contributes to the complexity and diversity of the live world.

To advance our understanding of chromatin replication, I have focused on developing new technologies. To study the distribution patterns of chromatin proteins and the structure of DNA replication forks I developed Enrichment and Sequencing of Protein-Associated Nascent DNA (eSPAN) (1-4). eSPAN has expanded our understanding of eukaryotic chromatin replication, such as the role of the DNA replication clamp proliferating cell nuclear antigen (PCNA), which is specifically removed from the lagging DNA strand when replication forks stall (1). I have also developed Genomic Enrichment of ssDNA and Sequencing (GESS-seq), an innovative dsDNase enzyme-based assay to analyze DNA replication fork structures that will be employed in this proposal. By combining a double-strand (ds)DNase enzyme with single-strand (ss)DNA sequencing, this new technology can help elucidate the mechanism by which the leading and lagging strands of DNA are coupled (or uncoupled) during DNA replication. This novel application of dsDNase to genome DNA structure analysis represents a significant advancement in the field of DNA replication.

By leveraging the methods, I have developed to interrogate chromatin replication, I will address *three long-standing questions in molecular biology*, corresponding to the three specific objectives in this proposal: (1) What is the mechanism by which the leading and lagging DNA strands are coupled during chromatin replication? (2) What are the biochemical and functional strand preferences of chaperones for newly produced H3-H4 histones (Caf1, Asf1, Rtt106)? (3) What are the DNA strand preferences of transcription factors during the DNA replication process? Answering these questions is crucial because these processes ensure the integrity of chromatin replication and thus the faithful transmission of genetic material from one generation to the next. Addressing them will contribute to our understanding of the mechanisms responsible for DNA replication and to my *long-term research career goal* of becoming a leading expert in chromatin replication research, unraveling the intricate mechanisms that govern this fundamental process.

While completing my research training and embarking on my career as a principal investigator (PI), I became passionate about educating the next generation of scientists. In this proposal, I have integrated my research focus on yeast genetics and chromatin with my commitment to fostering future scientists' education. My educational objectives are to (1) create an epigenomics training program for undergraduates participating in the Hormel Institute (HI)'s existing research experience programs, and (2) develop a yeast genetics training program for high school students completing science fair projects. For both programs, I will prioritize recruiting members of underrepresented groups to align with my *long-term educational career goal*: to empower students from diverse backgrounds and equip them with the necessary skills to excel in biological research. By nurturing their scientific curiosity and promoting critical thinking, I aspire to advance scientific knowledge and contribute to the growth of a diverse, innovative, and inclusive scientific community.

In short, an NSF CAREER grant would allow me to make major scientific and educational contributions to the STEM research community that I could not otherwise make, playing a pivotal role in my career development. The insights derived from the proposed experiments will help unravel the intricate DNA strand-specific binding preferences and functions of histone chaperones and transcription factors. This potentially includes documenting transcription factors sequentially binding to both replicating DNA strands. Our findings and the contribution of GESS-seq will also help pave the way for further investigation of essential chromatin factors, including chromatin remodeling factors and chromatin modifiers. Finally, this project will engage more than 100 trainees in scientific research, including 5 graduate students, 50 undergraduate participants, and 50 high school students.

2. BACKGROUND

Leading and lagging DNA strands coordination during DNA replication (Objective 1). DNA replication is a fundamental process in all living organisms, ensuring the faithful transmission of genetic information from one generation to the next. In the late 1960s, the Japanese scientist Reiji Okazaki proposed the concept of discontinuous DNA replication (5), wherein one (lagging) strand of the double helix is synthesized in short segments, while the other (leading) strand is synthesized continuously. Decades later, how the two replication strands coordinate with each other remains uncertain.

Some progress has been made in identifying the molecular mechanisms that coordinate the leading and lagging strands in DNA replication. The trombone model and replisome hypothesis propose that different DNA polymerase complexes cooperate to carry out DNA replication (6). Accordingly, researchers have discovered that DNA polymerase (Pol) ϵ and δ are responsible for leading and lagging strand synthesis, respectively; the divisional labor model further supports these models (7,8). In addition, we have learned that coordination of leading and lagging strand synthesis requires the eukaryotic sliding clamp PCNA (9). In yeast, uncoupling of the two DNA strands during replication is a rare occurrence observed exclusively under extreme stress conditions and limited to cell cycle checkpoint mutants (2,10). Specifically, under conditions of intense stress, such as hydroxyurea treatment, we have observed that the lagging strand exhibits significantly higher movement speed than the leading strand in checkpoint mutants (2). As a consequence, cells cannot survive these conditions (10,11).

During my postdoc at the Mayo Clinic, I developed eSPAN (Fig. 1) (1), a strand-specific method for studying DNA replication proteins. Using eSPAN in yeast, I confirmed the strand preference of several key DNA replication proteins, including Pol δ , Pol ϵ , DNA ligase, and minichromosome maintenance complex (MCM) helicase (1). I also found the DNA replication clamp PCNA prefers the lagging strand under normal conditions but is unloaded from the lagging strand when exposed to replication stress (1-4).

Based on our current understanding, uncoupling of the leading and lagging strands during DNA replication

is rare, due to the essential nature of Pol δ and Pol ϵ . Furthermore, checkpoint pathways regulate DNA replication when replication forks stall (2). If the leading and lagging strands at the DNA replication fork are uncoupled, it remains uncertain whether these checkpoint pathways would be activated when a replication fork stalls—or what the consequences for the cell would be. In preparation for our work in **Objective 1**, we have isolated a DNA Pol I mutant that exhibits DNA replication strand uncoupling. This mutant will be instrumental in *investigating the mechanisms underlying coupling DNA strands*.

The DNA strand-specificity of histone chaperones (Objective 2). An integral aspect of cellular proliferation is the

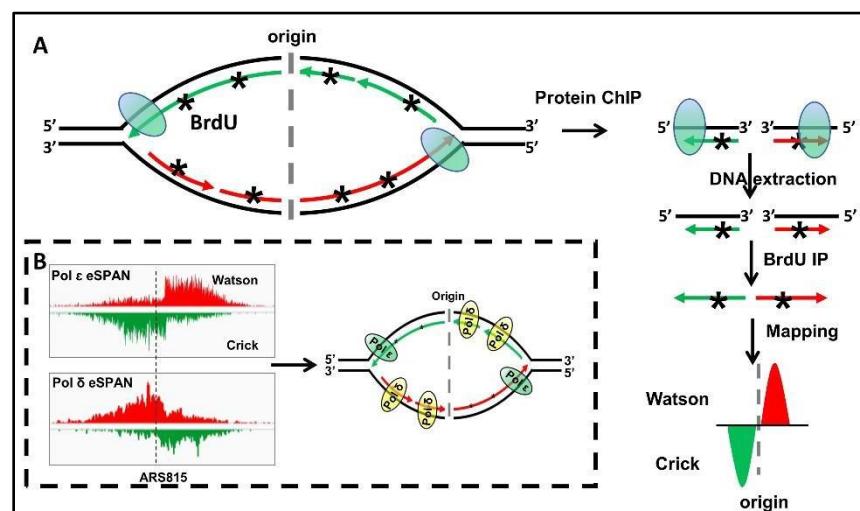


Fig. 1. eSPAN can detect proteins' strand preference. (A) Steps: Early S-phase cells with BrdU-labeled nascent DNA are subjected to chromatin immunoprecipitation (ChIP) targeting a replication-associated protein. Nascent ssDNA associated with the targeted protein is then enriched by BrdU immunoprecipitation (BrdU-IP). After ssDNA library construction and sequencing, sequenced reads are mapped to the Watson (red) or Crick (green) strand of the reference genome. A leading strand protein is shown. (B) Representative Pol ϵ and Pol δ eSPAN results. Left: Mapping data at a single origin (ARS815). Right: Cartoon representation of strand-specific distribution of Pol ϵ and Pol δ .

accurate transmission of information across generations. This includes not only the linear sequence of

genomic DNA, but also the chromatin structure that encodes epigenetic information. The maintenance and transmission of chromatin structure remains an elusive area within the field of epigenetics. The nucleosome, the basic unit of chromatin, is composed of 147 base pairs of DNA nucleotides plus histone octamers comprising two copies each of the core histones H2A, H2B, H3, and H4.

One important branch of chromatin regulation research is understanding nucleosome recycling during DNA replication. Nucleosome assembly, particularly the formation of the H3-H4 tetramer, is vital for compacting and organizing DNA, regulating gene expression, enabling epigenetic modifications, and contributing to cellular identity and development. These processes are fundamental to the function and maintenance of eukaryotic cells and essential for the overall health and survival of an organism. In each cell cycle, half the histone molecules are newly synthesized and the other half are recycled. Formation and deposition of the H3-H4 tetramer is a rate-limiting step in nucleosome assembly. Parental H3-H4 tetramers do not split during DNA replication—coupled nucleosome assembly (12–15), so they never mix with new tetramers (14). By contrast, H2A-H2B dimers appear to dissociate from the parental octamer and randomly reincorporate into newly assembled nucleosomes (16,17).

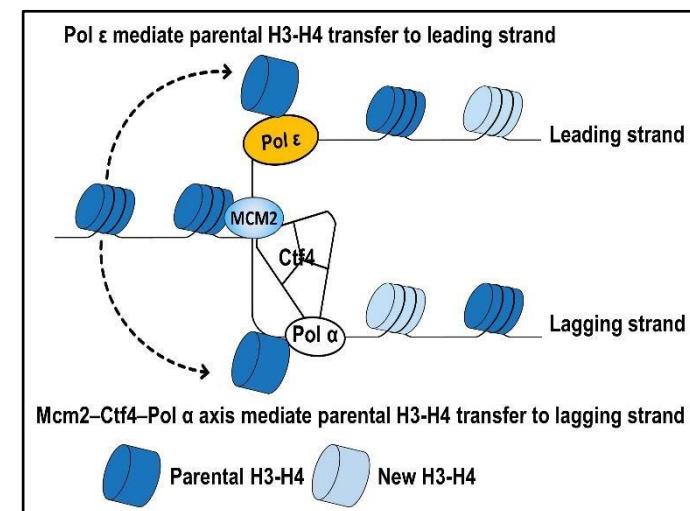


Fig. 2. Two distinct parental histone H3-H4 transfer pathways for the leading and lagging DNA strands during chromatin replication. Parental histone H3-H4 tetramers (dark blue) are efficiently transferred to newly synthesized DNA by Polymerase (Pol) ε subunit Dpb3/Dpb4 and the Mcm2-Ctf4-Pol α axis following the DNA replication fork. Newly synthesized histone H3-H4 tetramers (light blue) are deposited in the empty space left by the parental histone tetramer.

polymerase), this bias for the lagging strand is markedly enhanced (18). Pol ε thus appears to mediate recruitment of parental nucleosomes to the leading strand (**Fig. 2**). We also found that parental H3-H4 tetramers are transferred to the lagging strand by the Mcm2-Ctf4-Pol α complex (19). As a consequence, gene silencing at subtelomeric regions and the mating type locus was disturbed in the Dpb3/Dpb4 and Mcm2 mutants. These data on parental histone H3-H4 strand preference inform our hypotheses about strand bias for new-histone H3-H4 chaperones in **Objective 2**.

New H3-H4 tetramer assembly and deposition are highly regulated by assistant proteins, known as histone chaperones. Three major chaperones participate in replication-coupled nucleosome assembly of new-histone H3-H4 tetramers in yeast: chromatin assembly factor 1 (Caf1), anti-silencing factor 1 (Asf1) and regulator of Ty1 transposition factor 106 (Rtt106). **Caf1**, which is conserved among all eukaryotes, binds to the histone H3-H4 tetramer and mediates replication-coupled nucleosome assembly by interacting with PCNA (20,21). Caf1 mutations affect gene silencing in eukaryotic organisms, from yeast to mammals (22–24). **Asf1** presents the newly synthesized histone H3-H4 tetramer to Rtt109-Vps75 for acetylation of histone H3K56, after which the Asf1-bound histone H3-H4 tetramer is transferred to Caf1 or Rtt106 (25,26). Asf1 and Caf1 are only partly redundant, as double Asf1 and Caf1 mutant yeast cells show greater gene silencing than Asf1 mutants (27). **Rtt106**, which is unique to yeast, enhances the silencing defects of Caf1 but not Asf1 mutants (28,29). These DNA replication-coupled chromatin regulators are important for maintaining chromatin stability, making them an active focus of research. However, *how these histone chaperones*

coordinate during the DNA replication process and whether they have a role in replication strand division is unclear. We will address these questions in **Objective 2**, improving our understanding of how chromatin structure is maintained during DNA replication, gene expression, and epigenetic regulation.

The DNA strand-specificity of transcription factors (Objective 3). In a cell, DNA serves not only as the template for DNA replication but also as the blueprint for gene transcription. As key regulatory proteins, transcription factors play a pivotal role in orchestrating gene expression by influencing the transcription process. These factors can be broadly categorized into two groups: site-specific transcription factors, which bind to specific DNA sequences near the gene's promoter region, and general transcription factors that are required for the initiation of transcription at most genes (30). The interplay between transcription factors and DNA replication has been the subject of extensive research. Notably, studies have revealed the intricate connection between histone acetylation and gene transcription during DNA replication. Histone acetylation, a crucial chromatin modification, is known to maintain consistent gene expression levels throughout the DNA replication process (31). A recent report in yeast demonstrated that a specific histone H3 modification, H3K56Ac, can inhibit recruitment of general transcription factors to replicating DNA strands. Interestingly, this modification does not appear to affect the binding of site-specific transcription factors (32). These findings suggest that H3K56Ac exerts selective control over general transcription factors during DNA replication, potentially influencing a cell's overall gene expression profile during this critical cellular process. Building upon these results, we predict that general transcription factors may display a DNA strand preference in parental-histone-transfer-defective yeast mutants (*dpb3Δ* and *mcm2-3a*) (18,19). Testing this hypothesis relates to the asymmetric nature of DNA replication and may offer insights into asymmetric cell division, with implications for cell fate determination and tissue development. Thus, in **Objective 3**, we will pursue this intriguing research question, exploring the impact of transcription factors and histone modifications on DNA replication asymmetry and cellular division.

3. PRELIMINARY DATA

3A. A new method, genomic enrichment of ssDNA and sequencing (GESS-seq), to characterize the replication fork structure. Previous studies of DNA replication location and structure have used labeling with thymidine analogs, such as BrdU or EdU, 2D gel analysis, protein ChIP or ChIP-seq, or electronic microscopy (33-36). Although these methods paved the way for DNA replication research, they are labor-intensive or high cost. Recently, we designed a new approach using a shrimp-derived dsDNase (37) to detect the ssDNA region present during DNA replication (Fig. 3A). First, genomic DNA is extracted from yeast cell samples. Next, the extracted DNA is treated with dsDNase, which binds ~30,000 times more efficiently to dsDNA than ssDNA(37). It digests dsDNA into small nucleotides (~8 bp), but ssDNA regions of replication forks are resistant. These remaining ssDNA fragments can be analyzed with qPCR or high-throughput sequencing to determine which areas of the genome host replication forks in a given sample. More important, this method maintains the sequence direction of ssDNA fragments, allowing us to learn more about the distinct processes occurring at the leading and lagging strands of each replication fork.

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To validate GESS-seq, we performed a variety of tests. Budding yeast has two groups of replication origins: early (~134 total) and late (~245 or more total) (11). Hydroxyurea (HU) inhibits DNA replication by inhibiting ribonucleotide reductase. After 0.2M HU treatment, only early origins fire in WT yeast; late origins are inhibited by the cell-cycle checkpoint. However, in the cell-cycle checkpoint defective *rad53-1* mutant, later replication origins also fire after HU treatment. Thus, we expected GESS-seq to show that early replication origins fire in both WT and *rad53-1* mutant strains after HU treatment, but late replication origins fire only in *rad53-1* mutant strains. In the qPCR analysis shown in Fig. 3B, ARS607 and ARS607+2kb (ARS607 and 2kb away from ARS607 locus specific PCR primers) are located within an early replication origin. As expected, after HU treatment, the ARS607+14k primer pair was located in the unreplicated region in both WT and *rad53-1* mutant strains. By contrast, ARS501 is a late replication origin. As expected, it was enriched only in the *rad53-1* mutant. In addition, as expected, G1-phase samples did not display ssDNA enrichment at any tested loci, since the yeast in these samples were not replicating. These data indicate dsDNase efficiently produces DNA samples enriched for the ssDNA region of the replication fork. Our primary mapping findings from this experiment (Fig 3C) confirm G1-phase samples do not show any clear replication-related peaks. Moreover, as expected, after HU treatment, both WT and *rad53-1* mutant strains show clear peaks at early replication regions (origins 101, 102, 106). However, only the *rad53-1* mutant

shows clear peaks at later origins (origins 103, 104, 105). Finally, our GESS-seq data match our data from BrdU-IP-ssSeq, our previous method to track newly synthesized DNA (1-3).

In the checkpoint *rad53-1* mutant, we detected clear lagging strand bias for late but not early replication origins (**Fig 3D**). These results indicate the replication fork structure is different for early and late origins in this checkpoint mutant. We propose a replication fork structure that explains these findings for the *rad53-1* mutant: the late origin has a larger new DNA gap on the leading strand than the early origin (**Fig 1E**). Our BrdU-IP-ssSeq data show both early and late origins have strand bias. However, our GESS-seq data show bias only at the late origin. The undetected strand bias at the early origin may be explained by a broad ssDNA region at the early origin, which masks the strand bias observed with BrdU-IP-ssSeq. In short, these

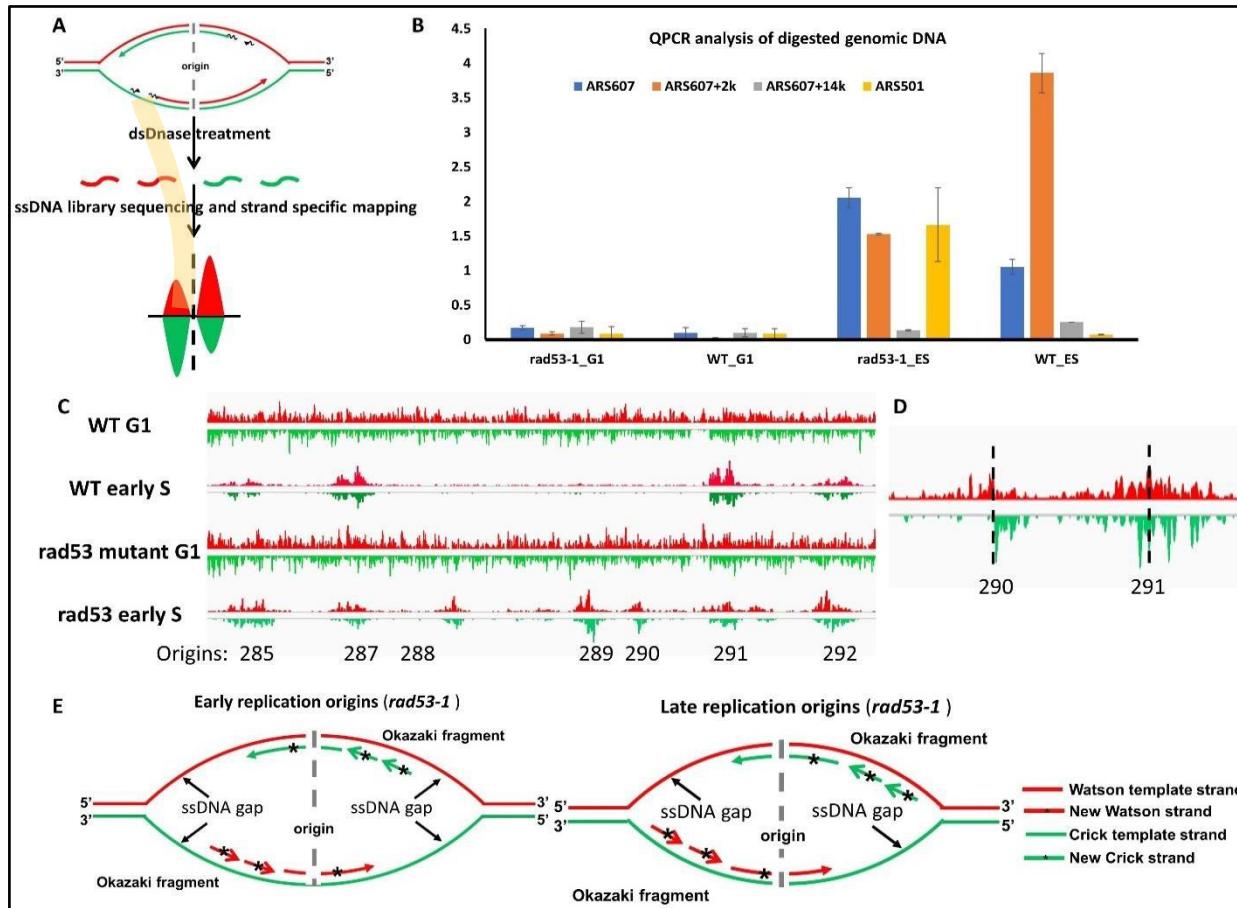


Fig. 3. GESS-seq shows replication fork structures differ at early and late replication origins in the *rad53* checkpoint mutant yeast strain. (A) Schema for GESS-seq. Total DNA in early S-phase cells is extracted and treated with a dsDNA-specific enzyme. Recovered ssDNA is then used for library construction and sequencing. Sequenced reads are mapped to the Watson (red) and Crick (green) strands of the reference genome. (B) qPCR analysis of recovered ssDNA at ARS607 (early) and ARS501 (late) replication origins in yeast. (C) Snapshot of GESS-seq mapping. Signals represent normalized sequence read densities for Watson (red) and Crick (green) strands. (D) Representative example: Enlarged view of origin 290 (late) and 291 (early) from early S-phase sample of *rad53-1* mutant yeast. (E) Model of DNA replication fork structures at early and late replication origins in *rad53-1* mutant.

findings show GESS-seq provides information that cannot be obtained from previous DNA replication structure analysis tools, including our BrdU-IP-ssSeq method (2). GESS-seq and BrdU-IP-ssSeq detect the template and newly synthesized strand, respectively, resulting in distinct, complementary outcomes.

3B. In Pol1 yeast mutant, leading and lagging strands are uncoupled during DNA replication. Previously, we showed the lagging strand DNA polymerase, Pol1 (*pol1-4A*), is involved in parental histone

transfer in yeast (18). In this study, a Pol1 mutation (*pol1_C1372S, F1396S*) was spontaneously generated during our Pol1 gene tagging experiment. To perform BrdU-IP-ssSeq of this mutant (Fig. 4A), G1-arrested cells were released into fresh medium with BrdU and samples were collected at 30 and 40 mins. For each sample, chromatin was fragmented, DNA was extracted, and newly synthesized DNA (labeled with BrdU) was immunoprecipitated. Then, the recovered ssDNA sequencing library was constructed, maintaining ssDNA direction information (ie, sequence data was mapped to the Watson or Crick strand of the yeast genome). In WT cells from both time points, BrdU-labeled DNA was distributed nearly symmetrically on the Watson/Crick strands, indicating equal replication speeds. However, in the 30-min Pol1 mutant sample, the leading strand appeared to replicate faster (ie, have more BrdU-labeled DNA) (Fig. 4B,C). These findings indicate the leading and lagging strands were uncoupled in the replication origin region (Fig. 4D). Moreover, this uncoupling appears to have occurred only at the replication start region, as this phenotype was not present in the 40-min mutant sample. It is also possible the uncoupling phenotype was persistent but could not be detected by BrdU-IP-ssSeq at 40 min due to the broader BrdU track at this time point.

In sum, previously we reported the lagging strand replicates faster than the leading strand in checkpoint mutant cells grown under replication-stressed conditions (2). Here, we report the opposite strand bias in the Pol1 mutant grown under normal conditions. In **Objective 2**, our goal is to understand the uncoupling control mechanism responsible for this phenomenon, as it safeguards genome stability and maintains genetic inheritance. We will employ GEES-seq to test whether this phenomenon is widespread in the genome or limited to DNA replication origin regions, a task that was not feasible with BrdU-IP-ssSeq.

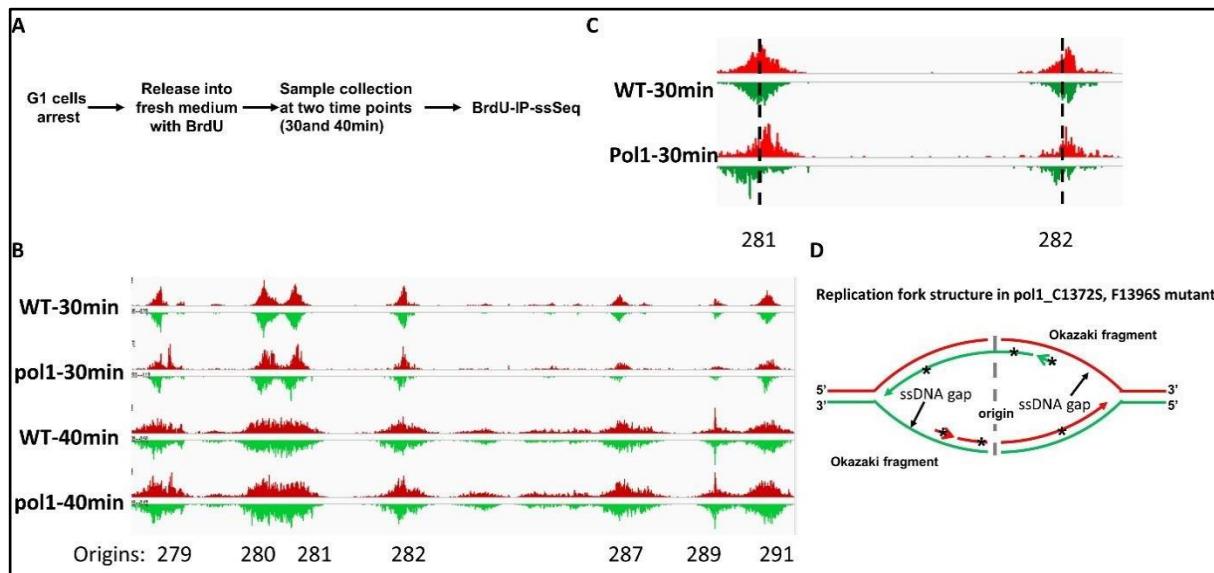


Fig. 4. In *pol1* yeast mutant (*pol1_C1372S, F1396S*), leading and lagging strands are uncoupled during DNA replication. (A) Procedure for BrdU-IP-ssSeq. (B) Snapshot of BrdU IP-ssSeq signals for 30- and 40-min samples from WT and Pol1 mutant cells. In the 30-min sample for Pol1 mutant cells, more BrdU-labeled DNA occurs on the leading strand. Signals represent normalized sequence read densities for the Watson (red) and Crick (green) strands. (C) Enlarged view of origins 281 and 282 for the 30-min samples from (B). (D) Model showing DNA replication fork structure of the Pol1 mutant, with ssDNA gap on newly synthesized lagging strand as leading and lagging strand uncouple.

3C. We have used genetic interactions to infer the strand preference of chaperones for new-histone H3-H4 tetramers during chromatin replication. Newly assembled chromatin is composed of half parental and half newly synthesized H3-H4 tetramers. In yeast, three chaperones of new-histone H3-H4 tetramers—**Caf1, Asf1, and Rtt106**—are reported to be involved in replication-coupled nucleosome assembly (23,24,38,39). Whether they have a replication strand preference or specific roles on the two strands is unknown (39). Due to technical difficulties, we have not been able to directly investigate the strand preference of these chaperones. However, we surmise the following: (A) Multiple H3-H4 tetramer assembly pathways cooperate in DNA replication, so a defect in a single pathway will be partially complemented by an alternative pathway, but disruptions to multiple pathways will have an additive effect; this assumption is

supported by extensive previous work (13,39-41). (B) Mutation of any chaperone of new histones will lead to moderate chromatin instability. (C) We have identified two pathways involved in recycling parental histone H3-H4 tetramers during DNA replication: a leading strand, Dpb3/Dpb4-mediated pathway and a lagging strand, Mcm2-Ctf4-Pol a-mediated pathway (18,19). If a new-histone chaperone prefers the lagging strand, we expect a *dpb3Δ* mutation to exacerbate the chromatin instability present in a new-histone chaperone mutant, as *dpb3Δ* mutants have a defect in transferring parental histones to the leading strand. (D) We expect an *mcm2-3A* mutation to have an additive effect on chromatin instability in a lagging-strand chaperone mutant, as *mcm2-3a* mutants have a defect in transferring parental histones to the lagging strand. We used this rationale to design an experiment to identify each new-histone chaperone's strand preference using the highly sensitive Cre-Reported Altered States of Heterochromatin (CRASH) assay to measure chromatin instability in mutants (42-44) (**Fig 5A**).

The results were as follows: **Caf1**'s largest subunit, Cac1, is also its catalytic subunit. The *dpb3Δ/cac1Δ* mutant showed less silencing loss than the *cac1Δ* mutant, whereas the *mcm2-3A/cac1Δ* mutant showed more silencing loss than the *cac1Δ* mutant (**Fig. 5B,C**). Based on the strong replication strand preference of parental histone H3-H4 tetramers in the *dpb3Δ* and *mcm2-3A* mutants, we predict Caf1 has a lagging strand preference for new histone H3-H4 tetramer assembly (**Fig. 5F**). **Rtt106** showed similar results: the *dpb3Δ* mutation partially complemented the *rtt106Δ* mutation, but the *mcm2-3A* mutation had an additive effect in the *rtt106Δ* mutant (**Fig. 5E**). Thus, we predict Rtt106 has a lagging strand preference (**Fig 5F**). The results for **Asf1** showed the opposite. An *mcm2-3a* mutation partially complemented an *asf1Δ* mutation, and a *dpb3Δ* mutation had an additive effect in the *asf1Δ* mutant (**Fig. 5D**). We predict Asf1 has a leading strand preference (**Fig 5F**). These genetic interaction results prompt us to propose models (**Fig 5F**) to elucidate the strand-specific roles of Caf1, Asf1, and Rtt106. In **Objective 2**, we will rigorously test and strengthen this model, which opens new possibilities for manipulating chromatin stability.

4. PROPOSED RESEARCH ACTIVITIES

4A. Objective 1. Investigate the mechanism by which leading and lagging strands are coupled during DNA replication. While investigating the mechanisms of parental histone transfer, we discovered a Pol1 mutant (*pol1_C1372S, F1396S*) in which the leading and lagging DNA strands are decoupled during DNA replication (**Section 3B**). Currently, PCNA is thought to play a pivotal role in the coupling of leading and lagging strands. Depleting PCNA in vitro results in slower leading strand synthesis and extended lagging strand extension (9). Previously, we showed that under replication stress conditions, lagging strand synthesis in cell cycle checkpoint mutants in yeast progresses faster than leading strand synthesis (2). Under these extreme conditions, DNA strand uncoupling occurs, and the mutant cells die. DNA strand uncoupling in the *pol1_C1372S, F1396S* mutant differs, as it occurs under normal growth conditions and cells remain viable. Indeed, we have investigated several DNA polymerase mutants in the past, including *pol1-4A*, *pol2-04*, and *pol3-01* (2,19), and none have exhibited the uncoupling phenotype seen in the *pol1_C1372S, F1396S* mutant. DNA replication strand uncoupling is intriguing as it provides insights into the accuracy of DNA synthesis and repair. Thus, in this objective, we will use the *pol1_C1372S, F1396S* mutant to uncover the mechanism for coupling leading and lagging strands in DNA replication.

First, we will determine which point mutation, *pol1-C1372S* or *pol1-F1396S*, is responsible for the uncoupling phenotype (**Experiment 1**); it is possible both are required. Pol1 functions in initiating and elongating Okazaki fragments (45), which plays a critical role in maintaining genetic inheritance. Not surprisingly, we have also noticed growth retardation in this mutant (**Experiment 2**), which could be caused by an inefficient DNA replication process or activation of the cell cycle checkpoint. Our experiments in this objective will provide the data to distinguish between these possibilities (**Experiment 3**). Finally, we will use GESS-seq to characterize the replication fork structure of this mutant (**Experiment 4**).

Experimental design. (1) *Determining the mutation responsible for the DNA uncoupling phenotype.* Our Pol1 mutant (*pol1_C1372S, F1396S*) contains two point mutations. Given the high frequency of yeast homologous recombination, it will be straightforward to generate two individual mutants: *pol1_C1372S* and *pol1_F1396S*. We will obtain a DNA fragment containing the site-specific mutation, a drug resistance

selection marker, and a short flanking homologous arm. This DNA fragment will be introduced into yeast cells, and drug-resistant colonies will be selected and subjected to Sanger sequencing. Single mutants will

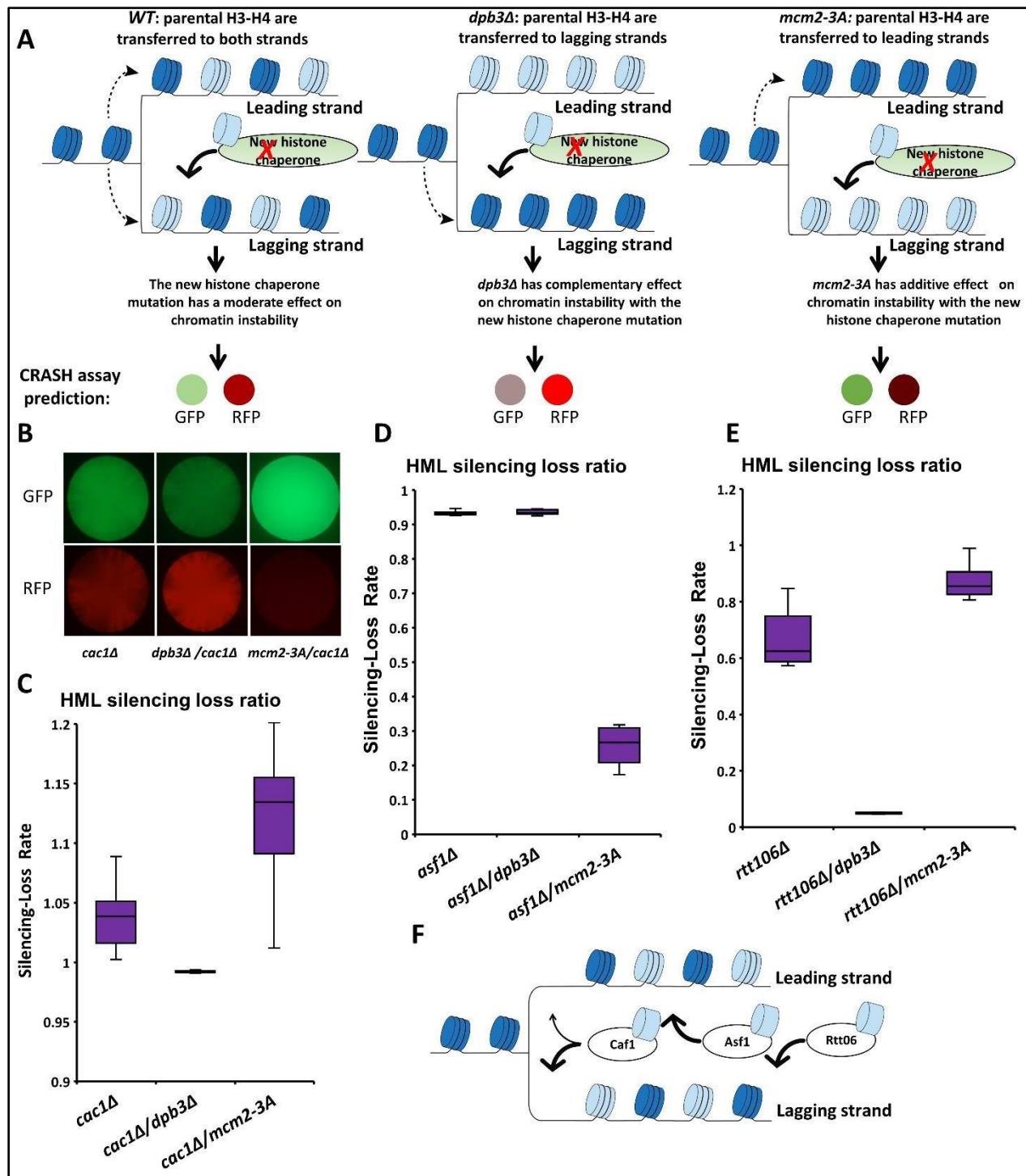


Fig. 5. Strand preference of chaperones for new-histone H3-H4 tetramers during chromatin replication, inferred from genetic testing in yeast. (A) Experiment rationale. (B) Representative fluorescence images of colonies derived from *cac1Δ*, *dpb3Δ/cac1Δ*, and *mcm2-3A/cac1Δ* mutants, obtained using the CRASH assay. Strains contain LoxP-RFP-LoxP-GFP cassette at *URA3* and *HMLα::cre* at the silenced HML locus. A bright GFP channel and dark RFP channel indicate loss of silencing. (C) Silencing loss rates for *cac1Δ* mutants from (B). (D-E) Silencing loss rates for *asf1Δ* and *rtt106Δ* mutants (C) Model showing replication strand preferences for Caf1, Asf1, and Rtt106 based on CRASH assay data. Arrow thickness reflects relative preference.

be tested for the uncoupling phenotype using BrdU-IP-ssSeq (**Fig. 4**).

(2) *Determining the growth rate of the pol1_C1372S, F1396S, pol1_C1372S, and F1396S mutants.* We have observed growth retardation relative to WT cells in the *pol1_C1372S, F1396S* mutant. Here, we will compare the growth rate of all three mutants to WT cells and to one another under various conditions. (A) Cell growth dot assay: Replication-component mutants are often sensitive to DNA replication inhibitors (e.g., HU) and DNA damage agents (e.g., methyl methanesulfonate, or MMS). We will dot serially diluted yeast cells onto yeast cell culture plates with or without HU and MMS to determine the relative growth rate under normal and stress conditions. (B) DNA content analysis by flow cytometry: G1 arrested cells will be released into fresh rich culture medium (YPD). Samples will be collected every 20 min throughout the cell culture cycle. This flow cytometry assay will monitor DNA replication speed in a single cell cycle. Compared to WT cells, we anticipate that the mutant cells will display growth retardation and reduced DNA replication speed.

(3) *Investigating the relationship between activation of the S-phase checkpoint pathway and the DNA replication uncoupling phenotype.* Uncoupling of the leading and lagging DNA strands during replication results in a stretch of ssDNA on the template strand of the lagging strand. This ssDNA can activate the S-phase cell cycle checkpoint pathway (46), which preserves the function and structure of stalled DNA replication forks and prevents chromosome fragmentation. Understanding the relationship between activation of the S-phase checkpoint pathway and DNA replication uncoupling will offer a mechanistic explanation for how synthesis of DNA replication strands is coordinated. In yeast, S-phase checkpoint activity can be monitored by analyzing phosphorylated Rad53 levels (47) via western blot. We will investigate the relationship between DNA strand uncoupling and the checkpoint pathway by analyzing the genetic interaction between the *rad53-1* mutation (an S-phase checkpoint defective mutant) and our *pol1* mutations. Yeast tetrad dissection method will be used in this analysis. Diploid yeast cells (*rad53-1/+; pol1_C1372S, F1396S/+*) will be dissected into four spores using a yeast dissection microscope, and the resulting haploid cells' growth phenotype will be monitored. If the S-phase checkpoint pathway is essential or important for growth of the *pol1* mutant, the *rad53-1/pol1_C1372S, F1396S* mutant will grow slower than the *pol1_C1372S, F1396S* mutant or even exhibit lethality, resulting in smaller colonies or empty spots on the segregated tetrad dissection. Thus, smaller colonies or empty spots would indicate that the DNA replication uncoupling will activate the S-phase checkpoint pathway.

(4) *Using GEES-seq to characterize the replication fork structure in the pol1_C1372S, F1396S mutant.* In our preliminary analysis, we observed uncoupling of the leading and lagging strands in the *pol1_C1372S, F1396S* mutant during DNA synthesis at an early (30 min) but not late (40 min) time point (**Section 3B**). Two possible explanations exist: (A) The leading strand progresses faster than the lagging strand at both time points, but the BrdU-IP-ssSeq method does not detect the difference at the later time point due to an extended BrdU track; or (B) Uncoupling only occurs near the replication start site, and lagging strand synthesis quickly catches up. (A) would indicate the uncoupling occurs during the elongation stage, while (B) would suggest it is limited to the fork initiation stage. It may also suggest that Pol1 plays different roles in DNA replication initiation and elongation. To distinguish between these possibilities, we developed GEES-seq, which uses a specific dsDNase to enrich ssDNA regions (**Section 3A**) and can potentially detect strand bias in genomic regions far from the replication origin. We will employ GEES-seq to investigate replication fork structure in the *pol1_C1372S, F1396S* mutant to provide insights into the uncoupling phenomenon, using procedures similar to those used in the BrdU-IP-ssSeq experiment from **Section 3B**. Data analysis. The GEES-seq sequencing analysis pipeline will follow the same procedures as the BrdU-IP-ssSeq analysis. The key distinction lies in the final data interpretation and modeling. Sequence data from BrdU-IP-ssSeq (already obtained) represents newly synthesized DNA, whereas data from GEES-seq represents all ssDNA, whether newly synthesized or part of the parental template. Our final model of DNA replication fork structure will be proposed based on analysis of both types of data, providing a comprehensive understanding of replication dynamics in the *pol1_C1372S, F1396S* mutant.

Potential pitfalls and alternative strategies: If the *pol1_C1372S* and *F1396S* mutations exhibit additive growth retardation and replication strand uncoupling, we will construct the model based on the Pol1 structure rather than focusing solely on the point mutations only.

Integrated educational plan. Quinn Dickinson from the Bioinformatics and Computational Biology (BICB) program of University of Minnesota will analyze the GESS-seq data.

Expected outcomes: By investigating the *pol1_C1372S, F1396S* mutant, we anticipate gaining valuable insights into the mechanism that couples leading and lagging strands during DNA replication. Understanding the role of Pol1 in the initiation and elongation phases of DNA replication could have implications for genome stability and genetic inheritance. Confirming the relationship between DNA strand uncoupling and the S-phase checkpoint pathway may shed light on how cells preserve replication fork function and prevent genomic instability. Applying GESS-seq to study our Pol1 mutant will further enhance our understanding of replication dynamics. Overall, the findings from Objective 1 will contribute to a broader understanding of DNA replication mechanisms and their significance in maintaining genomic stability.

4B. Objective 2. Study the biochemical and functional strand preferences of chaperones for newly produced H3-H4 histones (Caf1, Asf1, Rtt106). Half of H3-H4 tetramers are synthesized de novo, while the other half originate from recycled parental histones (16,17). In yeast, Caf1, Asf1, and Rtt106 participate in nucleosome assembly for new-histone H3-H4 tetramers. In our previous work, we identified two parental histone chaperone transfer pathways, one for the leading strand and one for the lagging strand (18,19). It remains unknown whether the new-histone H3-H4 chaperones prefer the leading or lagging strand during chromatin replication. It is important to establish strand preference because these histone chaperones play a crucial role in regulating chromatin dynamics and maintaining genome integrity. Our preliminary data suggest Caf1 and Rtt106 mainly function on the lagging strand, whereas Asf1 primarily functions on the leading strand (**Section 3C**). In this objective, we will more rigorously test whether these new-histone H3-H4 chaperones (Caf1, Rtt106, and Asf1) have replication strand preferences.

Experimental design. (1) *Investigate strand preferences for parental and new-histone H3-H4 chaperones using the CRASH assay.* In our preliminary studies on strand preference, we tested several genetic interactions using the CRASH assay, including for *cac1Δ-dpb3Δ, cac1Δ-mcm2-3a; rtt106Δ-dpb3Δ, rtt106Δ-mcm2-3a; and asf1Δ-dpb3Δ, asf1Δ-mcm2-3a* (**Section 3C**). Here, we will determine the strand preference for one additional factor, this one in the parental histone chaperone pathway, by introducing the *dpb4Δ* mutation. This addition will allow us to test and solidify our strand preference models of histone chaperones (**Fig 5F**). We predict results for the *dpb4Δ* mutant will mimic those for the *dpb3Δ* mutant.

(2) *Confirm strand preferences for parental and new-histone H3-H4 chaperones using loss of silence at a subtelomeric region.* We will employ loss of silence at a subtelomeric region. In addition to investigating genetic interactions at the mating type locus (as in the CRASH assay), we will examine them at a subtelomeric region—the end of the right arm of chromosome VII—utilizing the URA3 marker (48). It is important to validate our strand preference findings with interactions at another genome location because multiple gene silence pathways are involved in chromatin instability, some of which are locus dependent. URA3 expression at this telomere region varies between cells, resulting in differential growth on media lacking uracil or containing fluoroorotic acid (FOA), a drug that kills cells expressing URA3. We will use this system to test genetic interactions between *cac1Δ-dpb3Δ, cac1Δ-mcm2-3a; rtt106Δ-dpb3Δ, rtt106Δ-mcm2-3a; and asf1Δ-dpb3Δ, asf1Δ-mcm2-3a*. As an example, on uracil-deficient plates, we predict *cac1Δ/dpb3Δ* mutants will display slower growth than *cac1Δ* mutants, whereas *cac1Δ/mcm2-3a* mutants will display faster growth. We predict the reverse pattern on FOA plates.

(3) *Perform strand-specificity analysis of new-histone H3-H4 chaperones Caf1, Rtt106, and Asf1 using eSPAN.* Our eSPAN method (1) allows us to directly test a target protein's physical strand preference. We will perform eSPAN on Caf1-5flag, Rtt106-5flag, and Asf1-5flag strains, which are already available in my laboratory, using cells arrested in early S phase with HU. Under these conditions, only ~2 kb DNA around the replication origin will be replicated. We will validate ChIP (the first step of eSPAN) using qPCR primers for positive (ARS607) and negative (ARS607+14k) loci (1,26). For the remaining steps, we will follow the standard eSPAN protocol (1). We predict the results will support our preliminary findings: Caf1 and Rtt106 prefer the lagging strand, whereas Asf1 prefers the leading strand.

(4) *Explore the functional genetic interaction between PCNA and parental histone H3-H4 chaperones.* Recruitment of Caf1 to the DNA replication fork occurs via PCNA (21). PCNA mutations (*pol30-8* or *pol30-79*) can disrupt the PCNA-Caf1 interaction and completely inhibit Caf1 chromatin recruitment (39). In this

experiment, we will use the CRASH assay to test whether these PCNA mutations mimic the *cac1Δ* mutation when combined with *dpb3Δ* or *mcm2-3A* mutation. We predict the *dpb3Δ/pol30-879* will show lower levels of silencing than the *cac1Δ* mutant, whereas the *mcm2-3A/pol30-879* mutant will show greater levels of silencing. We expect these findings to further validate our model, in which Caf1 has a lagging strand preference for new-histone H3-H4 tetramer assembly and this preference depends on PCNA.

Potential pitfalls and alternative strategies. Given my previous experience with the CRASH and subtelomeric loss-of-silence assays, I do not anticipate potential pitfalls during these experiments. However, the results obtained at the mating type locus and subtelomeric region may differ. This would indicate that these histone chaperones' strand preferences are locus-dependent and we would modify our model accordingly. We may also encounter challenges using eSPAN, due to low ChIP efficiency resulting from the indirect interaction of new histone H3-H4 chaperones with DNA. If this occurs, we will explore approaches to increase ChIP efficiency, such as native ChIP or a two-step crosslinking protocol (49,50).

Integrated educational plan. The genetic interaction assay can be segmented into smaller projects suitable for high school students (see **Section 6B**). They will conduct yeast dot assays, analyze the results, and present their findings at their science fairs. Simultaneously, two graduate students from the BICB program will be responsible for analyzing the histone chaperones' eSPAN data.

Expected outcomes. Our findings will shed light on how these histone chaperones regulate chromatin dynamics and genome integrity. The results may confirm our preliminary results, that Caf1 and Rtt106 mainly function on the lagging strand, while Asf1 primarily functions on the leading strand. Overall, these findings will improve our understanding of histone chaperones' roles in chromatin replication and regulation.

4C. Objective 3. Examine the DNA strand preferences of transcription factors during the DNA replication process. During DNA synthesis (S phase), gene dosage increases for partially replicated DNA while remaining the same for unreplicated regions. Previous studies have indicated that histone modification, specifically H3K56 acetylation by the acetyltransferase enzyme Rtt109, helps maintain consistent gene dosage by inhibiting gene transcription (31). Based on the asymmetric deposition of newly synthesized histone H3-H4 tetramers (marked by H3K56Ac) in DNA polymerase ε (*dpb3Δ* or *dpb4Δ*) mutants or MCM2 helicase (*mcm2-3A*) mutants (**Section 3C**), we hypothesize that transcription factors on the recently replicated DNA strand will exhibit near-symmetrical binding in WT cells but asymmetrical binding in *dpb3Δ* (or *dpb4Δ*) and *mcm2-3A* mutants. Confirming this prediction is correct could link parental histone transfer to asymmetric cell division, with important implications for cell fate determination and tissue development. To test our hypothesis, we will use eSPAN, the method we developed, to detect strand-specific binding of two site-specific transcription factors: **Reb1** and **Phd1**. Reb1 regulates transcription by all three yeast RNA polymerases and promotes the formation of nucleosome-free regions (51,52). Phd1 is a transcriptional activator of genes involved in yeast pseudohyphal growth (53). We selected these transcription factors because they are well characterized and their ChIP-seq data are available (54).

Experimental design. Transcription factor tagging. Reb1 has approximately 1700 binding sites across the yeast genome and Phd1 has 967, making them suitable targets for eSPAN. Reb1 will be tagged with 5 FLAG and PHD1 with 3HA (55,56). The general transcriptional factor RNA polymerase II will also be targeted, specifically the RPB3 component, which will be tagged with MYC-tag. The commercially available Millipore RNA Pol2-5P antibody (04-1572-I), which has previously been tested in our lab, will be used. Public ChIP-seq data is available (57) and will be used as a control.

Yeast strains. To ensure consistency, all gene tags (Reb-5FLAG, PHD1-3HA, RPB3-9MYC) will be genetically crossed into the WT, *dpb3Δ*, and *mcm2-3A* strains.

Procedure. We will collect samples (WT, *dpb3Δ*, and *mcm2-3A* cells) in a single experiment and store them at -80°C. Synchronized cells will be used to increase eSPAN efficiency. Yeast cells will be grown in YPD medium to exponential phase and then arrested in G1 phase using two doses of α factor for 3 hours at 25°C. Samples will then be released into fresh YPD medium containing BrdU. Two time points (30 min, 40 min) will be collected to cover most transcription factor binding sites and capture transcription factor binding after DNA replication fork formation. Samples collections will follow the standard eSPAN method (1,4).

Potential pitfalls and alternative strategies. Previously, our eSPAN data analysis focused on mapping at the replication fork (1). eSPAN requires predefined replication direction at specific locus. We analyzed only a ~5 kb region around replication origins using HU-arrested early S-phase samples. The replication direction is predetermined near strong replication origins. However, transcription factors are evenly distributed across the genome. The replication direction at a specific genomic locus will be over 90% uniform if it is near a replication origin, but ambiguous if the locus is far from the origin. To address this challenge, we will leverage previously mapped yeast Okazaki fragment data as a replication direction reference (58) and assign a penalty score based on Okazaki fragments' reads at each location for the final analysis. Given our extensive experience with eSPAN, this additional data treatment step will be straightforward.

Integrated educational plan. Two graduate students from the BICB program will perform the experiments and data analysis.

Expected outcomes. By investigating the DNA replication strand preferences of transcription factors using eSPAN, we will gain insights into the mechanisms that coordinate gene expression and DNA replication asymmetry. This research has the potential to unveil novel regulatory networks and shed light on the broader implications of DNA replication dynamics for cellular processes and organismal development.

5. INTELLECTUAL MERIT

This proposal harnesses the power of recent methodological advances, such as eSPAN and CRASH assays(1,42), to develop **GESS-seq**, a new technology to unravel chromatin replication in budding yeast. Thus far, the phenomenon of DNA replication strand uncoupling *in vivo* has received limited attention due to technical challenges. However, our preliminary data using BrdU-IP-ssSeq clearly demonstrate an uncoupling phenotype (**Section 3B**). Understanding the mechanisms underlying uncoupling is of paramount importance for gaining insights into genome instability, and this study will provide key knowledge in this area. In addition, although multiple new-histone H3-H4 chaperones have been identified(39), how they cooperate during chromatin replication remains elusive. Our proposed strand division model (**Section 3C**) will shed light on the functional diversity among these chaperones and elucidate the molecular mechanisms underlying their roles in chromatin replication. Finally, previous research has shown that interference between transcription and replication during the DNA replication process can impact genome stability and DNA repair(32). However, how transcription factors associate with the two DNA replication strands remains unclear. Our results regarding the strand preference of transcription factors will provide novel insights into this process and may uncover the chromatin-based mechanisms underlying asymmetric cell division. Collectively, these contributions will advance our understanding of chromatin replication.

6. INTEGRATED EDUCATIONAL PLAN

The intricate relationship between chromatin structure and gene expression has made epigenetics an essential component of nearly every branch of biological research. As a professor, I have observed firsthand how this exciting field sparks students' passion for biology. This integrated educational plan capitalizes on the growing interest in epigenomics to empower undergraduates and high school students from diverse backgrounds to excel in biological research. It comprises two evidence-based components: creating (1) an epigenomics module for undergraduates participating in the Hormel Institute (HI)'s research experience programs and (2) a training program that allows high school students to complete sophisticated science fair projects. Both programs will foster students' scientific curiosity, enhance their critical thinking and problem-solving skills, and prepare a new, more diverse generation of biology researchers.

6A. Educational Objective 1: Add an epigenomics training module to the Hormel Institute's current research experience programs for undergraduates. Undergraduates who engage in research have a significantly higher GPA than those who do not, even after controlling for scholastic aptitude (59) and they perform better in graduate school (60). Research experiences are also the single most effective intervention for promoting the persistence of undergraduates from underrepresented groups in STEM (61). The HI offers two research training programs for undergraduates: the Summer Undergraduate Research Experience (SURE) program and the Student Training Experience Program (STEP). Every year, the **10-week SURE program** allows ~25 junior or senior undergraduates from across the country to work with HI scientists on biomedical research projects as paid summer interns. Students share their findings with the HI community

in research posters and presentations. The program also provides low-cost, on-campus housing and professional development and teambuilding activities, consistent with best practices in summer research experiences (62) (63). HI collaborates with local colleges to recruit diverse trainees and advertises the program nationally and internationally. As a result, ~60% of recent SURE participants have been women and ~40% have come from underrepresented groups. In the new **STEP program**, HI labs host University of Minnesota at Rochester (UMR) undergraduates for 1 year. STEP interns work in the lab for 90 hours/semester, allowing them to gain practical research experience and apply the knowledge they have gained from coursework. This Fall, the programs' first semester, five interns will participate; the program will expand in the future, with students from underrepresented groups being prioritized.

To further enrich these programs, we will integrate an epigenomic training section into each program to provide specialized knowledge and research opportunities in this rapidly advancing field. The *Epigenomic Training Program* will consist of two parts: lectures and lab workshops that will meet once a week, for 6 weeks (**Table 1**). A comprehensive series of lectures will cover fundamental concepts in epigenomics. Lectures and labs will include interactive activities, group discussions, and Q&A sessions to encourage active participation. This kind of active learning facilitates a deeper understanding of the subject matter (64) and also narrows achievement gaps for underrepresented students in STEM (65).

Table 1. Epigenomic course topics

Week	Session	Example Topics & Activities
1	Lecture: Introduction to epigenetics and epigenomics	Historical overview and significance of epigenetics, epigenetic modifications, epigenomics techniques and their applications
2	Lecture: Techniques and technologies in epigenomics	High-throughput sequencing technologies, bisulfite sequencing, ChIP-seq, RNA-seq, data analysis tools.
3	Lecture: Epigenomics and gene regulation	Chromatin structure and gene regulation, effect of DNA methylation and histone modifications on gene expression
4	Lecture: Epigenetic inheritance and development	Transgenerational epigenetic inheritance, effect of epigenetic regulation on development, DNA replication and epigenetics
5	Lab: ChIP-seq part 1	Sample preparation, chromatin shearing and immunoprecipitation, DNA purification and qPCR analysis.
6	Lab: ChIP-seq part 2	ChIP DNA end-repair, adaptor ligation, library amplification & quantification

Quality mentoring is a critical component of a positive research experience (62,66), particularly for students from underrepresented groups (63). Thus, two graduate teaching assistants (TAs) will assist the students in the lectures and workshops. These TAs, selected for their research excellence, enthusiasm for mentoring, and lived experience as students from underrepresented groups, will serve as near-peer mentors and role models for the undergraduates. Their participation in group discussions, Q&A sessions, and informal discussions about their lives as graduate students will be especially valuable. Of note, near-peer mentoring improves the interest and engagement of mentees while simultaneously contributing to the personal, educational, and professional growth and persistence in science of the near-peer mentors (67) (68). Near-peer mentoring also improves mentees' sense of belonging in science and bolsters their positive science identities (69). It is thus a powerful strategy for providing students with individual-level support while also benefiting the maximum number of trainees (ie, students and their near-peer mentors).

All SURE and STEP students will be encouraged to attend the first, introductory Epigenomics lecture, where they will receive complimentary lunch and snacks. We will also invite other interested undergraduate and graduate students to attend, including those working at HI or attending UMR. Participation in subsequent lectures and labs will be voluntary and based on individual interests and needs. We anticipate all 25 SURE and >5 STEP students, as well as ~20 additional students, will participate in the first lecture. We expect 10 students to participate in the subsequent lectures and labs. To incentivize students to complete the program, we will provide certificates to all participants who finish the whole program. At the end of the program, we will also distribute a resource sheet with additional research opportunities, fellowships, grad programs, and

job opportunities at the Mayo Clinic's sequencing facility and epigenomic service core, as well as in other epigenomics-related labs. In this way, we will empower participants to continue their educational journey, pursue research opportunities, and explore potential career paths in STEM. Throughout, we will foster a sense of community and connectivity, assuring students that the knowledge and skills they gain can be further nurtured and applied in the broader scientific community. To facilitate the offering of similar epigenomics training programs in other locations, we will freely offer our curricula on the HI website. We will advertise the availability of these materials with posters and presentations on the program and its curricula at relevant meetings, such as the HI Symposium and UMR Research and Education Symposium.

6B. Educational Objective 2: Develop a new yeast genetics training program for high school students interested in completing a science fair project. Participating in a science fair has significant benefits for high school students. Even after controlling for pre-existing differences in attitudes, students who advance to a regional science fair show substantial gains in science self-efficacy, interest, and value perceptions, whereas students who do not participate in a science fair show declines in all three variables over the same time period (70). Moreover, undergraduates who engage in scientific research frequently participated in high school science fairs (71), emphasizing the lasting foundation for science success that these early research experiences build. Unfortunately, many students interested in doing a science fair project are hampered by a lack of resources and direction (72). In addition, students from underrepresented groups are not only less likely to participate in science fairs; when they do participate, they are less likely to receive help from parents, scientists, or teachers, putting them at an unfair disadvantage (73).

To ensure high school students from underrepresented groups have the opportunity to engage in a well-resourced, mentored science fair project, we will develop a genetic training program at HI where these students can learn the basics of experimental work with yeast. Yeast are simple to cultivate, grow rapidly, and are easy to manipulate genetically. Moreover, findings from yeast experiments have real-world relevance but do not pose ethical quandaries. While completing their projects, students will learn the fundamentals of scientific research, laboratory techniques, and critical thinking. Under the guidance and mentorship of our experienced research team, 10 local students a year will have the opportunity to select a research topic within the realm of yeast biology and conduct experiments in our well-equipped laboratory. Our community outreach and education manager, Kelly Vinclette (M.Ed.), will help enroll students and arrange their activities. Each year, the 3-month program will begin in November, providing students with adequate time to plan, conduct, and complete their research projects for science fairs (which usually take place in February). The 10 students will work in two small groups of five, each group supervised by undergraduate or graduate near-peer mentors. Students will present their discoveries at their school science fairs, allowing them to share their findings with their peers and the wider community. In addition, to hone their scientific communication skills, students will work with each other and their near-peer mentors to create short videos about their findings, which they will have the option of posting to YouTube. Finally, to help students transition to college-level biology, their near-peer mentors will stay in touch after the program ends, to offer encouragement and advice as they choose high school classes, apply to college, and make decisions about their future study and career trajectories. Students will also be provided with a resource sheet that provides information about colleges with strong epigenomics and genetics departments, scholarship opportunities, tips for applying to college, and more.

To recruit students from underrepresented groups, we will collaborate with local schools, particularly Austin High School, which has a diverse student population (~40% from underrepresented groups). Every Fall, we will conduct yeast genetic and epigenetic experimental demonstrations during visits to local high schools and also during fieldtrips in which high school biology classes come visit the HI. We will engage students in fun and easy experiments such as microscopically dissecting the segregation of yeast genetic traits and observing epigenetic instability in colored colonies. Ms. Avihe Kaliponka, an undergraduate from Namibia who is currently working in our lab through the SURE program, will assist in these recruitment efforts and serve as a role model for students from underrepresented groups interested in pursuing research careers.

In short, this program will foster students' passion for scientific inquiry, develop their critical thinking and problem-solving skills, and provide invaluable experience in presenting their work. It will be conducted outside regular school hours or during designated project periods, minimizing disruption to students' academic commitments, and our lab will help students secure transportation to the HI as necessary. The

program's small size reflects these young students' need for individual attention and a conducive learning environment, but we expect participants will serve as ambassadors for scientific research among their peers.

Evaluation. By incorporating an epigenomic training section into existing research experience programs for undergraduates and introducing a yeast genetic training program for high school students, our goal is to provide budding researchers with valuable educational opportunities, foster a love for scientific exploration, and cultivate the next generation of biology researchers. Evaluation specialist John N. Murray (M.A.), from UMN's Extension Department of Youth Development, will formally evaluate our progress toward these broader goals, as well as toward completing the objectives described above. Progress will be evaluated with pre-and post-intervention student surveys about the helpfulness of each program and their intentions to continue studying biology and pursuing research careers, and with metrics such as the number of students who complete each program, including the percentage who belong to underrepresented groups. Every year, we will review data with the evaluator to improve our offerings for the following year.

7. BROADER IMPACTS

This project will yield substantial benefits for the research community and society at large. By integrating data from biochemical, genetic, and genomic approaches, we will propose novel mechanisms and models that significantly advance our understanding of chromatin replication. This project will also pioneer an innovative technology, GESS-seq, that the broader scientific community can use to investigate the DNA replication fork with a level of precision never before possible. This will open new areas of exploration in the field of chromatin replication research., and the new scientific knowledge and tools produced will further two of NSF's Strategic Objectives, advancing the frontiers of research and enhancing research capability.

In addition, a key objective of this project is to train and mentor a diverse group of 5 graduate students, 50 undergraduate students, and 50 high school students in microbial biology, genetics, epigenomics, and bioinformatics. Postdoctoral fellows and graduate students will play an integral part in carrying out the proposed research. They will gain hands-on experience with state-of-the-art techniques, such as GESS-seq, and contribute to advancing scientific knowledge in chromatin replication. In addition, our novel training programs for undergraduates and high school students will equip talented students from underrepresented groups with the research experience and professional support they need to pursue further study in biology and, if they desire, careers in research. Our work with trainees will further two additional NSF strategic objectives: to ensure accessibility and inclusivity and unleash STEM talent for America.

Finally, by disseminating our findings through publications, conference presentations, and collaborations, we will share the knowledge and insights gained with the scientific community, contributing to the field's collective understanding of genome integrity, regulation, and chromatin biology.

8. RESULTS FROM PRIOR NSF SUPPORT

The PI has no prior NSF awards to report.

9. TIMELINE

Objective	Year 1	Year 2	Year 3	Year 4	Year 5
1	Genetic, biochemical analysis of Pol1 mutant (QD,CY)		Sequencing, analysis of genetic mutants (QD)		Manuscript preparation (QD, TBN1, CY)
	Sequencing, analysis of genetic mutants (QD)				
2	Yeast stain construction and eSPAN experiment (TBN1, CY)		Genomic sequencing, data analysis (TBN1, TBN2)		Manuscript preparation (TBN1, TBN2, CY)
3	Yeast strain construction and genetic analysis (TBN3, CY)		Genomic sequencing, data analysis (CY, TBN3, TBN4)		Manuscript preparation (TBN3, TBN4, CY)

CY, Chuanhe Yu (PI); QD, Quinn Dickinson (graduate student); TBN1-4, graduate students yet to be named.

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**SUMMARY
PROPOSAL BUDGET**

YEAR 1

		FOR NSF USE ONLY		
		PROPOSAL NO.		DURATION (months)
		2339605		Proposed Granted
		AWARD NO.		
ORGANIZATION University of Minnesota-Twin Cities				
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu				
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months		Funds Requested By proposer
		CAL	ACAD	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		1.8		16,760
2.				
3.				
4.				
5.				
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)		0.0		0
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		1.8		16,760
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (0) POST DOCTORAL SCHOLARS		0.0		0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0		0
3. (2) GRADUATE STUDENTS				22,144
4. (1) UNDERGRADUATE STUDENTS				11,648
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0
6. (0) OTHER				0
TOTAL SALARIES AND WAGES (A + B)				50,552
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				24,887
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				75,439
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)				
TOTAL EQUIPMENT				0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000
2. INTERNATIONAL				0
F. PARTICIPANT SUPPORT COSTS				
1. STIPENDS \$ 0				
2. TRAVEL 0				
3. SUBSISTENCE 0				
4. OTHER 0				
TOTAL NUMBER OF PARTICIPANTS (0)			TOTAL PARTICIPANT COSTS	0
G. OTHER DIRECT COSTS				
1. MATERIALS AND SUPPLIES				25,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500
3. CONSULTANT SERVICES				0
4. COMPUTER SERVICES				0
5. SUBAWARDS				0
6. OTHER				2,500
TOTAL OTHER DIRECT COSTS				30,000
H. TOTAL DIRECT COSTS (A THROUGH G)				107,439
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) MTDC (Rate: 59.0, Base: 94329)				
TOTAL INDIRECT COSTS (F&A)				55,654
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				163,093
K. FEE				0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				163,093
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$		
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

**SUMMARY
PROPOSAL BUDGET**

YEAR 2

		FOR NSF USE ONLY		
		PROPOSAL NO.		DURATION (months)
		2339605		Proposed Granted
		AWARD NO.		
ORGANIZATION University of Minnesota-Twin Cities				
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu				
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months		Funds Requested By proposer
		CAL	ACAD	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		1.8		17,095
2.				
3.				
4.				
5.				
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)		0.0		0
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		1.8		17,095
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (0) POST DOCTORAL SCHOLARS		0.0		0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0		0
3. (2) GRADUATE STUDENTS				22,587
4. (1) UNDERGRADUATE STUDENTS				11,648
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0
6. (0) OTHER				0
TOTAL SALARIES AND WAGES (A + B)				51,330
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				25,122
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				76,452
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)				
TOTAL EQUIPMENT				0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000
2. INTERNATIONAL				0
F. PARTICIPANT SUPPORT COSTS				
1. STIPENDS \$ 0				
2. TRAVEL 0				
3. SUBSISTENCE 0				
4. OTHER 0				
TOTAL NUMBER OF PARTICIPANTS (0)			TOTAL PARTICIPANT COSTS	0
G. OTHER DIRECT COSTS				
1. MATERIALS AND SUPPLIES				25,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500
3. CONSULTANT SERVICES				0
4. COMPUTER SERVICES				0
5. SUBAWARDS				0
6. OTHER				2,500
TOTAL OTHER DIRECT COSTS				30,000
H. TOTAL DIRECT COSTS (A THROUGH G)				108,452
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) MTDC (Rate: 59.0, Base:95342)				
TOTAL INDIRECT COSTS (F&A)				56,252
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				164,704
K. FEE				0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				164,704
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$		
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

**SUMMARY
PROPOSAL BUDGET**

YEAR 3

		FOR NSF USE ONLY		
		PROPOSAL NO.		DURATION (months)
		2339605		Proposed Granted
		AWARD NO.		
ORGANIZATION University of Minnesota-Twin Cities				
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu				
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months		Funds Requested By proposer
		CAL	ACAD	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		1.8		17,437
2.				
3.				
4.				
5.				
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)		0.0		0
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		1.8		17,437
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (0) POST DOCTORAL SCHOLARS		0.0		0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0		0
3. (2) GRADUATE STUDENTS				23,039
4. (1) UNDERGRADUATE STUDENTS				11,648
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0
6. (0) OTHER				0
TOTAL SALARIES AND WAGES (A + B)				52,124
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				25,362
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				77,486
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)				
TOTAL EQUIPMENT				0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000
2. INTERNATIONAL				0
F. PARTICIPANT SUPPORT COSTS				
1. STIPENDS \$ 0				
2. TRAVEL 0				
3. SUBSISTENCE 0				
4. OTHER 0				
TOTAL NUMBER OF PARTICIPANTS (0)			TOTAL PARTICIPANT COSTS	0
G. OTHER DIRECT COSTS				
1. MATERIALS AND SUPPLIES				25,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500
3. CONSULTANT SERVICES				0
4. COMPUTER SERVICES				0
5. SUBAWARDS				0
6. OTHER				2,500
TOTAL OTHER DIRECT COSTS				30,000
H. TOTAL DIRECT COSTS (A THROUGH G)				109,486
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) MTDC (Rate: 59.0, Base:96376)				
TOTAL INDIRECT COSTS (F&A)				56,862
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				166,348
K. FEE				0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				166,348
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$		
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

**SUMMARY
PROPOSAL BUDGET**

YEAR 4

		FOR NSF USE ONLY		
		PROPOSAL NO.		DURATION (months)
		2339605		Proposed Granted
		AWARD NO.		
ORGANIZATION University of Minnesota-Twin Cities				
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu				
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months		Funds Requested By proposer
		CAL	ACAD	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		1.8		17,786
2.				
3.				
4.				
5.				
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)		0.0		0
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		1.8		17,786
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (0) POST DOCTORAL SCHOLARS		0.0		0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0		0
3. (2) GRADUATE STUDENTS				23,500
4. (1) UNDERGRADUATE STUDENTS				11,648
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0
6. (0) OTHER				0
TOTAL SALARIES AND WAGES (A + B)				52,934
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				25,607
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				78,541
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)				
TOTAL EQUIPMENT				0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000
2. INTERNATIONAL				0
F. PARTICIPANT SUPPORT COSTS				
1. STIPENDS \$ 0				
2. TRAVEL 0				
3. SUBSISTENCE 0				
4. OTHER 0				
TOTAL NUMBER OF PARTICIPANTS (0)				0
G. OTHER DIRECT COSTS				
1. MATERIALS AND SUPPLIES				25,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500
3. CONSULTANT SERVICES				0
4. COMPUTER SERVICES				0
5. SUBAWARDS				0
6. OTHER				2,500
TOTAL OTHER DIRECT COSTS				30,000
H. TOTAL DIRECT COSTS (A THROUGH G)				110,541
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) MTDC (Rate: 59.0, Base:97431)				
TOTAL INDIRECT COSTS (F&A)				57,484
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				168,025
K. FEE				0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				168,025
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$		
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

**SUMMARY
PROPOSAL BUDGET**

YEAR 5

		FOR NSF USE ONLY		
		PROPOSAL NO.		DURATION (months)
		2339605		Proposed Granted
		AWARD NO.		
ORGANIZATION University of Minnesota-Twin Cities				
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu				
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months		Funds Requested By proposer
		CAL	ACAD	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		1.8		18,142
2.				
3.				
4.				
5.				
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)		0.0		0
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		1.8		18,142
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)				
1. (0) POST DOCTORAL SCHOLARS		0.0		0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0		0
3. (2) GRADUATE STUDENTS				23,970
4. (1) UNDERGRADUATE STUDENTS				11,648
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0
6. (0) OTHER				0
TOTAL SALARIES AND WAGES (A + B)				53,760
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				25,858
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				79,618
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)				
TOTAL EQUIPMENT				0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000
2. INTERNATIONAL				0
F. PARTICIPANT SUPPORT COSTS				
1. STIPENDS \$ 0				
2. TRAVEL 0				
3. SUBSISTENCE 0				
4. OTHER 0				
TOTAL NUMBER OF PARTICIPANTS (0)			TOTAL PARTICIPANT COSTS	0
G. OTHER DIRECT COSTS				
1. MATERIALS AND SUPPLIES				25,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				2,500
3. CONSULTANT SERVICES				0
4. COMPUTER SERVICES				0
5. SUBAWARDS				0
6. OTHER				2,500
TOTAL OTHER DIRECT COSTS				30,000
H. TOTAL DIRECT COSTS (A THROUGH G)				111,618
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) MTDC (Rate: 59.0, Base:98508)				
TOTAL INDIRECT COSTS (F&A)				58,120
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				169,738
K. FEE				0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				169,738
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$		
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY		
		INDIRECT COST RATE VERIFICATION		
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

**SUMMARY
PROPOSAL BUDGET**

Cumulative			
FOR NSF USE ONLY			
		PROPOSAL NO. 2339605	DURATION (months)
		Proposed	Granted
		AWARD NO.	
ORGANIZATION University of Minnesota-Twin Cities			
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR Chuanhe Yu			
A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)		NSF Funded Person-months	
		CAL	ACAD
		SUMR	Funds Requested By proposer
		87,220	Funds granted by NSF (if different)
1. Chuanhe Yu - Principal Inv		9.0	
2.			
3.			
4.			
5.			
6. () OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)			
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)		9.0	87,220
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)			
1. (0) POST DOCTORAL SCHOLARS		0.0	0
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)		0.0	0
3. (10) GRADUATE STUDENTS			115,240
4. (5) UNDERGRADUATE STUDENTS			58,240
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)			0
6. (0) OTHER			0
TOTAL SALARIES AND WAGES (A + B)			260,700
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)			126,836
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)			387,536
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING \$5,000.)			
TOTAL EQUIPMENT			0
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)			10,000
2. INTERNATIONAL			0
F. PARTICIPANT SUPPORT COSTS			
1. STIPENDS \$ 0			
2. TRAVEL 0			
3. SUBSISTENCE 0			
4. OTHER 0			
TOTAL NUMBER OF PARTICIPANTS (0)			0
G. OTHER DIRECT COSTS			
1. MATERIALS AND SUPPLIES			125,000
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION			12,500
3. CONSULTANT SERVICES			0
4. COMPUTER SERVICES			0
5. SUBAWARDS			0
6. OTHER			12,500
TOTAL OTHER DIRECT COSTS			150,000
H. TOTAL DIRECT COSTS (A THROUGH G)			547,536
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)			
TOTAL INDIRECT COSTS (F&A)			284,372
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)			831,908
K. FEE			0
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)			831,908
M. COST SHARING PROPOSED LEVEL \$ 0		AGREED LEVEL IF DIFFERENT \$	
PI/PD NAME Chuanhe Yu		FOR NSF USE ONLY	
		INDIRECT COST RATE VERIFICATION	
ORG. REP. NAME* Patrick Donnell		Date Checked	Date Of Rate Sheet
		Initials - ORG	

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

BUDGET JUSTIFICATION

A. SENIOR PERSONNEL:

Chuanhe Yu, Ph.D., Principal Investigator, 1.8 calendar months/15% effort. Dr. Yu will be primarily responsible for the conduct of the study in general and the strategy, interpretation and analysis of the data. They will work together to supervise the project, including scientific and administrative management and oversight, and manuscript preparation. Dr. Yu will serve as the contact PI for grant reporting purposes.

B. OTHER PERSONNEL:

Quinn Dickson, graduate assistant, (0.5 FTE) 3.0 calendar months/25% effort. Quinn Dickson, a graduate student in Bioinformatics and Computational Biology (BICB) program, has experience in bioinformatic data analysis and computational method development. He will perform the NGS sequence data analysis under the supervision of Dr. Yu.

TBN, graduate assistant, (0.5 FTE) 6.0 calendar months/50% effort. A graduate student in Bioinformatics and Computational Biology (BICB) program will be recruited to help this project under the supervision of Dr. Yu.

TBN, undergraduate assistant, (hourly) 14hrs/week at \$16.00/hr. An undergraduate student will do yeast genetic interaction assay, yeast dot assays and yeast mutation analysis.

C. FRINGE BENEFITS:

Fringe Benefits are calculated in accordance with UMN's federally negotiated fringe rate agreement dated May 2023. Fringe Rates are as follows: Academic: 37.1% Student Professional with GA Health: 25.1%

Graduate Student Tuition (\$13,110/year):

Tuition costs are calculated based on the graduate assistant effort percentage and the hourly tuition remission rate of \$22.41.

D. EQUIPMENT:

None

E. TRAVEL (\$2,000/year):

Travel costs in the amount of \$2,000 per year are requested to cover the cost of graduate students and Dr. Yu to present data at a national meeting, which would be an anticipated 4-day event. This will cover airfare (\$600), lodging (\$1,000), and meals/incidentals (\$400) \$100/day.

F. PARTICIPANT /TRINEE SUPPORT COSTS:

None

G. OTHER DIRECT COSTS:

G.1. Material and Supplies (\$25,000/year):

*Reagents and solvents for DNA, RNA and protein assays, including RNA and DNA extraction Kit, PCR Kit for gene expression, antibodies and relevant agents (\$5,000/year)

*Reagents and solvents for gene cloning, gene mutation and DNA sequencing library (\$15,000/year)

*Yeast cell culture media, additives and plasticware (\$3,000/year)

*Glassware, gloves, pipette tips and other consumables (\$2,000/year)

Total supplies and services= \$25,000/year

G.2. Publication Costs (\$2,500/year):

These funds are requested to cover project-related publication fees.

G.3. Workshop costs (\$500/year):

These funds will cover students attending the epigenomic workshops held at The Hormel Institute.

G.4. Core Services (\$2,000/year):

Flow cytometry (\$500/year)

Metabolic core (\$500/year)

Microscopy core (\$1,000/year)

Since the precise amount and allocation of these resources will vary in each year and will be adjusted to meet the demands of the work, these are provided as broad general cost estimates. We are confident about these estimates based on current studies of a broadly similar nature.

H. INDIRECT COSTS:

The University of Minnesota's federally negotiated rate for on-campus research at The Hormel Institute is 59.0%. A copy of UMN's current federally negotiated indirect costs rate agreement, approved in May 2023, can be found here: <https://research.umn.edu/units/oca/fa-costs/current-fa-rates>.

BUDGET NUMBER JUSTIFICATIONS:

The University of Minnesota defines the term "year" as the university fiscal year (July-June) for purposes of the NSF limitation on salary compensation for senior personnel. Calculation of the amount of salary requested in each budget period includes inflation effective July 1 of each fiscal year. Future years costs for personnel are escalated using a 2% rate of inflation which occurs every July 1. Per UMN's federally negotiated indirect costs rate agreement, tuition remission costs (\$13,110/year) are backed out of the total direct costs for the modified total direct costs (MTDC) base calculation. Indirect costs are based on UMN negotiated rates with the cognizant federal authority and are applied at a rate of 59.0% for The Hormel Institute for the entire project period using the approved MTDC formula (excludes equipment, capital expenditures, charges for patient care, student tuition remission, participant support costs, rental costs of off-site facilities, scholarships, and fellowships as well as the portion of each subaward greater than \$25,000).

	Year 1	Year 2	Year 3	Year 4	Year 5	TOTAL
Total Direct Costs	\$107,439	\$108,452	\$109,486	\$110,541	\$111,618	\$547,536
MTDC (TDC minus tuition)	\$94,329	\$95,342	\$96,376	\$97,431	\$98,508	\$481,986
Total Indirect Costs (59%)	\$55,654	\$56,252	\$56,862	\$57,484	\$58,120	\$284,372
TOTAL COSTS REQUESTED	\$163,093	\$164,704	\$166,348	\$168,025	\$169,738	\$831,908

FACILITIES & OTHER RESOURCES:

Laboratory: Dr. Chuanhe Yu's laboratory is located in Austin, Minnesota, at The Hormel Institute, University of Minnesota. The laboratory space, which includes culture/instrument rooms, is approximately 1150 sq ft, with access to adjacent cold rooms. The laboratory is equipped with state-of-the-art equipment. This includes one laminar flow hoods for cell culture, one chemical hood, one refrigerator, one -20°C freezer, one -80°C freezer, one isotemp incubator oven and one microwave oven, two tissue culture incubators, one high performance benchtop centrifuge for cell culture work, one Bioruptor® sonication machine (Diagenode), one Agilent fragment analyzer 2100, three microcentrifuges, two Eppendorf centrifuges, one Eppendorf thermal cycler, two protein and two nucleic acid electrophoresis systems, three water baths, one regular and one analytical weigh balances, two stirrer/hotplates, four power sources, one liquid nitrogen storage tank, one cell counter, one Millipore water purification system, one Nikon TMS inverted phase contrast microscope with computer & imaging software, four Bench Mark mixers, two platform shakers, one tissue homogenizer, one Lumina Light box, one UV trans-illuminator with SmartDoc 2.0 Imaging with orange filter. The lab is also equipped with four PCs, and a laser printer. The facilities available are outstanding and include everything needed to complete the proposed research project.

Office/Computer Dr. Yu has 120 sq. ft. of his own office space that is adjacent to his laboratory. Secretarial services are provided in a well-equipped central office. In addition to the usual office equipment, a Dell laptop computer attached to dual monitors/external keyboard, two desktop computers, and two laser printers are available for preparation of manuscripts, meeting posters, grants and reports. They are located in Dr. Chuanhe Yu's office and laboratory. The computers are equipped with statistical software, Windows, Microsoft Office, internet, e-mail, graphic and word-processing software. The computers are linked to the main University of Minnesota library where many scientific journals can be retrieved electronically. IT services are housed in The Hormel Institute.

Shared Facilities & Resources at the Hormel Institute

A key goal of the Hormel Institute, University of Minnesota is to provide access to technologies, services and scientific consultation that facilitate interaction and enhance scientific productivity. The shared resources highlighted below provide stability, reliability, cost-effectiveness, and quality control that would be difficult to achieve otherwise.

Research Environment The Hormel Institute is an academic research branch of the University of Minnesota and a research partner with the Mayo Clinic, Rochester. The institute's environment is one of collegial support that fosters collaborative, multidisciplinary approaches to understand tumor biology and, ultimately, the development of new therapeutic approaches. The Institute provides a great deal of institutional support for approximately 150 researchers. All faculty members are in the same facility and collaborate on several research projects. The research environment includes weekly journal clubs, several monthly seminars with leaders in the fields of cancer, chemoprevention, molecular design, and others.

The institute has recently undergone two major expansions, doubling in size, which demonstrates the research institute's constant growth. The institute now comprises 17 research groups, all studying different aspects of cancer biology. The current research building comprises ~40 research laboratories, conference rooms equipped with smart boards, state-of-the-art imaging equipment, and audio and video conferencing capabilities. There are also break-out rooms for scientific discussions, a conference room (seating 25), a seminar room (seating 150), and a new 250-seat auditorium equipped with innovative global communications technology.

Animal Facilities The Hormel Institute's Vivarium consists of 7,300 sq ft and is AAALAC-accredited. This state-of-the-art facility serves the institute's investigators and the animal research program by providing valuable husbandry services, veterinary care services, training and guidance. This is accomplished through

the utilization of trained and dedicated animal care professionals who are committed to the humane care and use of animals in biomedical research. The third-floor facility houses specific pathogen free (SPF) rodents and provides a procedural room for survival surgeries and varying procedures. The first-floor facility houses specific pathogen free (SPF) and conventional rodents. The first-floor facility provides procedures for solar ultraviolet light radiation, surgery, imaging, irradiation, animal biosafety level-2 (ABSL2)/chemical hazard use, and varying procedures. The process for obtaining access to these areas requires completing required online training curriculum, being listed on an active IACUC protocol and then acquiring in-house training. The animal facilities has two shared instruments.

- Bruker Carestream In-Vivo Xtreme Imager – Provides researchers with the ability to image live mice or rats using bioluminescence or fluorescence. A cooled camera is used to capture the images. System comes with X-Ray for background imaging. Instrument comes with fully integrated anesthesia so that animals are kept under anesthesia during imaging.
- Rad Source RS 2000 Biological Irradiator – Irradiator can be used for bone marrow irradiation in mice with at least a 94% dose uniformity without causing skin burns on the animals. This system can also be used to irradiate cells.

Shared Instrument Core

The Shared Instrument Core at the Hormel Institute has a large variety of instrumentation covering a wide range of research interests. The core is managed by Todd Schuster, Senior Researcher. Staff is available to provide training, troubleshoot, and to assist in the operation if needed.

Other

General facilities at The Hormel Institute include cold rooms, beta and gamma counters, various centrifuges, low-temperature freezer room, Molecular Dynamics Storm 840 Imaging System, Leica Inverted Fluorescence microscope, Nikon CS1 confocal microscope, an impressive new Cryo-Electron Microscope, Real-Time PCR, Flow cytometry, cell sorter, protein crystallization robotics and access to Argonne Laboratories, Labsystems Luminoskan microplate reader, Fluoroskan microplate reader, electron microscopy laboratory, GC/MS, HPLC. Electronics and a fully outfitted and staffed machine shop.

Administrative Support The Hormel Institute provides administrative services in a central office. These services include purchasing, general administrative support, and accounting. Additionally, The Hormel Institute has a Research Development Office staffed with Grants and Contracts Professionals who provide high-level support with budget preparation, grant submissions, preparing progress reports and research development activities. Investigators have access to grant editors and peer-review programs. Investigators have easy access to computer labs and comprehensive IT support on-site. The institute also has a staffed research library and access to the University of Minnesota's library resources

Other shared resources at the University of Minnesota include bioinformatics and biostatistics support, which provides members with a broad range of expertise in data management and biostatistics; histopathology services which provide pathology support and expertise for animal models; and a mouse genetics lab which provides comprehensive laboratory, tissue culture, and administration capabilities. The recently established Genome Engineering Shared Resource was created to offer services that cover the full range of gene editing technologies and is staffed by experts. In addition, the University of Minnesota has a comprehensive array of research support services. The facilities have been developed to reflect large instrumentation needs and meet the needs for emerging technologies. Examples of shared resources relevant to cancer research: the centers for Genomics and Proteomics (Mass Spec); electron microscopy; flow cytometry/cell sorting; image analysis; mouse genetics (including transgenics and gene-targeted mice) and other model organism cores (zebrafish, flies); NMR spectroscopy; Open Biosystems RNAi libraries; and centralized tissue procurement for human subjects research.

The Mayo Clinic (Shared Resources) Additionally, The Hormel Institute's faculty have shared privileges at the Mayo Clinic in nearby Rochester, Minnesota. Dr.Chuanhe Yu is also a Mayo Research Collaborator and can access all Mayo shared multiple institutional cores that are available to support this project, including Epigenomics Development Laboratory (EDL) of the Mayo Clinic Center for Individualized Medicine Epigenomics Program. The equipment in EDL includes a UCD-400 Bioruptor Twin sonicator (Diagenode) and a Covaris S220 Focused Ultrasonicator for chromatin fragmentation, an Advanced Analytical Fragment Analyzer Automated CE System, an Invitrogen Qubit 2.0 fluorometer, an Eppendorf BioPhotometer plusUV/Vis photometer, two Diagenode SX-8G IPStar Compact Automated Systems for chromatin immunoprecipitation (ChIP) studies and library preparation, a Bio-Rad iQ5 Real-Time PCR and a Bio-Rad Digital Droplet PCR machine, a Qiagen PyroMark Q24 pyrosequencer, a LI-COR Odyssey Fc imaging system, a HydroTech Pump Gel Drying System, as well as general wet lab equipment including centrifuges and balances, pH meter, refrigerators, freezers (-20 °C and -80 °C) and a water purification system. The EDL also has a cell/tissue culture room equipped with a class IIA/B3 biosafety hood suitable for work with recombinant DNA and replication-deficient retroviral and lentiviral vectors, 2 cell/tissue culture incubators, a phase-contrast microscope and liquid N₂ freezers for cell storage. Molecular modeling software (Accelrys DS Protein Bundle: including DS Analysis, DS Biopolymer, DS CHARMM, DS Modeler, DS Protein Families, DS Protein Health, DS Protein Refine, DS Sequence Analysis and DS Standalone) is also available.

COLLABORATIONS RELATED TO THIS NSF-CAREER APPLICATION AND NOT INCLUDED IN THE BUDGET:

Collaborations in this application primarily focus on the educational aspects of the proposal. Our integrated educational plan's first objective aims to incorporate an epigenomics training module into the existing research experience programs for undergraduates at the Hormel Institute (HI). These programs, known as the Summer Undergraduate Research Experience (SURE) program and the Student Training Experience Program (STEP), are overseen by **Kelly Vincelette** (M.Ed.), our community outreach and education manager. Through her collaboration, we will enhance these programs and provide valuable training opportunities to young scientists. The second objective of our integrated educational plan involves establishing a new yeast genetics training program for high school students interested in pursuing science fair projects. This initiative program involves the recruitment and training of high school students. Ms. Kelly Vincelette will also be a valuable collaborator in this endeavor. To ensure the success of our educational goals, timely evaluation is crucial. To facilitate this, we have enlisted the expertise of **John N. Murray** (M.A.), the evaluation specialist from the Extension Department of Youth Development at the University of Minnesota. Mr. Murray will conduct formal evaluations to assess our progress towards achieving broader educational objectives and meeting specific project milestones.

NSF BIOGRAPHICAL SKETCH

Provide the following information for the Senior personnel.
Follow this format for each person. **DO NOT EXCEED 3 PAGES.**

IDENTIFYING INFORMATION:

NAME: Yu, Chuanhe

POSITION TITLE: Assistant Professor

ORGANIZATION AND LOCATION: Hormel Institute-University of Minnesota, Austin, MN, United States

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	DATE RECEIVED	FIELD OF STUDY
Mayo Clinic, Rochester, Minnesota, USA	Postdoctoral Fellow	2012 - 2015	Epigenomics
National Cancer Institute-Frederick, Frederick, Maryland, USA	Postdoctoral Fellow	2010 - 2012	Epigenetics
Iowa State University, Ames, Iowa, USA	PHD	12/2009	Genetics
Sun Yat-Sen University, Guangzhou, Guangdong(China), China	MS	07/2003	Biochemistry and molecular biology
Sun Yat-Sen University, Guangzhou, Guangdong(China), China	BS	07/2000	Microbiology

Appointments and Positions

- 2019 - present Assistant Professor, Hormel Institute-University of Minnesota, Austin, MN, United States
- 2018 - 2019 Assistant Professor of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine and Science, Mayo Clinic, Rochester, MN, USA
- 2015 - 2018 Research Associate, Mayo Clinic, Rochester, MN, USA
- 2012 - 2014 Postdoctoral Fellow, Mayo Clinic, Rochester, MN, USA
- 2010 - 2012 Postdoctoral Fellow, National Cancer Institute-Frederick, Frederick, MD, USA

Products**Products Most Closely Related to the Proposed Project**

1. Yu C, Gan H, Han J, Zhou ZX, Jia S, Chabes A, Farrugia G, Ordog T, Zhang Z. Strand-specific analysis shows protein binding at replication forks and PCNA unloading from lagging strands when forks stall. Mol Cell. 2014 Nov 20;56(4):551-63. DOI:S1097-2765(14)00749-7
2. Gan H, Yu C, Devbhandari S, Sharma S, Han J, Chabes A, Remus D, Zhang Z. Checkpoint kinase Rad53 couples leading- and lagging-strand DNA synthesis under replication stress. Mol Cell. 2017 Oct 19;68(2):446-455.e3. DOI:S1097-2765(17)30669-X
3. Yu C, Gan H, Serra-Cardona A, Zhang L, Gan S, Sharma S, Johansson E, Chabes A, Xu RM,

- Zhang Z. A mechanism for preventing asymmetric histone segregation onto replicating DNA strands. *Science*. 2018 Sep 28;361(6409):1386-1389. DOI:science.aat8849
4. Gan H, Serra-Cardona A, Hua X, Zhou H, Labib K, Yu C, Zhang Z. The Mcm2-Ctf4-Pol α axis facilitates parental histone H3-H4 transfer to lagging strands. *Mol Cell*. 2018 Oct 4;72(1):140-151.e3. DOI:S1097-2765(18)30740-8
 5. Serra-Cardona A, Yu C, Zhang X, Hua X, Yao Y, Zhou J, Gan H, Zhang Z. A mechanism for Rad53 to couple leading- and lagging-strand DNA synthesis under replication stress in budding yeast. *Proc Natl Acad Sci U S A*. 2021 Sep 21;118(38) DOI:10.1073/pnas.2109334118

Other Significant Products, Whether or Not Related to the Proposed Project

1. Yu C, Gan H, Zhang Z. Both DNA polymerases δ and ϵ contact active and stalled replication forks differently. *Mol Cell Biol*. 2017 Nov 1;37(21) DOI:MCB.00190-17
2. Yu C, Zhang J, Pulletikurti V, Weber DF, Peterson T. Spatial configuration of transposable element Ac termini affects their ability to induce chromosomal breakage in maize. *Plant Cell*. 2010 Mar;22(3):744-54. DOI:tpc.109.070052
3. Yu C, Bonaduce MJ, Klar AJ. Defining the epigenetic mechanism of asymmetric cell division of *Schizosaccharomyces japonicus* yeast. *Genetics*. 2013 Jan;193(1):85-94. DOI:genetics.111.137109
4. Serra-Cardona A, Duan S, Yu C, Zhang Z. H3K4me3 recognition by the COMPASS complex facilitates the restoration of this histone mark following DNA replication. *Sci Adv*. 2022 May 6;8(18):eabm6246. DOI:10.1126/sciadv.abm6246
5. Tian C, Zhou J, Li X, Gao Y, Wen Q, Kang X, Wang N, Yao Y, Jiang J, Song G, Zhang T, Hu S, Liao J, Yu C, Wang Z, Liu X, Pei X, Chan K, Liu Z, Gan H. Impaired histone inheritance promotes tumor progression. *Nat Commun*. 2023 Jun 10;14(1):3429. DOI: 10.1038/s41467-023-39185-y

Synergistic Activities

1. Development of eSPAN Tool for DNA Replication and Chromatin Replication Studies: I designed and developed the eSPAN method, a powerful tool for detecting DNA strand-specific binding at DNA replication forks. The design strategy was published in *Mol Cell* 2014 (Yu C, Gan H, Han J, Zhou ZX, Jia S, Chabes A, Farrugia G, Ordog T, Zhang Z. Strand-specific analysis shows protein binding at replication forks and PCNA unloading from lagging strands when forks stall. *Mol Cell* 2014; 56: 551-63. DOI: S1097-2765(14)00749-7), and the detailed method was published in *Genome Instability: Methods and Protocols* 2018 (Yu C, Gan H, Zhang Z. Strand-Specific Analysis of DNA Synthesis and Proteins Association with DNA Replication Forks in Budding Yeast. *Methods Mol Biol* 2018; 1672: 227-38. DOI: 10.1007/978-1-4939-7306-4_17). This method was highlighted in *Mol Cell* and recommended by F1000. Several research laboratories, including those outside the US such as in the UK and China, have successfully used this method in their publications, contributing to significant advancements in DNA replication and chromatin research.
2. Development of a Chromosome Rearrangement Tool with Transposable Elements: My research focused on genome rearrangements induced by Ac/Ds transposable elements, a pioneering work initiated by Nobel Laureate Barbara McClintock. Through collaborative efforts, my colleagues and I discovered a new alternative transposition mechanism responsible for chromosome

breakage and major rearrangements. Leveraging this novel mechanism, we developed a chromosome rearrangement tool for chromosome engineering in plants (Zhang J, Yu C, Krishnaswamy L, Peterson T. Transposable elements as catalysts for chromosome rearrangements. *Methods Mol Biol.* Volume 701. 2010/12/25 ed, 2011: 315-26.). I demonstrated the tool's applicability in rice chromosome engineering (Yu C, Han F, Zhang J, Birchler J, Peterson T. A transgenic system for the generation of transposon Ac/Ds-induced chromosome rearrangements in rice. *Theor Appl Genet* 2012; 125: 1449-62. DOI: 10.1007/s00122-012-1925-4). The development of this tool opens up new possibilities for genetic manipulation in plants and holds potential implications for crop improvement and research.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Yu, Chuanhe in SciENcv on 2023-07-22 16:00:15

Other Personnel Biographical Information

Data Not Available

Effective 01/30/2023 NSF CURRENT AND PENDING (OTHER) SUPPORT OMB-3145-0058

*Name: Chuanhe Yu

ORCID ID (Optional):

*Position Title : Assistant Professor

*Organization: Regents of the University of Minnesota

*Location: The Hormel Institute, Austin, MN, United States

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §§6605. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§3729-3733 and 3802.

Signature

(Please type out full name): Chuanhe Yu

Date: 07/25/2023

***Required fields**

Note: NSF has provided 15 project/proposal and 10 in-kind contribution entries for users to populate. Please leave any unused entries blank.

Projects/Proposals Section:

According to 42 U.S.C. §§ 6605, Current and Pending (Other) Support (A) means all resources made available, or expected to be made available, to an individual in support of the individual's research and development efforts, regardless of (i) whether the source of the resource is foreign or domestic; (ii) whether the resource is made available through the entity applying for a research and development award or directly to the individual; or (iii) whether the resource has monetary value; and (B) includes in-kind contributions requiring a commitment of time and directly supporting the individual's research and development efforts, such as the provision of office or laboratory space, equipment, supplies, employees, or students. If the time commitment or dollar value is not readily ascertainable, reasonable estimates should be provided.

Projects/Proposals

1.*Project/Proposal Title : Role of nucleosome dynamics in epigenetic inheritance

*Status of Support : Current Pending

Proposal/Award Number (if available): R01 GM130588

*Source of Support: NIH

*Primary Place of Performance : The Hormel Institute, University of MN, Austin, MN

*Project/Proposal Start Date (MM/YYYY) : 02/2019

*Project/Proposal End Date (MM/YYYY) : 01/2024

*Total Award Amount : \$ 1,309,800.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2020	6.00	4. 2023	6.00
2. 2021	6.00	5.	
3. 2022	6.00		

*Overall Objectives : To test this hypothesis, I will identify the proteins involved in parental histone (H3-H4)2 segregation during DNA replication (Aim 1); and investigate the molecular mechanism whereby polymerase ε and the DNA helicase contribute to parental nucleosome assembly (Aim 2). The proposed studies will elucidate the mechanisms of the parental (H3-H4)2 tetramer transfer process.

*Statement of Potential Overlap : None. There is no budgetary or scientific overlap.

Projects/Proposals

2.*Project/Proposal Title : CAREER: Deciphering strand-specific mechanisms of chromatin replication using a yeast model

*Status of Support : Current Pending

Proposal/Award Number (if available): NSF22586

*Source of Support: National Science Foundation

*Primary Place of Performance : The Hormel Institute, University of MN, Austin, MN

*Project/Proposal Start Date (MM/YYYY) : 02/2024

*Project/Proposal End Date (MM/YYYY) : 01/2029

*Total Award Amount : \$ 831,908.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2024	1.80	4. 2027	1.80
2. 2025	1.80	5. 2028	1.80
3. 2026	1.80		

*Overall Objectives : Specific objectives: (1) investigate the mechanism by which leading and lagging strands are coupled during chromatin replication, (2) study the biochemical and functional strand preferences of chaperones for newly produced H3-H4 histones (Caf1, Asf1, Rtt106), and (3) examine the DNA strand preferences of transcription factors during the DNA replication process. Throughout, STEM graduate and undergraduate students will lead

*Statement of Potential Overlap : None. There is no budgetary or scientific overlap.

In Kind Contributions

Required fields*In-Kind Contributions Section:**

In this section, disclose ALL in-kind contributions related to current and pending support. In-kind contributions include, but are not limited to, office/laboratory space, equipment, supplies, and employee or student resources. See the table entitled, NSF Pre-award and Post-award Disclosures Relating to the Biographical Sketch and Current and Pending Support for instructions on in-kind support for use on the project being proposed.

Please enter your support entries so they are grouped together based on the "Status of Support" and are in the order of Current to Pending from top to bottom.

In Kind Contributions

1.*Status of Support : Current Pending

*In-Kind Contribution Start Date (MM/YYYY) : 06/2023 *In-Kind Contribution End Date (MM/YYYY) : 08/2023

*Source of Support : The Hormel Institute - SURE Interns

*Summary of In-Kind Contribution : SURE Interns lab placement. One intern student, 40 hours per week for 10 weeks, at \$16.00/hr. From May 30, 2023 until August 4, 2023.

*Person-Month(s)(or Partial Person-Months) Per Year Associated with the In-Kind Contribution

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	2.30	4.	
2.		5.	
3.			

*U.S. Dollar Value of In-Kind Contribution: \$ 6,400

The chromatin-based mechanism of Camptothecin resistance in yeast.

*Overall Objectives :

*Statement of Potential Overlap : None. There is no budgetary or scientific overlap.

Data Management Plan

I. Types of Data:

This proposal will generate three types of data:

- (a) Yeast Strains and Other Materials: Novel yeast strains and recombinant plasmids generated in Dr. Yu's lab will be documented with genotype information and their source of origin.
- (b) Genomic Data: A substantial amount of genomic sequencing data will be generated during the proposed study.
- (c) Protocols or Methods: Both previously furnished protocols and new protocols will be generated during this study.

II. Data Storage and Preservation:

- (a) Yeast Strains and Other Materials: Novel yeast strains and recombinant plasmids will be properly stored in Dr. Yu's lab with well-maintained records.
- (b) Genomic Data: The data generated by this project will be deposited and stored in local databases on computers in the PI lab, registered to the Hormel Institute-University of Minnesota. Backups of the data will be kept on local data storage devices. Data log files will be maintained to ensure traceability and will contain the date, experimental conditions, and processed data. Lab notebooks will be regularly updated and securely stored in a central location within the designated labs. Any paper copies of processed data or related information will be affixed to the lab notebooks.
- (c) Protocols or Methods: All protocols and methods will be hand-documented in the lab notebooks, including the related results and conclusions. The protocol will be regularly updated and stored within Yu's lab.

III. Data and Metadata Formats:

- (a) Yeast Strains and Other Materials: Detailed information about novel yeast strains and recombinant plasmids generated in our laboratory will be described in further publications.
- (b) Genomic Data: The genomic sequencing data generated in this study will be deposited into GenBank, and the corresponding link will be freely shared for research purposes once the relevant paper has been accepted for publication. Raw data before publication will be stored in local server will be limited to the students and PIs involved in the project, as well as the computer administrators at the Hormel Institute and University of Minnesota.
- (c) Protocols or Methods: All protocols, results, and conclusions, including experimental conditions, will be described in journal publications and conference presentations.

IV. Access to Data and Data Sharing Practices and Policies:

- (a) Yeast Strains and Other Materials: Novel yeast strains and recombinant plasmids generated in our laboratory will be made available to those requesting them for research purposes in accordance with NSF and University of Minnesota policies. Published strains and other reagents can be made available to the research community by requesting them from Yu's lab.
- (b) Genomic Data: The genomic sequencing data generated from this study will be deposited into GenBank and shared freely for research purposes once the corresponding paper has been accepted for publication. Raw data before publication will be shared with all project personnel. Access to local databases will be restricted to the students and PIs involved in this project and the designated computer administrators at the Hormel Institute and University of Minnesota.
- (c) Protocols or Methods: All protocols, results, and conclusions, including experimental conditions, will be described in journal publications and conference presentations. Before publication, the protocols or methods will be internally shared within Yu's lab and University of Minnesota.

V. Roles and Responsibilities:

All datasets generated during this project will be licensed under the Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA) license agreement. The full agreement is available at <http://creativecommons.org/licenses/by-nc-sa/3.0/legalcode>.

VI. If this grant is funded, the PI, Dr. Chuanhe Yu, will be responsible for all data management arrangements.

Postdoctoral Mentoring Plan

Data Not Available

July 21, 2023

Dear CAREER Panel Members,

I am writing to enthusiastically recommend Dr. Chuanhe Yu for an NSF CAREER award. Dr. Yu is an Assistant Professor and the leader of the epigenetic information inheritance section at The Hormel Institute (HI), University of Minnesota since December 2019. I have been the Executive Director of The Hormel institute since September 2020

Chuanhe received his PhD in genetics from Iowa State University in 2009 and began his epigenomic training at the Mayo Clinic in 2012. Dr. Yu's groundbreaking research in the field of epigenetics has made significant contributions to our understanding of DNA replication and inheritance of epigenetic information encoded in histone proteins. He designed and developed the eSPAN method (enrichment and sequencing of protein-associated nascent DNA), which has provided unprecedented opportunities to study these processes. Notably, Dr. Yu made a remarkable discovery using eSPAN, *i.e.*, when replication forks stall in response to DNA damage, the replication clamp PCNA is unloaded from lagging strands. This discovery, along with the design of the eSPAN method, was published in *Molecular Cell* and received recognition from F1000Prime. In his proposed NSF CAREER project titled '*CAREER: Deciphering strand-specific mechanisms of chromatin replication using a yeast model.*' Dr. Yu has introduced another new tool: GEES-seq (Genomic Enrichment of ssDNA and sequencing), which can detect abnormal DNA replication fork structures. Leveraging these transformative techniques, Dr. Yu plans to conduct a series of experiments that will elucidate the mechanisms of DNA replication strand coupling and the DNA replication strand preferences of histone H3-H4 tetramer chaperones and gene transcriptional factors on the DNA replication fork. By addressing these previously understudied aspects, his research will significantly advance our understanding of chromatin replication.

At The Hormel Institute, Dr. Yu has demonstrated exceptional leadership and collaboration. His expertise in yeast genetics and epigenetics has fostered multiple research collaborations within our Institute and beyond. For instance, he has collaborated with Dr. Gasper J. Kitange (HI faculty), to study the function of a prp19 gene in DNA repair. Additionally, Dr. Yu has collaborated with Dr. Yibin Deng from the Department of Urology (UMN) on a drug screening project using a fast-growing yeast model. He has also established strong working relationships with other research groups interested in DNA replication and repair, both within our university and externally. These collaborations create an excellent research environment that will support Dr. Yu's proposed research.

Dr. Yu's commitment to conducting novel research with broad impact is evident throughout his career. He has developed innovative techniques such as eSPAN and others, which have significantly contributed to the fields of DNA replication and epigenomics. His research has resulted in groundbreaking discoveries, including the finding that parental nucleosomes are nearly evenly transferred to newly synthesized DNA replication strands, resolving more than 40 long-standing controversies in the field. His work has been published in the leading journal *Science*, and he has authored several methodology and book chapters that provide valuable protocols and instructions for utilizing his methods. Dr. Yu's research productivity and novelty demonstrate his potential to make further important contributions through his proposed CAREER research.

*FRSMed is not a medical qualification. Dr. Clarke is also an AAAS Fellow



In addition to his research accomplishments, Dr. Yu has shown a strong commitment to education. In his proposed CAREER project, he aims to develop a new educational program for high school students, providing them with essential knowledge and hands-on experience in yeast genetics. This program will foster students' passion for scientific inquiry, develop critical thinking skills, and provide invaluable research experience. Furthermore, Dr. Yu plans to expand the epigenomic training section within our existing undergraduate research programs (SURE (Summer Undergraduate Research Experience) and STEP (UMR/HI Student Training Experience Program)), allowing students to gain firsthand experience with advanced equipment and techniques. These educational initiatives align perfectly with our institutional goals and the NSF's outreach efforts.

Dr. Yu's qualities extend beyond his research and educational pursuits. He possesses exceptional personal skills, a collaborative spirit, and discipline. During his time at the Hormel Institute, he has successfully recruited talented students and staff into his laboratory, currently supervising a diverse team. He has published numerous peer-reviewed papers in leading journals, demonstrating his research success, and I anticipate continued excellence in his future endeavors.

In summary, I wholeheartedly support Dr. Yu's application for an NSF CAREER award. **I certify that he is eligible to submit an NSF-CAREER proposal in 2023.** I strongly urge you to support Dr. Yu with an NSF CAREER award and help launch the career of an exceptionally bright, bold, and creative young investigator. Please do not hesitate to reach out if you require any further information.

Sincerely,



Dr. Robert Clarke
Executive Director, The Hormel Institute
I. J. Holton Chair in Cancer Research
Professor of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Submitted/PI: Chuanhe Yu /Proposal No: 2339605
UNIVERSITY OF MINNESOTA

University of Minnesota Extension

*475 Coffey Hall
1420 Eckles Avenue
St. Paul, MN 55108-6070
www.extension.umn.edu*

Dear Program Director and/or Reviewer,

If the proposal submitted by Dr. Chuanhe Yu entitled CAREER: Deciphering strand-specific mechanisms of chromatin replication using a yeast model is selected for funding by the NSF, it is my intent to collaborate and/or commit resources as detailed in the Project Description or the Facilities, Equipment or Other Resources section of the proposal.

Sincerely,



John N. Murray, M.A.

Evaluation Specialist
Extension Department of Youth Development
612.626.3825
murr0328@umn.edu

UNIVERSITY OF MINNESOTA

The Hormel Institute
801-16th Ave, NE
Austin, MN 55912
507-437-9637

July 18, 2023

Dear Program Director and/or Reviewer,

If the proposal submitted by Dr. Chuanhe Yu entitled CAREER: Deciphering strand-specific mechanisms of chromatin replication using a yeast model is selected for funding by the NSF, it is my intent to collaborate and/or commit resources as detailed in the Project Description or the Facilities, Equipment or Other Resources section of the proposal.

Sincerely,



Kelly Vincellette, M Ed
Community Outreach & Education Manager
The Hormel Institute, University of Minnesota