Replication in the Vicinity of Absolute Blocks to Replication

Michael Seidman
LMG, NIA, NIH

- Marina Bellani
- Jing Huang
- Manikandam Paramasivam

LG, NIA, NIH
- Weidong Wang
- Chen Ling

UC Riverside
- Yinsheng Wang
- Shuo Liu

Northwestern University
- Arun Kalliat Thazhathveetil

VU University/ Amsterdam
Johan de Winter
The replication fork is driven by helicases

Strategies for responding to replication challenge imposed by DNA adducts
How do cells deal with replication blocks?

1. Avoid them
   - Remove them before a fork encounter
   - Multiple DNA repair pathways

2. Repair after block

Problem: delay completion of replication
complex genomes with multiple origins
50-100,000
How do cells cope with replication blocks?

3. Bypass lesion and continue synthesis

Unwind
Bypass synthesis
How do cells cope with replication blocks?

4. Uncouple replication and repair

Unwind

Restart synthesis

Post Replication Repair

Rupp, 1968
The replication fork is driven by helicases

Interstrand crosslinks present a major challenge to the replication apparatus
Considered absolute blocks to replication

Do these models describe encounters with genomic ICLs in mammalian cells?

DNA Interstrand Crosslink (ICL) repair during replication

- Single Fork
  - Unhooking
  - Translesion Synthesis
  - Extension of leading daughter strand
  - Removal of crosslink remnant
  - Recombinational repair to restore the fork
  - Replicate-repair-replicate

- Double Fork
  - (Walter lab)
  - Unhooking
  - Translesion Synthesis
  - Extension of leading daughter strand
  - Removal of crosslink remnant
  - Recombinational repair to restore the fork
  - Replicate-replicate-repair
Trimethyl Psoralen forms a high proportion of ICLs

4,5′,8-trimethylpsoralen + UVA
Digoxigenin-tagged TMP

TMP

Digoxigenin

4,5',8-trimethylpsoralen

D-TMP

10

1

Angelicin

D-Ang

ONLY MAs
Cells incubated with Thymidine analogs

Visualization of replication tracks on DNA Fibers

DNA combing [Fiber analysis]

Immunofluorescent detection
Immuno quantum dot detection of Dig-TMP on a DNA fiber

- CldU: immunofluorescence
- Dig-TMP: immunoquantum dot

CldU → Dig-TMP + UVA → Comb DNA fibers / Detect

Overnight
Possible replication patterns in the vicinity of ICLs

Single Fork

Double Fork

DIG-TMP +UVA → CldU → Replication patterns
A minority of replication tracts encounter an adduct Dig-Angelicin
Replication encounters with D-Ang MAs

D-Ang/UVA or D-TMP/UVA

ClodU 1 h

Replication patterns

single sided

double sided

D-Ang

MA

→

and/or
Replication fork encounters with D-Ang MAs

D-Ang/UVA or D-TMP/UVA

ClidU 1 h

Replication patterns

Wt

Frequency (%)

0 20 40 60 80 100 120

D-Ang

single sided

double sided

D-Ang

MA

and/or
Replication encounters with D-TMP ICLs

D-Ang/UVA or D-TMP/UVA

ClidU 1 h

Replication patterns

Frequency (%)

D-Ang D-TMP

Wt

Single sided

Double sided

D-Ang

MA

and/or
Replication encounters with D-TMP ICLs

D-Ang/UVA or D-TMP/UVA

CldU 1 h

Replication patterns

Wt

Frequency (%)

0 20 40 60 80 100 120

D-Ang D-TMP

Single sided

Double sided

D-Ang

MA

D-TMP

ICL

and/or
Double sided events dominate in repair deficient cells.
Are the ICLs intact at the time of fork encounters?

Are parental strands covalently linked at the time of the fork encounter(s)?
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?

ERCC1−/− cells $\xrightarrow{\text{CldU} \text{ several replication cycles}}$ IdU one replication cycle

$\xrightarrow{\text{D-TMP/UVA}}$

$\xrightarrow{\text{EdU} \quad \text{1 h pulse}}$

shearing

*
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?

ERCC1- cells → CldU (several replication cycles) → IdU (one replication cycle) → D-TMP/UVA → EdU (1 h pulse) → shearing.

* CldU
IdU
D-TMP
EdU
merge

**
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?

ERCC1<sup>−/−</sup>

- EdU-IdU
- EdU-CldU
- EdU-CldU-IdU

Frequency (%)

Dig-signal on fiber cell treatment

UVA
Are ICLs intact at the time of fork encounters?

ERCC1

Frequency (%)

- UVA  + D-Ang /UVA

Dig-signal on fiber cell treatment

EdU-IdU
EdU-CldU
EdU-CldU-IdU
Are ICLs intact at the time of fork encounters?
Are ICLs intact at the time of fork encounters?

Most Dig-TMP adducts are intact ICLs
Parental strands are crosslinked at the time of fork encounter(s)
The timing of DTMP/UVA ICL unhooking

**XPD -/- cells**

- How long does it take for %BRG to decline?

**XPD^+ cells**

- CldU → How long it takes for %BRG to decline?
- CldU → several replication cycles
- IdU → one replication cycle
- D-TMP/UVA
- EdU 
  - 1 h pulse
  - cold chase
  - sample at different timepoints
  - How long does it take for %BRG to decline?
Unhooking of DIG-TMP ICLs at the fork takes >6 hours.
Are double sided patterns the result of dual fork stalling at an ICL?

Two sequential pulses to visualize the direction of the replication fork

DIG-TMP/UVA → ClDU 20m → IdU 20m → Replication patterns

ICL

dual fork collision
Replication in the vicinity of ICLs

A. DIG-TMP/UVA → CldU 20m → IdU 20m → Replication patterns
   - single fork

B. double fork

C. New origin?

-frequency (%)

- ERCC1+:
  - Single Fork
  - Double Fork
  - New origin?
Replication in the vicinity of ICLs

DIG-TMP/UVA → ClidoU 20m → IdoU 20m → Replication patterns

A
- Single fork

B
- Double fork

C
- New origin?

D
- Fork traverse

Frequency (%)

ERCC1⁺
Equivalent results in repair proficient cells
What is the time cost of traverse?

Dig-pso/UVA Double pulse
Duration of traverse

±DIG-TMP/UVA
±DIG-Ang/UVA

CldU → IdU

Replication patterns

DT40

IdU tract length (μm)

+ -

D-TMP
6 min

+ -

D-Ang
1.2 min

fiber with DIG
cells treated with traverse time

p<0.001

NS
What drives replication traverse of ICLs?

ICLs are absolute blocks to HELICASES

DNA TRANSLOCASES can move along DNA without unwinding

FANCM
translocase activity
recruited to ICLs only in S phase
Influence of FANCM translocase activity on traverse

DIG-TMP

- Wt
- FancM<sup>-/-</sup> + FANCM wt
- FancM<sup>-/-</sup>
- FancM<sup>-/-</sup> + FANCM D203A

Frequency (%)
FancM protein is important for traverse of ICLs
Replication traverse of ICLs, but not MAs, is promoted by FANCM translocase activity.
Are the FA core proteins required for replication traverse?
Deficiency in FA core proteins does not influence the frequency of replication patterns.

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**FancF**

- **DIG-TMP**
  - Frequency (%)
  - Single Fork
  - Double Fork
  - New Origin
  - Fork Traverse

**FancE**

- **DIG-TMP**
  - Frequency (%)
  - Single Fork
  - Double Fork
  - New Origin
  - Fork Traverse

**FancA**

- **DIG-TMP**
  - Frequency (%)
  - Single Fork
  - Double Fork
  - New Origin
  - Fork Traverse

**FancG**

- **DIG-TMP**
  - Frequency (%)
  - Single Fork
  - Double Fork
  - New Origin
  - Fork Traverse
Replication fork traverse of ICLs is mediated by FANCM in the context of the FANCM-MHF complex.

**Graph:**
- **X-axis:** Single Fork, Double Fork, New Origin, Fork Traverse
- **Y-axis:** Frequency (%)
- **Legend:**
  - Black: Wt
  - White: Mhf1<sup>−/−</sup>
  - Red: Mhf1<sup>−/−</sup> + MHF1 wt
  - Blue: Mhf1<sup>−/−</sup> + MHF1 K73A,R74A

**Statistical Analysis:**
- Single Fork: p<0.001
- Double Fork: NS
- New Origin: p<0.001
- Fork Traverse: p<0.001

**Blot Analysis:**
- **MHF1−/−:**
  - MHF1<sup>1K73AR74A</sup>
  - MHF1 wt
  - Wt

**Relative FANCM Level:**
- 0.6
- 1.7
- 1
- 1

**Diagram:**
- Replication fork highlighting MHF1/2 and FANCM interactions.
ATR/ATRIP at replication impediments
Replication patterns in cells deficient for ATR

Dominated by single sided patterns
ATR is required for Replication Traverse of ICLs, not MAs
ATR/ATRIP is essential for replication traverse of ICL
MCM-2 is phosphorylated by ATR in response to psoralen/UVA

<table>
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<tr>
<th>ATR i</th>
<th>TMP/UV</th>
<th>Chromatin</th>
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- pMCM2 S108
- MCM2
A kinase resistant FANCM mutant = a FANCM null

FANCM(S1045A)
Replication Fork encounters with an ICL

Single fork stalling

DNA Pol

CMG

CDC45-MCM2-7-GINS

ATR

FANCM

MHF1/2

FANCM promotes traverse of the ICL by the replication apparatus

Replication Traverse

Dual fork stalling
Replication restart is much faster than repair

**Single Fork Stalling**
- ICL
- DNA Pol
- CMG
- FANCM
- MHF1/2

- ~20%
- ~6 minutes

**Dual Fork Stalling**
- ~15%

**Unhooking**
- > 6 Hours

**Replication Traverse**
- ~60%

**Post Replication Repair**
The Replication Imperative:

Complete replication! Repair later

Single fork stalling

ICL

DNA
Pol

APR

CMG

CDC45-MCM2-7-GINs

FANCM

MHF1/2

CMG

FANCM promotes traverse of the ICL by the replication apparatus

Replication Traverse

Dual fork stalling

Post Replication Repair