Plan Overview

A Data Management Plan created using HKUL DMPTool

Title: Pre-Replication Complex Melts Origin DNA in Human Cells

Creator: Jian Li

Affiliation: The University of Hong Kong

Principal Investigator: Nan Liu

Contributor: Shangyu Dang, Chunlong Chen

Template: HKU Template

Project abstract:

Eukaryotic DNA replication is initiated from multiple sites distributed along each chromosome. Each initiation event is tightly regulated to ensure a faithful inheritance of genetic information during each cell division cycle. This initiation is achieved through a temporal process from replication licensing to origin firing. Replication licensing begins during the period from late mitosis to early G1 phase, where the origin recognition complex (ORC1–6) recognizes and binds origin DNA, serving as a platform to promote the assembly of pre-replicative complex (pre-RC) with the help of CDC6 and CDT1. As a result, two copies of MCM2-7 hexamers are loaded in a head-to-head orientation to form the MCM2-7 double hexamer (MCM-DH) encircling duplex DNA, poised for further activation. As cells enter S phase, both kinases and additional factors work in concert to activate the MCM-DH and transform it into two active CMG (CDC45–MCM–GINS) helicases, upon which two sister replisomes are established to drive bidirectional DNA synthesis.

A critical step in replication initiation is the unwinding of origin duplex DNA to generate single-stranded templates, and the mechanism of helicase-mediated DNA melting is the research focus in the field of DNA replication. Currently, how the human double hexamer (MCM-DH)

complex unwinds origin DNA remains completely unclear. To address this long-strand question, we determined a 2.59-Å cryo-EM structure of endogenously purified human MCM-DH bound to origin DNA. Our structure reveals that the MCM-DH untwists and stretches the tightly captured DNA strands within the constricted central channel. As a result, the duplex DNA is disrupted nearly half a helical turn, leading to the separation of a base pair at the hexamer interface and forming an initial open structure (IOS). Mutations of key amino acids involved in IOS formation and maintenance compromises MCM-DH assembly and/or stability on chromatin, ultimately impairing replication initiation. These findings uncover an intrinsic mechanism that couples MCM-DH formation with initial melting of origin DNA in human cells.

Given the fact that origin DNA is tightly bound by MCM-DH assembly, we isolated those origin DNA fragments and mapped the genome-wide distribution of MCM-DH using next-generation sequencing. We found that MCM-DHs form broad clusters that coincide with early initiation zones in early-replicating domains. In contrast, the density and distribution of these clusters are higher and more extensive in late-replicating domains, challenging the notion that origin firing efficiency is determined solely by MCM-DH abundance. Furthermore, MCM-DH positioning is mutually exclusive with actively transcribed regions, suggesting that transcription restricts helicase loading to intergenic regions. This spatial separation likely minimizes transcription-replication conflicts, thereby helping maintain genome stability.

In summary, our integrated structural, functional, and genomic analyses provide comprehensive insights into the role of MCM-DH in regulating DNA replication initiation. These findings also offer a structural framework for developing therapeutic strategies against replication-associated diseases using MCM2-7 as drug targets.

Start date: 01-01-2020

End date: 11-30-2025

Last modified: 12-03-2025

Copyright information:

Pre-Replication Complex Melts Origin DNA in Human Cells

Data Collection

What data will you collect or create?

The project will generate laboratory research data files. That will include the cryo-EM micrographs, negative staining images, FACS data and other cellular and biochemical data from different instruments.

How will the data be collected or created?

The cryo-EM micrographs will undergo analysis to reconstruct the cryo-EM 3D density map and build atomic model. The FACS data will present the cell progression profile. The WB and other cellular data represents the functional assays.

Documentation and Metadata

What documentation and metadata will accompany the data?

The cryo-EM density map of hMCM-DH(WT/WT) and hMCM- DH(RL/WT) have been deposited in EMDB database (EMDB: EMD-32258) and (EMDB: EMD-33320). The atomic model of hMCM-DH(WT/WT) has been deposited to PDB (PDB: 7W1Y).

The hMCM-DH footprints-seq data have been deposited at GEO (accession number GSE202066).

Ethics and Legal Compliance

How will you manage any ethical issues?

No human or animal participant is involved in this project. Consent for data obtainment from openaccess publications are not necessary.

How will you manage copyright and Intellectual Property Rights (IP/IPR) issues?

Theoretically, supervisor and the relevant funder for this project will have the copyright. They will have the right to authorize the reusing.

Storage and Backup

How will the data be stored and backed up during the research? i. e. until stored in the final location (e.g. on your password protected laptop)?

I will make three copies of my dataset. Data files will be kept in my password-protected computer laptop and PC. I will also store a backup copy of the data files in a password-protected external hard drive which will be updated on a regular basis. Lastly, all data and files will be backed-up in a personal Onedrive as a back-up method.

How will you manage access and security?

The data is protected within the lab group, supervisors, and trusted collaborators upon my agreement.

Selection and Preservation

Which data are of long-term value and should be retained, shared, and/or preserved?

Data from this project will be used in publications, thesis writing, and conference presentations and will be retained and preserved as 'Data and code availability' in a paper that will be accessible to the public.

What is the long-term preservation plan for the dataset?

The data will be long-term preserved (as long as possible) as a public database.

Data Sharing

How will you share the data?

All deposited data files can be shared and freely downloaded.

Are any restrictions on data sharing? If yes, Why?

No.

Responsibilities and Resources

Who will be responsible for data management?

Data will be managed primarily by me and my supervisor.

What resources will you require to deliver your plan?

No additional resources are needed for me to deliver this DMP.