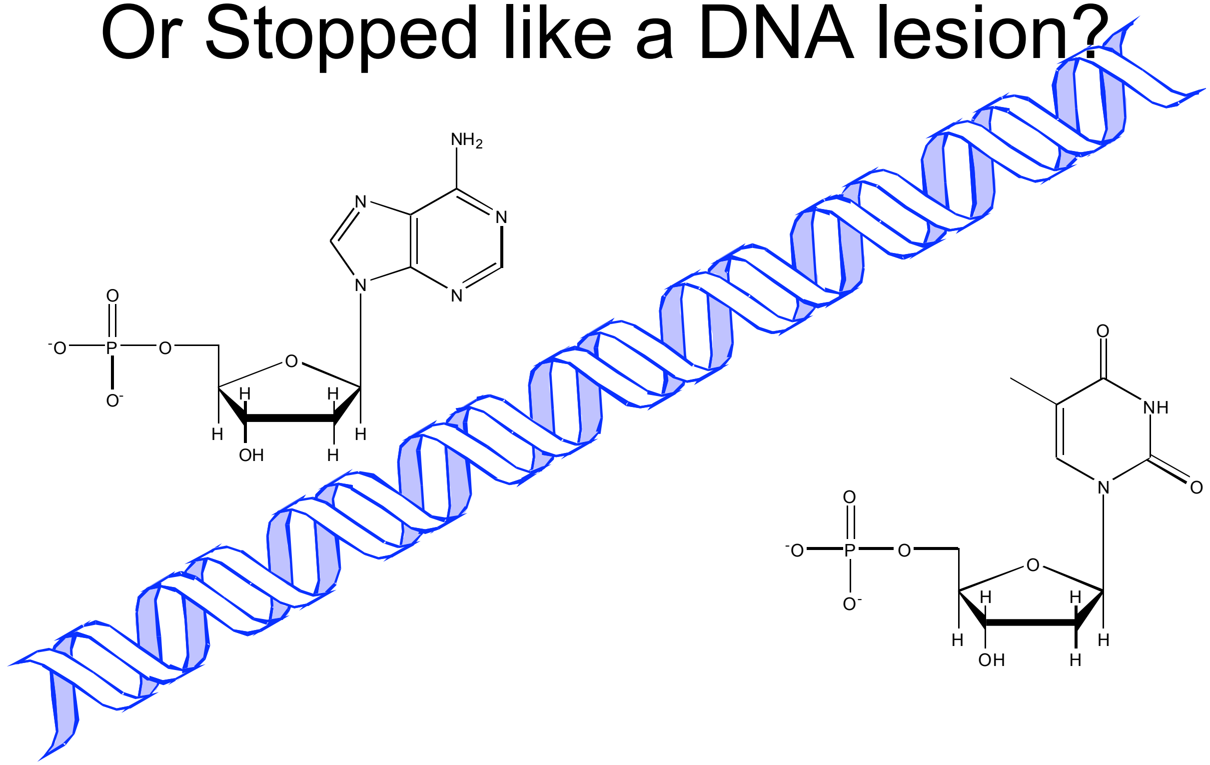
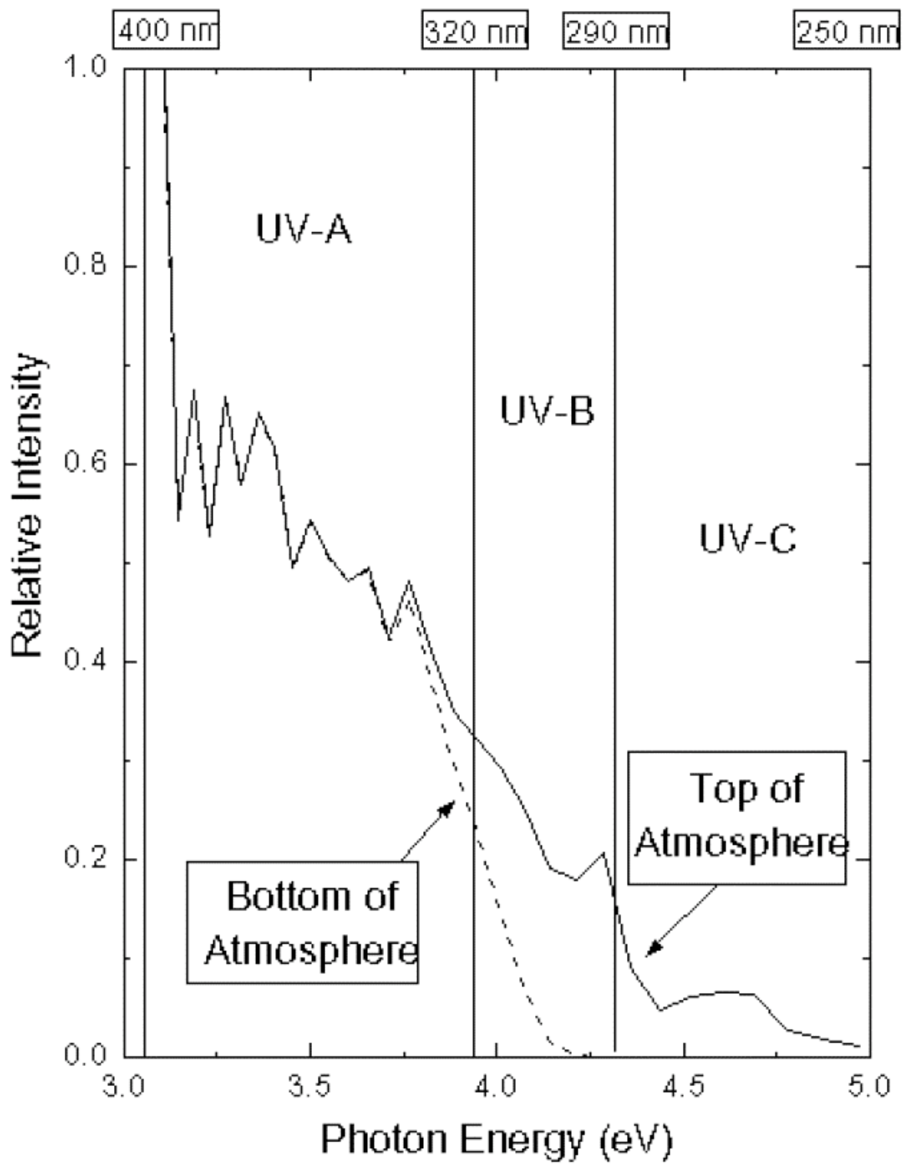
Rewrite the textbooks on DNA Replication

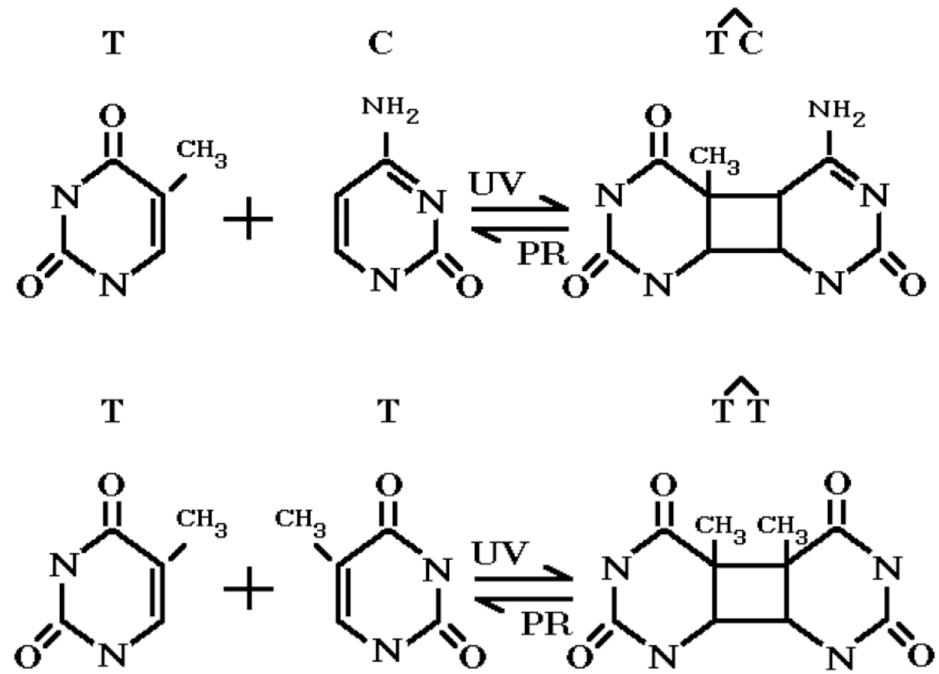
Unraveling the truth (like a helicase)

Or Stopped like a DNA lesion?



BCMB625: Adv. Molec. Bio.





Beth A. Montelone, Ph. D., Division of Biology, Kansas State University
http://www-personal.ksu.edu/~bethmont/mutdes.html

In 1967…

Dean Rupp & Paul Howard-Flanders asked…

"What would happen to the DNA if bacteria lacking NER are allowed to go on growing in medium containing ^{3}H-Thymidine after exposure to UV?"

1.) Replication Rate is virtually the same.
between wt and bacteria deficient in nucleotide excision repair (NER)

2.) DNA synthesized after UV was initially discontinuous
Via alkaline sucrose gradient centrifugation.

Bridges BA, ***DNA Repair*** (2005) v4:618-634
Rupp WD and Howard-Flanders P, ***J. Mol. Biol.*** (1968) v31:291-304

Discontinuities in the DNA synthesized in an Excision-defective Strain of *Escherichia coli* following Ultraviolet Irradiation

W. Dean Rupp and Paul Howard-Flanders

Radiobiology Laboratories
Yale University School of Medicine
New Haven, Connecticut, U.S.A.

(*Received 11 April 1967, and in revised form 18 October 1967*)

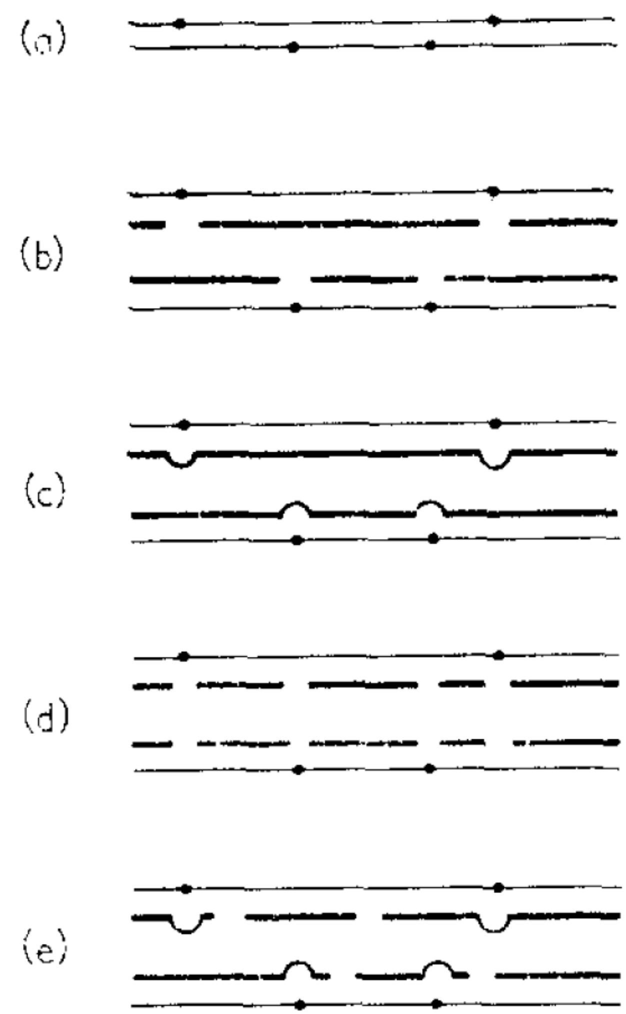


Fig. 9. Schematic model of possible structures resulting from the replication of DNA that contains unexcised dimers.

(a) Parental DNA with dimers.

(b) Gap in daughter strand at position of dimer in complementary parental strand.

(c) Daughter strands contain non-complementary material opposite dimers in parental strand.

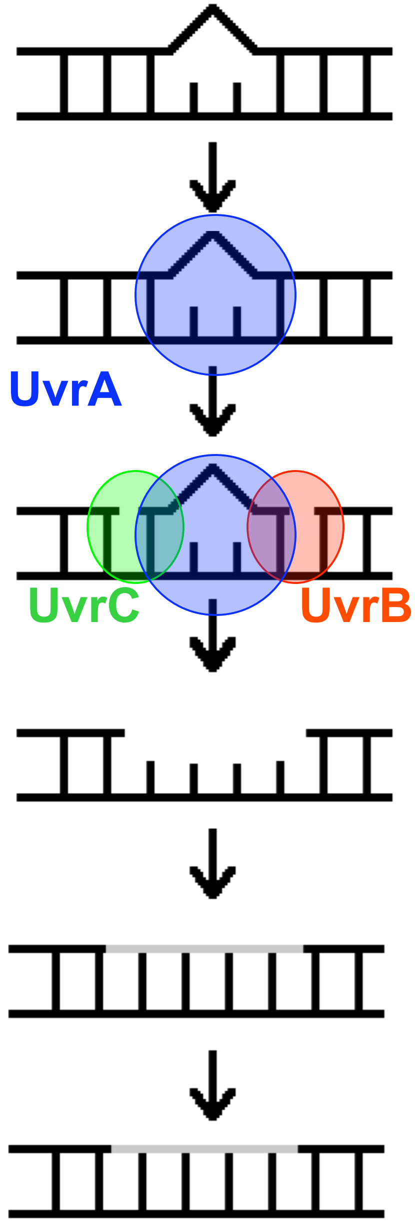
(d) Dimer in parental strand results in gaps on both daughter strands at that point.

(e) Daughter strands contain non-complementary material opposite dimers in parental strand and random gaps.

Nucleotide Excision Repair (NER)

E. coli	*S. cerevisiae*	*H. sapiens*
UvrA	Rad14	XP-A
“ B	“ 1	“ -F
“ C	“ 2	“ -G
“ D	“ 25	“ -B
	“ 4 COMPLEX	“ –C COMPLEX

NER (Nucleotide Excision Repair)



DNA with dimer

Dimer recognized
and DNA cut

E. coli cuts
12-nt's apart

uvrD (DNA helicase II) unwinds
Dimer excised

Gap filled by
DNA polymerase

Nick sealed by
DNA ligase

Modified from:
Beth A. Montelone, Ph. D., Division of Biology, Kansas State University
http://www-personal.ksu.edu/~bethmont/mutdes.html

As an aside:
To think about…

The Current Roster of DNA Polymerases

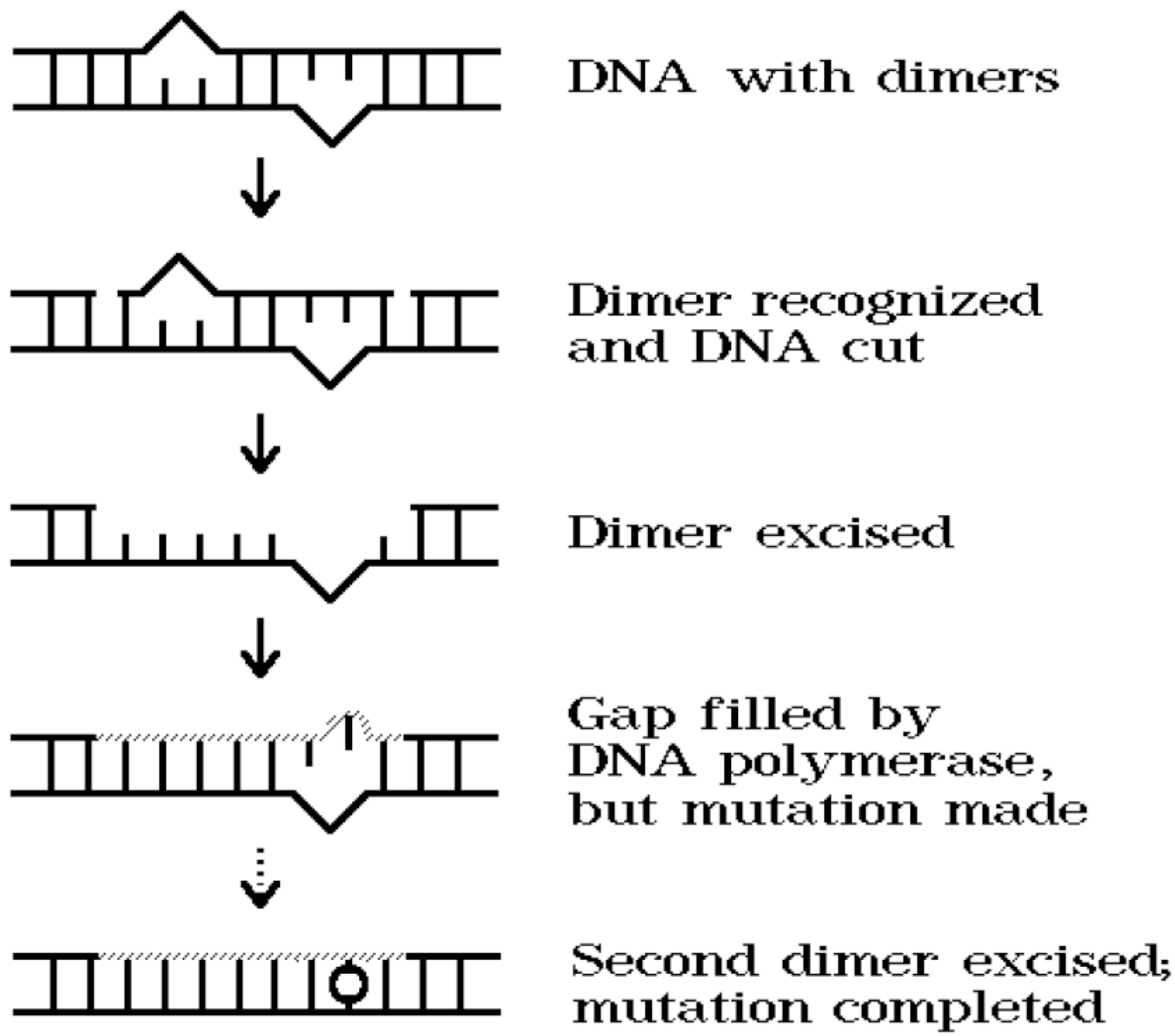
Greek Name	Human Name	Yeast Name	Proposed Function
α	POLA	*POL1*	Replication
β	POLB	—	BER; ss break repair
γ	POLG	*MIP1*	Mitochondrial replication; Mt BER
δ	POLD1	*POL3*	Replication
ϵ	POLE	*POL2*	Replication
ζ	POLZ	*REV3*	Bypass synthesis
η	POLH	*RAD30*	Bypass synthesis
θ	POLQ	—	Bypass synthesis
ι	POLI	—	Bypass synthesis (?)
κ	POLK	—	Bypass synthesis
λ	POLL	*POL4*	NHEJ
μ	POLM	—	NHEJ (?)
ν	POLN	—	Bypass synthesis
—	REV1	*REV1*	Bypass synthesis

Roswell Park:

DNA Repair

1. Direct Repair
2. BER (Base Excision Repair)
3. $\boxed{\text{NER}}$ (Nucleotide Excision Repair)
4. MMR (Mis-Match Repair)
5. $\boxed{\text{SOS Repair}}$ (Error-prone, “last-ditch” response)
6. DSBR (Double Strand Break Repair)
    - i.) Homologous Recombination
    - ii.) NHEJ (Non-Homologous End-Joining)

Mutagenic Repair
(trans-lesion synthesis)



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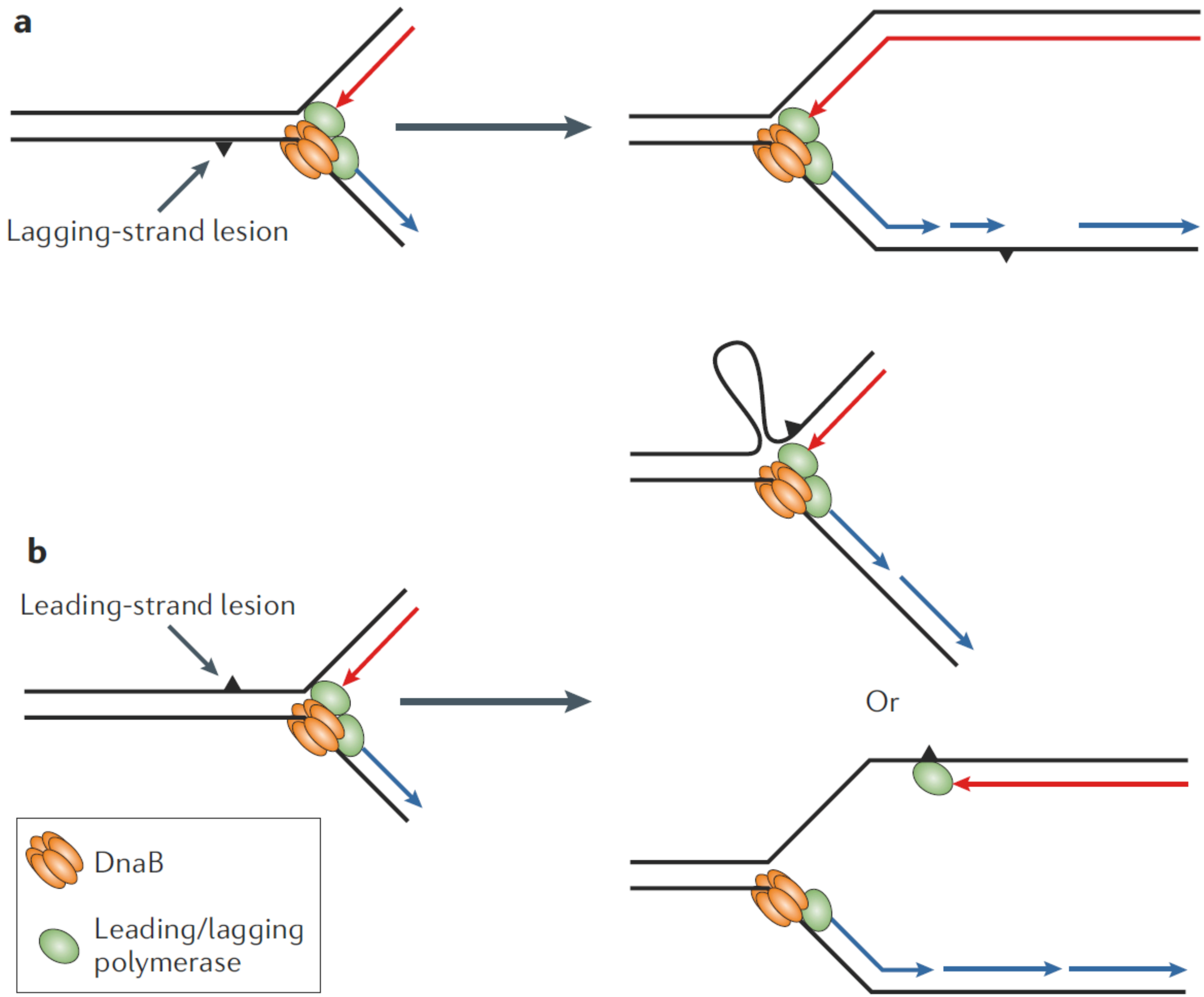
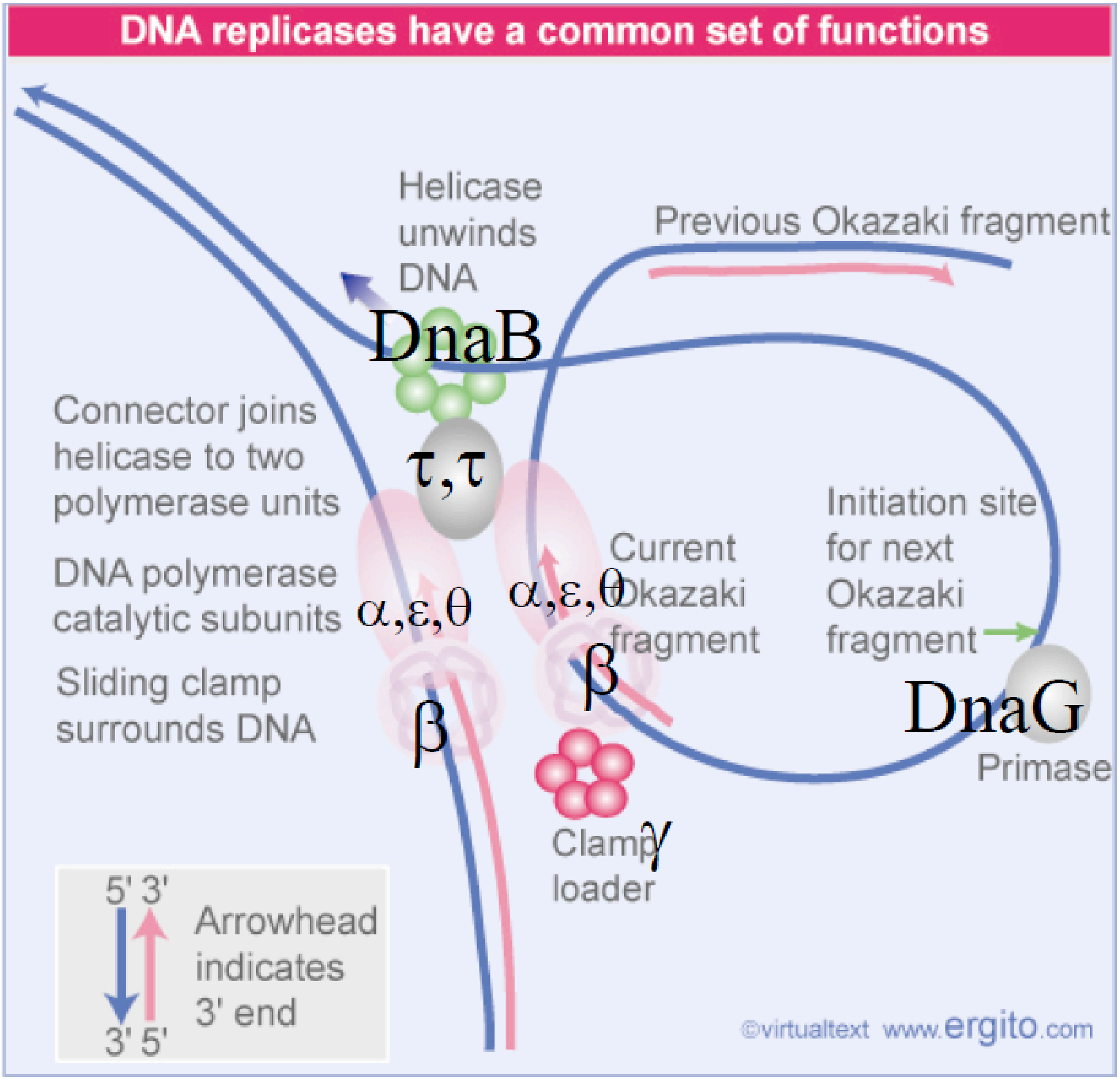


Nature Reviews, Molec. Cell Biol. (Dec2006) v7:933


DNA replicases have a common set of functions
Helicase unwinds DNA
DnaB
Previous Okazaki fragment
Connector joins helicase to two polymerase units
τ,τ
DNA polymerase catalytic subunits
α,ε,θ
α,ε,θ
Current Okazaki fragment
Initiation site for next Okazaki fragment
Sliding clamp surrounds DNA
β
β
DnaG
Primase
Clamp loader
γ
5' 3'
3' 5'
Arrowhead indicates 3' end
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Today's Papers look at a longstanding discrepancy

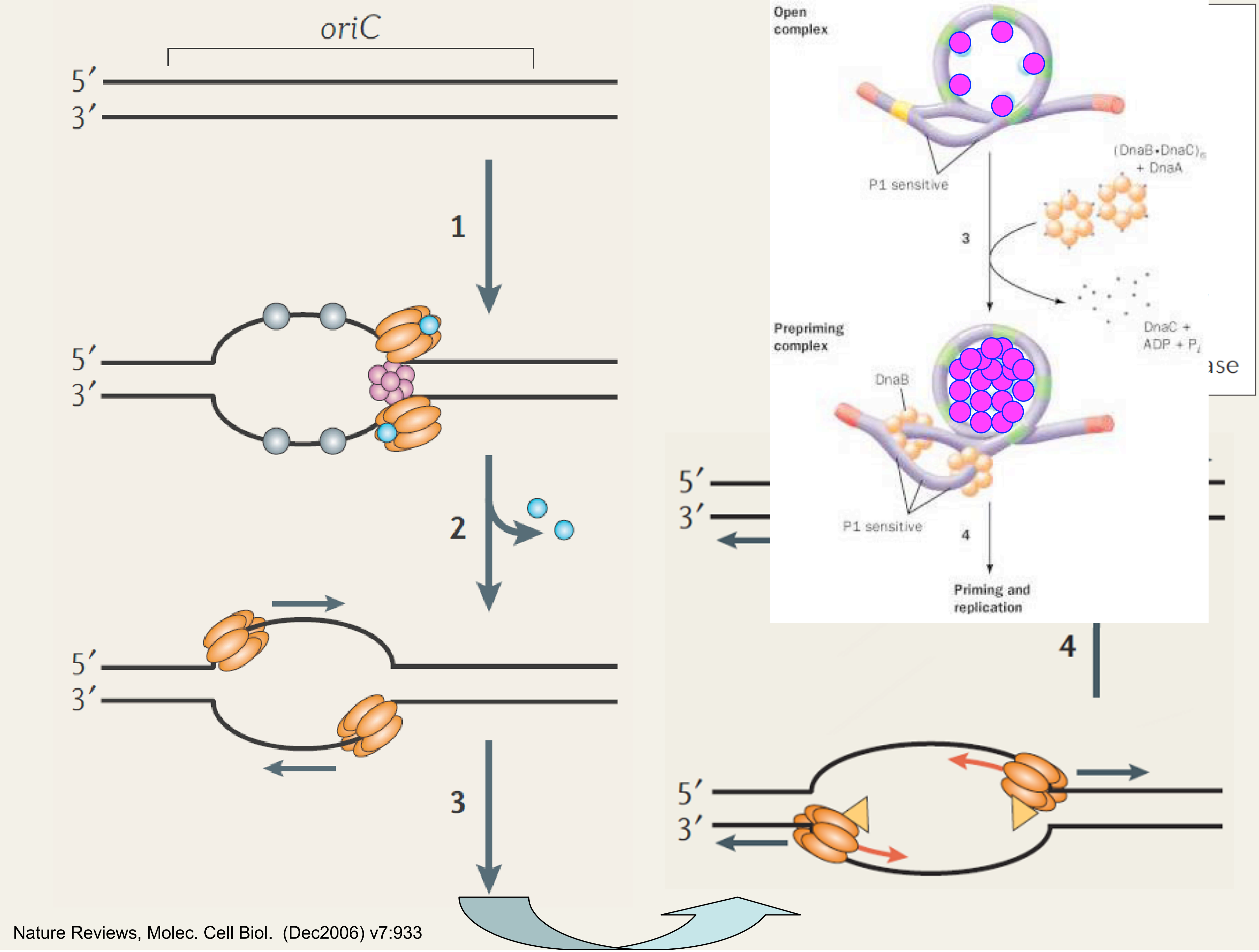
Okazaki & others found nascent strands being synthesized in a discontinuous fashion

IN CONTRAST…

"Biochemical reconstitutions of DNA clearly demonstrated that the leading strand is synthesized in a mechanistically continuous fashion, a disparity that has never been satisfactorily resolved."

The Primosome

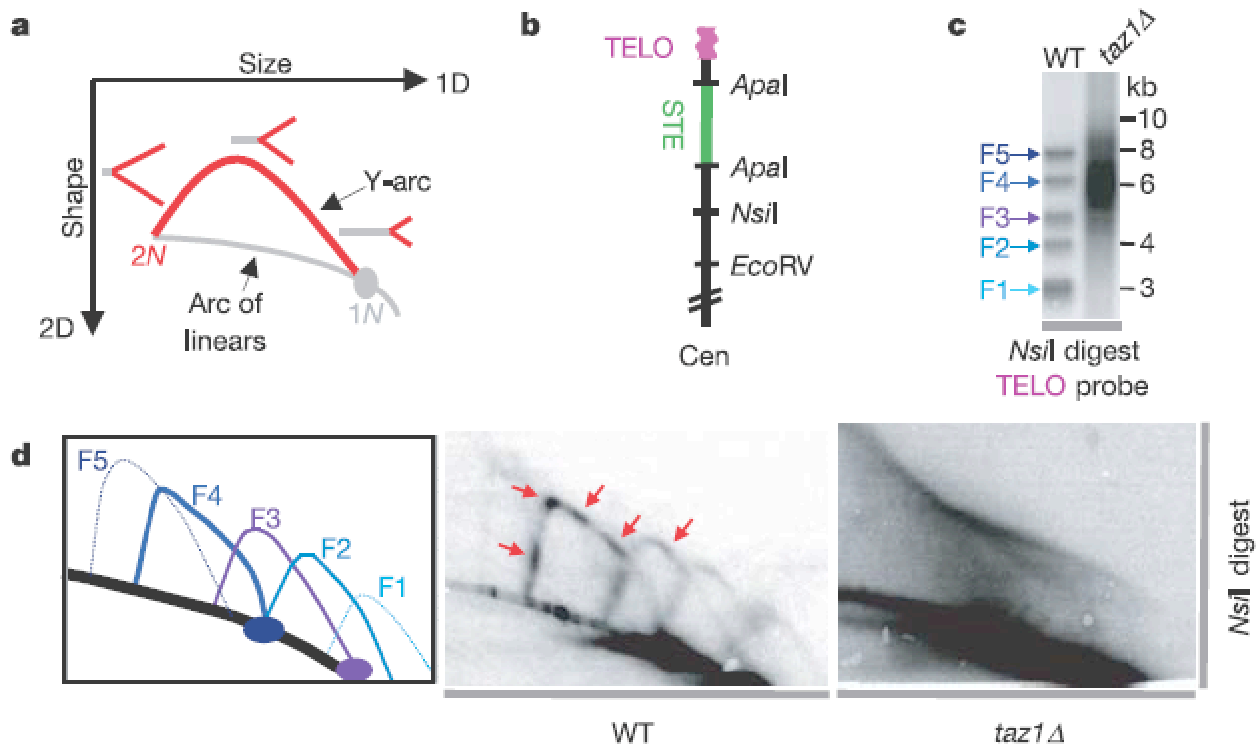
- Required for initiation
- Required to restart a stalled replication fork after DNA has been repaired.



Nature Reviews, Molec. Cell Biol. (Dec2006) v7:933

recA	DNA pairing; strand exchange;	binds w/ polarity unlike SSB
uvrD	DNA helicase II	
ssb	Single-strand binding protein	
ruvA	Holliday junction binding	
ruvB	5′-3′ junction helicase (member of: AAA+ helicases (ATPases associated with diverse cellular activities))	
ruvC	Holliday junction endonuclease	
polA	DNA polymerase I; repair DNA synthesis	
priA	3′-5′ helicase; restart primosome assembly	
dnaB	Restart primosome component (5′→3′ helicase)	
dnaG	Restart primosome component	

& some methodology



- Topic for Discussion Thursday: It appears in both papers that specialized translesion polymerases are needed. How broadly applicable are these proposed mechanisms (i.e., can we really assume that what occurs in a severely damaged DNA strand is the same process as “healthy” DNA synthesis? Are they specific to single-celled organisms which do not participate in the complex process of apoptosis that is found in multi-cellular organisms)?

Replication fork reactivation downstream of a blocked nascent leading strand

Ryan C. Heller[1] & Kenneth J. Marians[1,2]

NATURE|Vol 439|2 February 2006

How does Bacteria Deal with a Leading Strand Block?

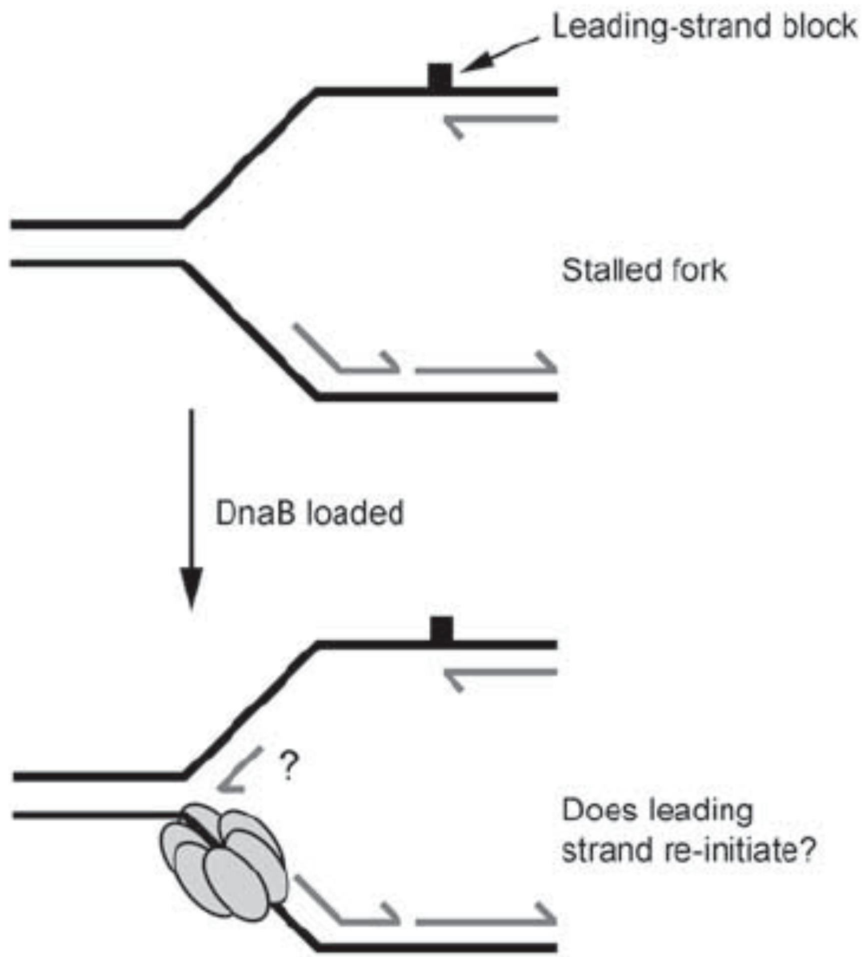
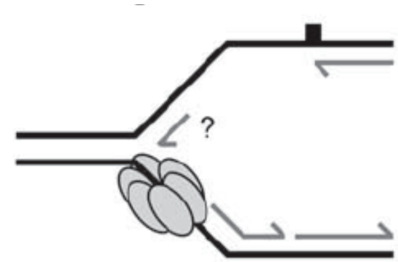


FIGURE 1

Priming of Leading Strand via PriC or PriA-Dependent Systems



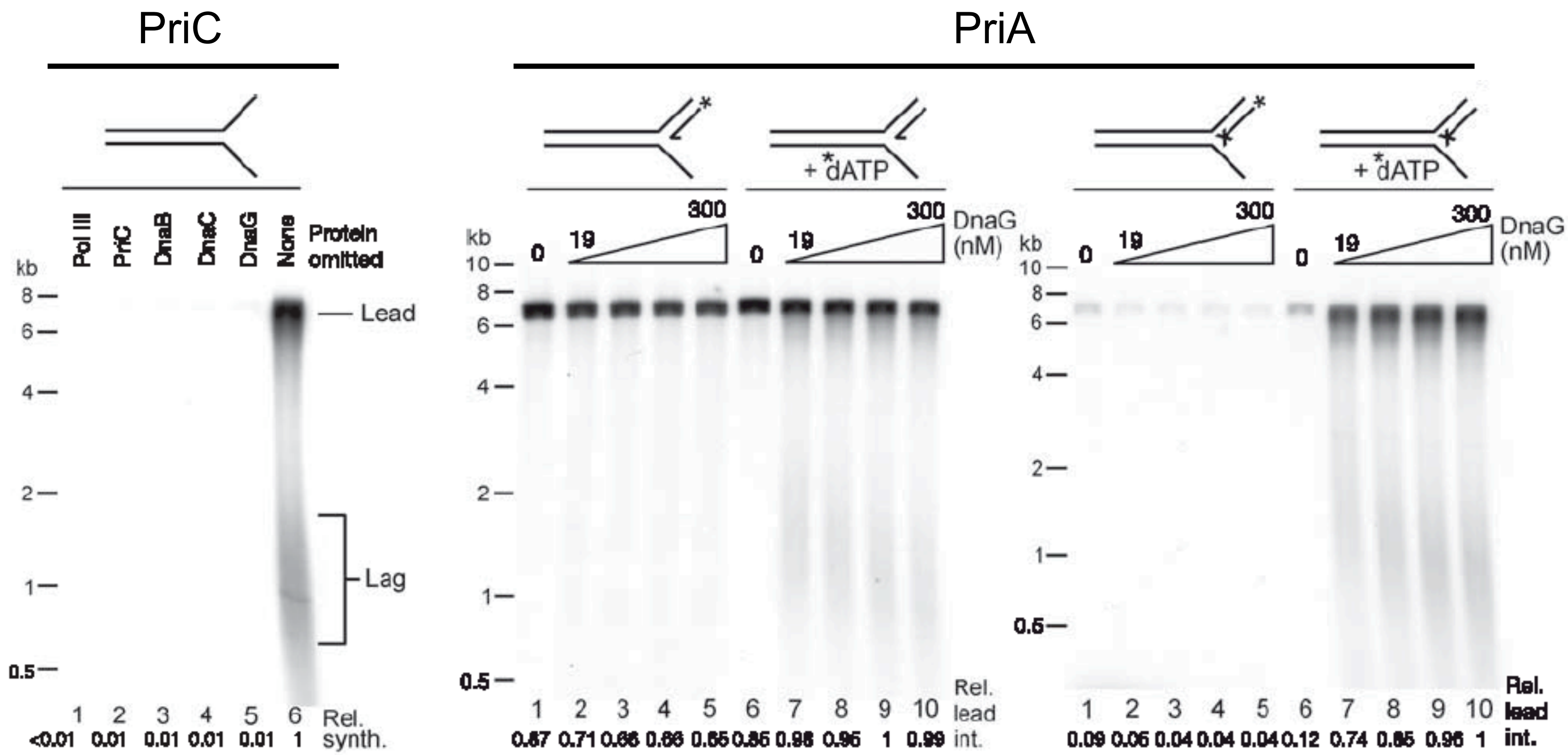


FIGURE 1

DnaG Priming and Interactions with DnaB

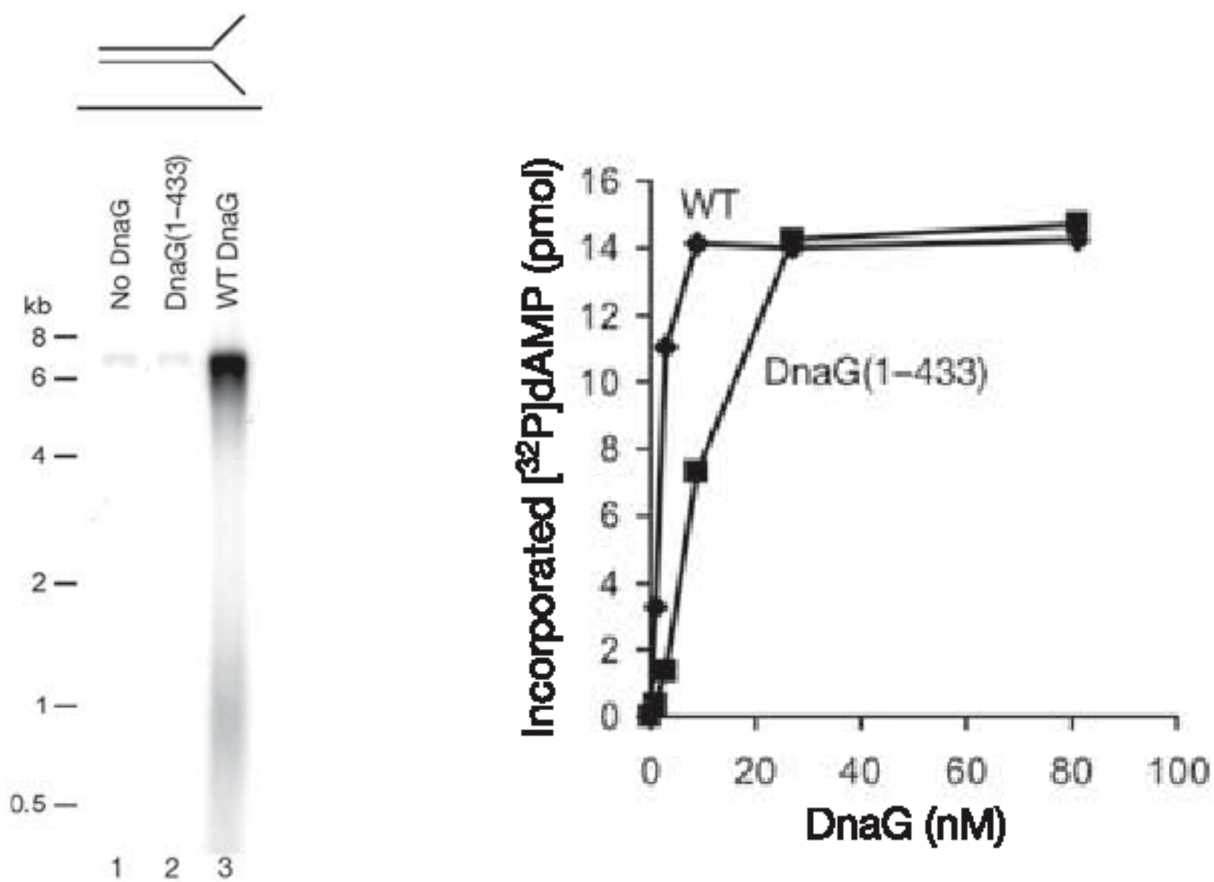


FIGURE 2

How Many DnaG Hexamers are Required for Restart of Replication?

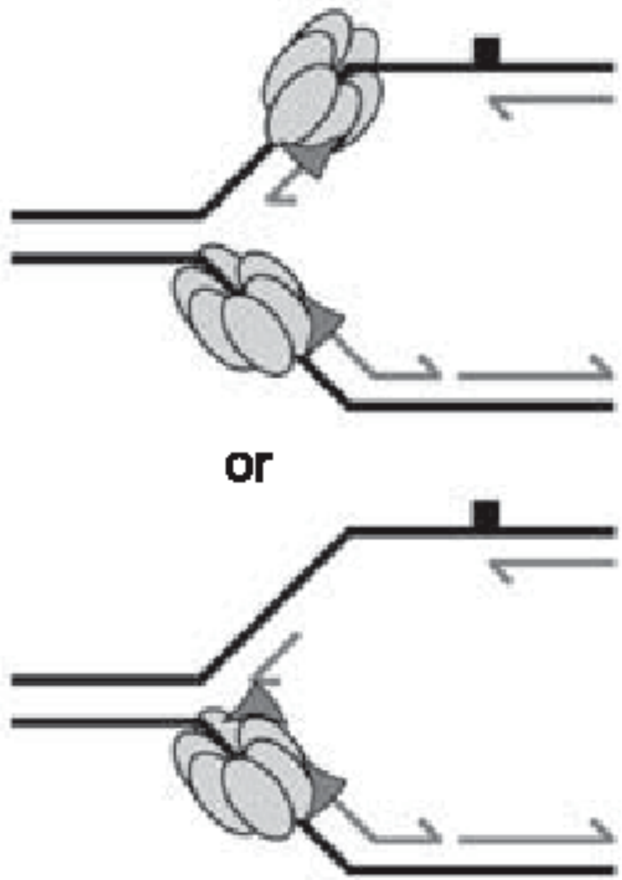


FIGURE 2

Modified Linear Template:
Fork 3'-Arm is Replaced with a Biotin Group

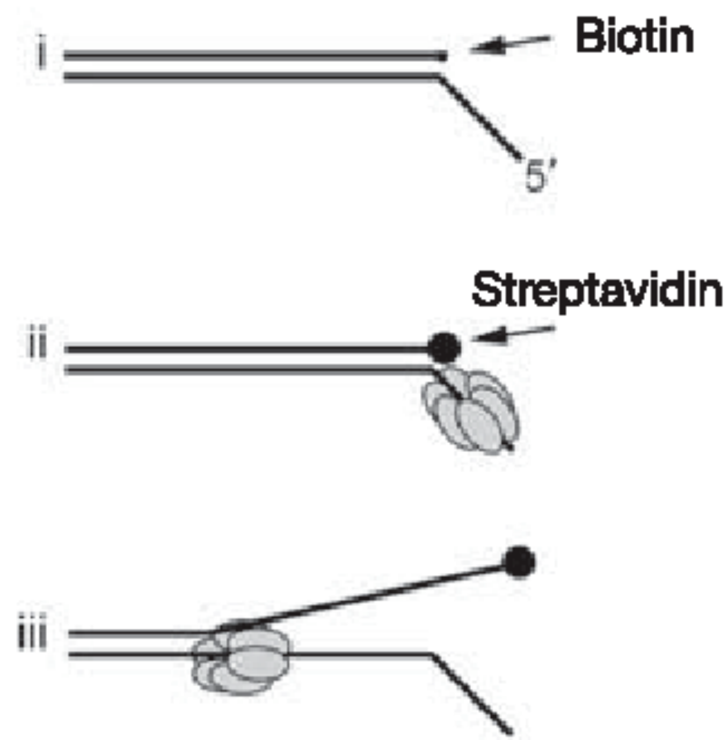


FIGURE 2

Replication Restart Systems

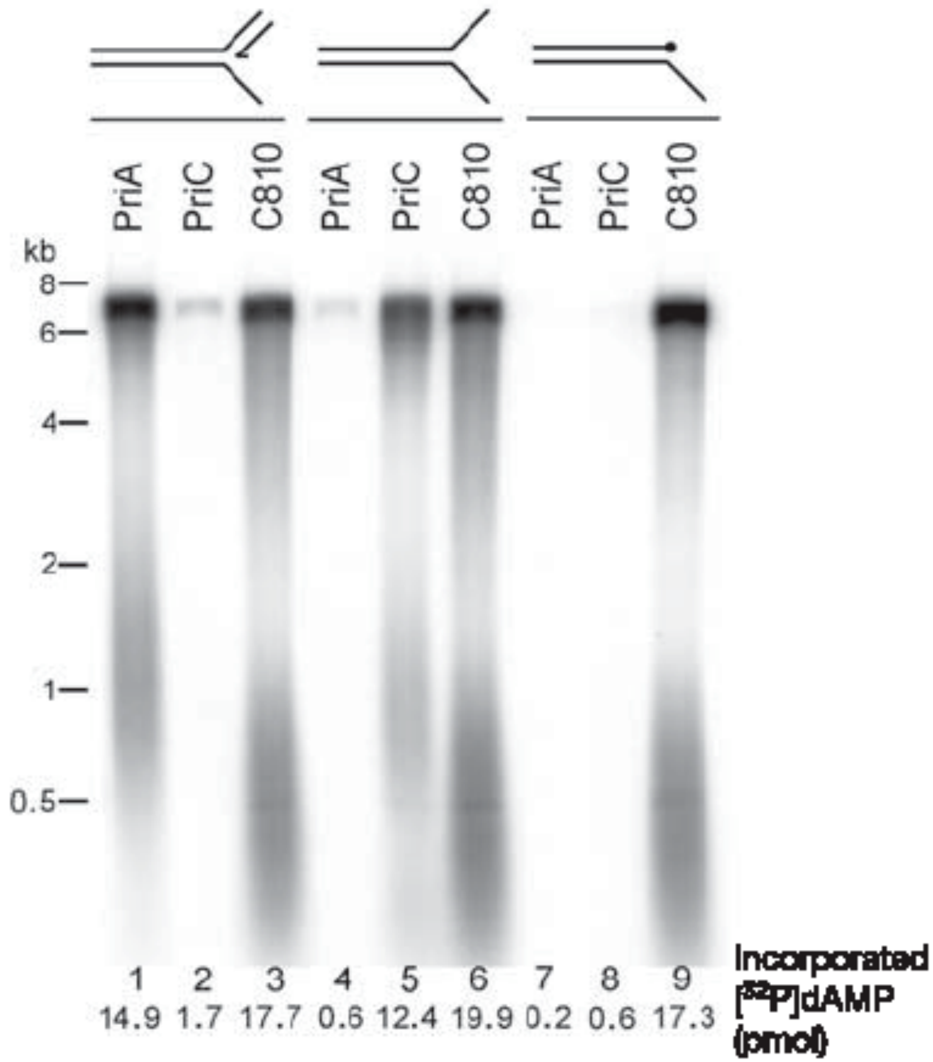


FIGURE 2

A Single DnaB Hexamer on the Lagging-Strand Template Coordinates Priming on Both Strands

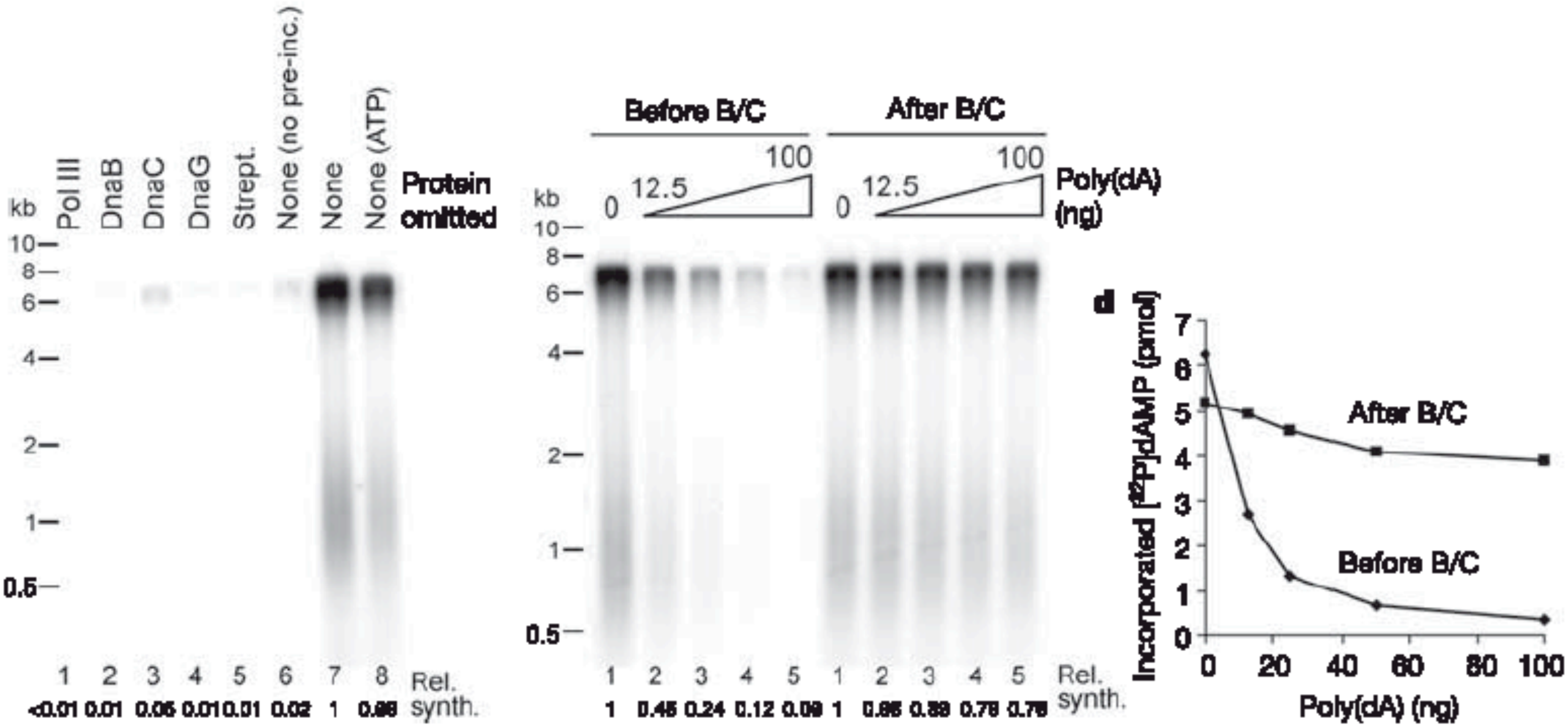


FIGURE 3

PriC-Dependent Restart of a Stalled Fork Generates Daughter Strand Gaps

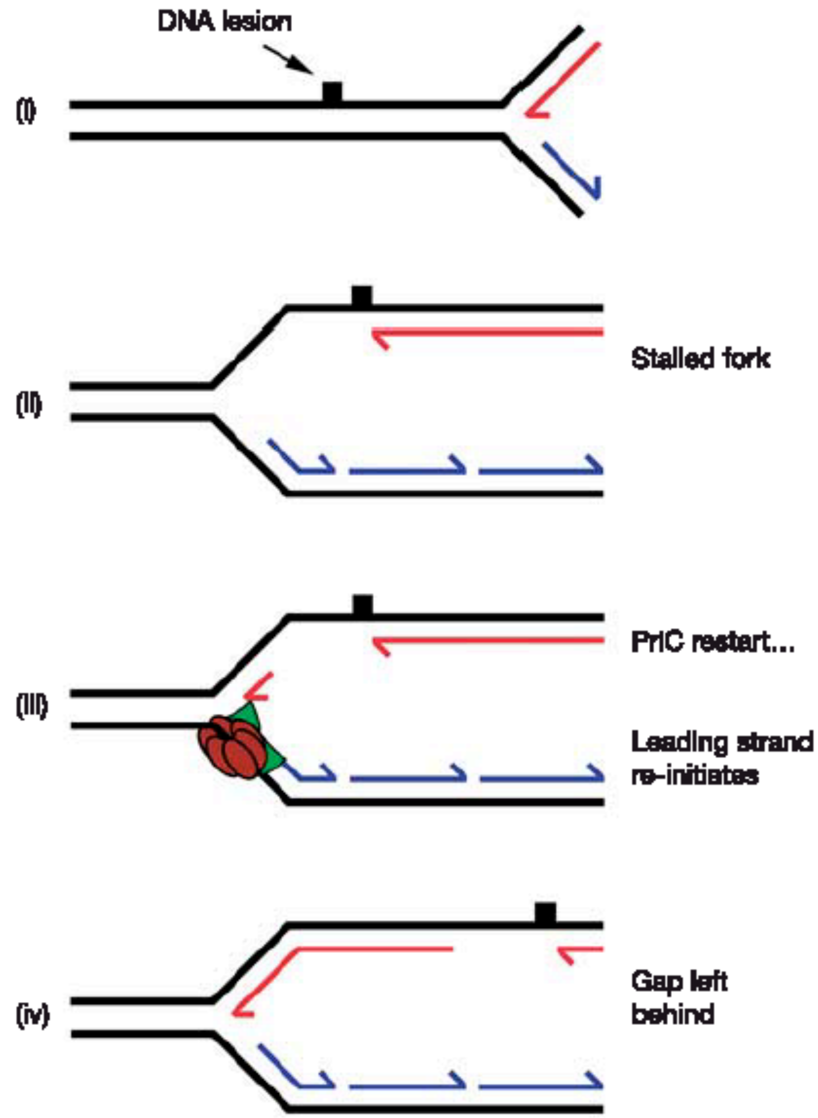


FIGURE 4

Conclusions – Heller & Marians

- Leading strand replication re-initiation occurs within bacteria
- Both PriA and PriC restart systems can prime the leading strand with the appropriate fork template
    - PriC is the main replisome restart machinery in lesion bypass
- A single DNA hexamer primes both the leading and the lagging strand

Multiple Mechanisms Control Chromosome Integrity after Replication Fork Uncoupling and Restart at Irreparable UV Lesions

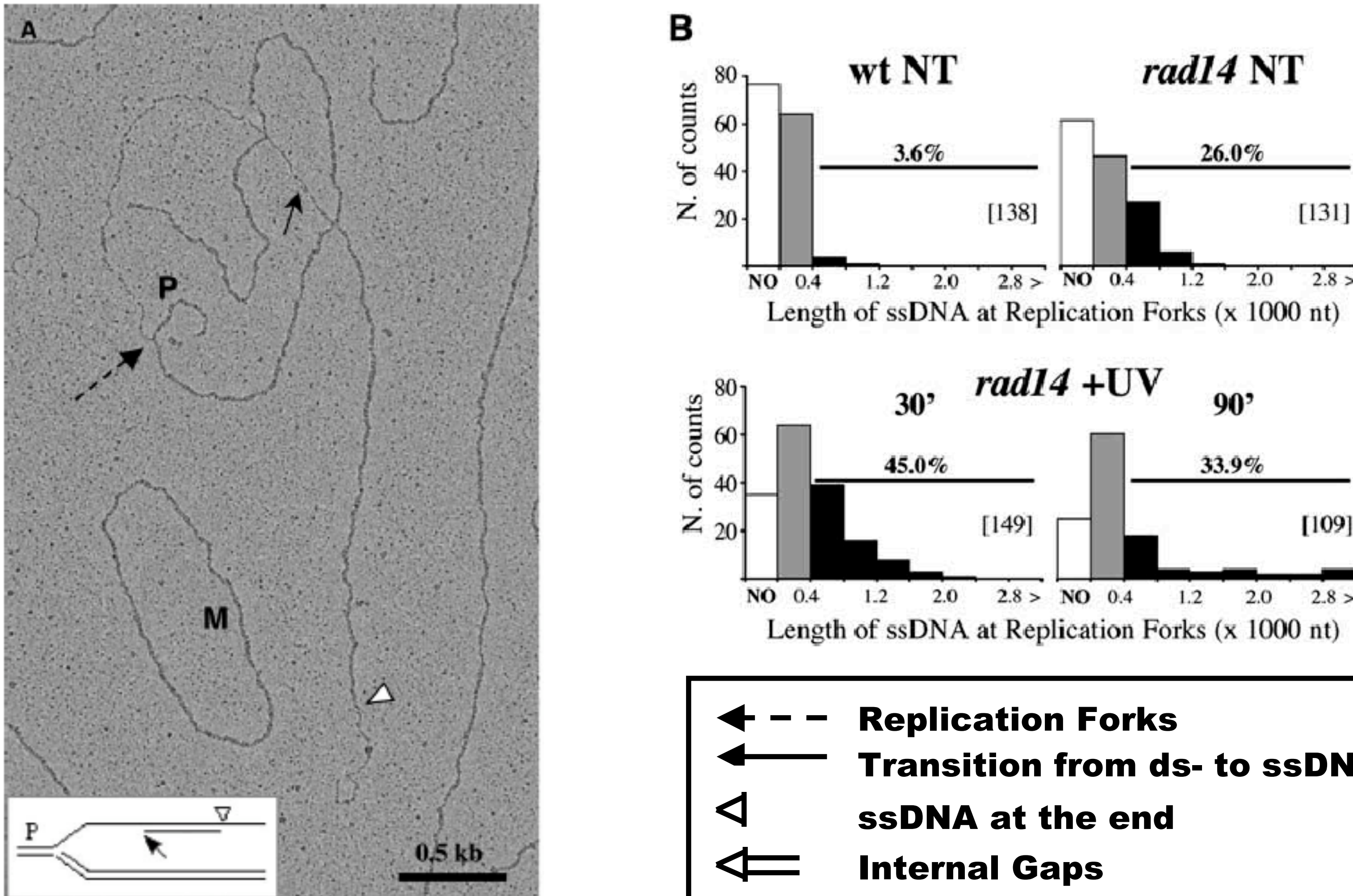
Massimo Lopes,[1,4,*] Marco Foiani,[2,3]
and José M. Sogo[1,*]

Molecular Cell *21*, 15–27, January 6, 2006

EM Experimental Design

- rad14 yeast cells (excision repair deficient)
    - presynchronized in G1
    - UV-irradiated (constant dose of 50J/m^2) and released from block into S phase
- Samples from UV or mock treated rad14 cells
    - Cross linked *in vivo* with psoralen after release from G1
    - Enriched for RIs by binding/elution from BND cellulose
    - EM under nondenaturing conditions
- Internal spread Markers (3.1kb)
    - Supercoiled under native conditions
    - Small single strand bubbles to compensate supercoiling
    - Internal control for DNA length measurements for both ss and dsDNA

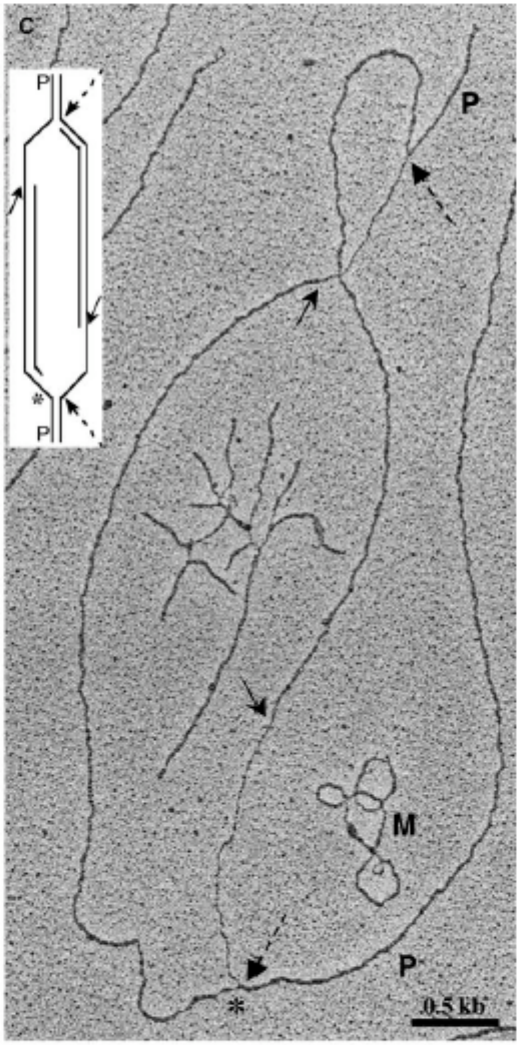
Uncoupling of Leading and Lagging Strand Synthesis at UV-Damaged Replication Forks

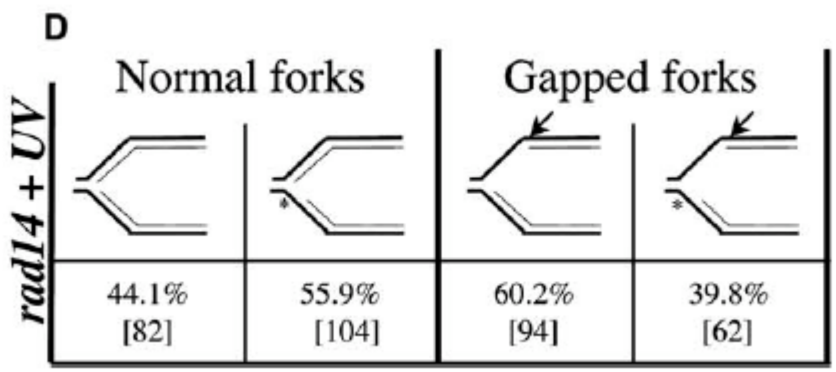


Replication Forks
Transition from ds- to ssDNA
ssDNA at the end
Internal Gaps
* Small ssDNA region at the fork
P Parental Unreplicated
M Internal Spreading Marker (3.1kb)

FIGURE 1

Uncoupling of Leading and Lagging Strand Synthesis at UV-Damaged Replication Forks





Replication Forks

Transition from ds- to ssDNA

ssDNA at the end

Internal Gaps

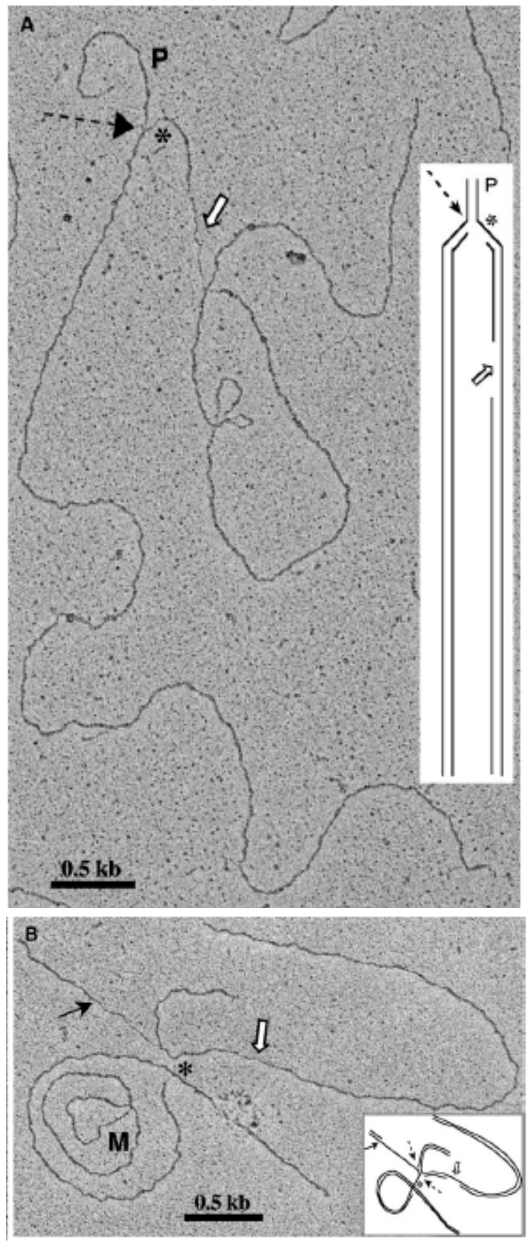
* Small ssDNA region at the fork

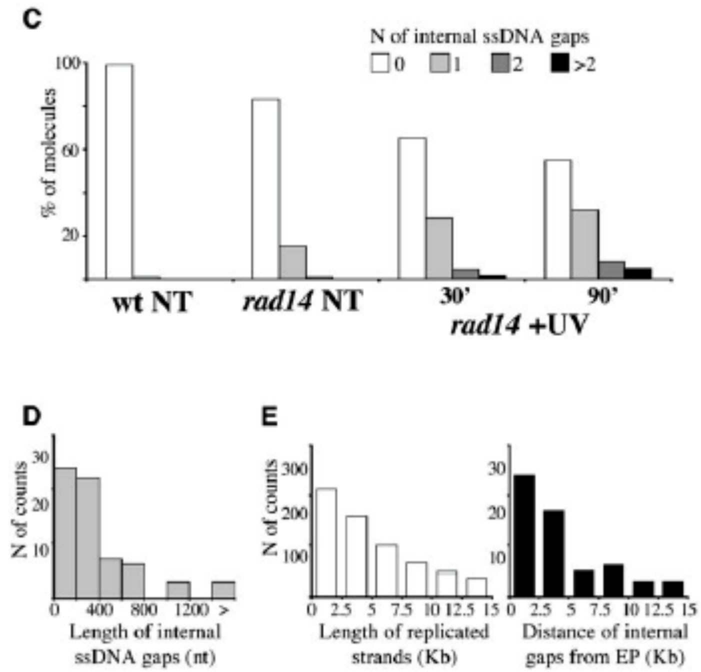
P Parental Unreplicated

M Internal Spreading Marker (3.1kb)

FIGURE 1

Small ssDNA Regions Accumulate along UV-Damaged Replicated Duplexes

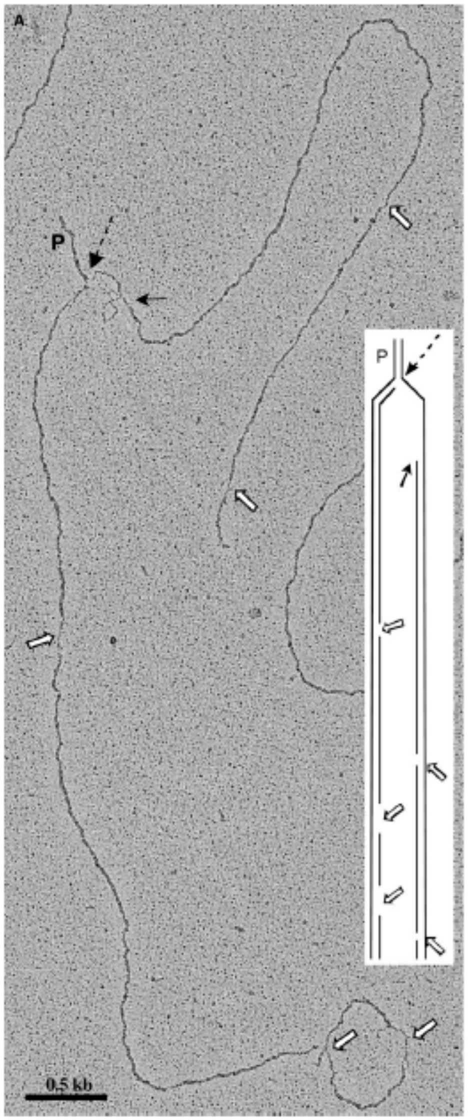




◄ – – –	Replication Forks
◄———	Transition from ds- to ssDNA
◁	ssDNA at the end
⇦	Internal Gaps
*	Small ssDNA region at the fork
P	Parental Unreplicated
M	Internal Spreading Marker (3.1kb)

FIGURE 2

Increased Internal Gaps in TLS Polymerase, Recombination and Checkpoint Mutants



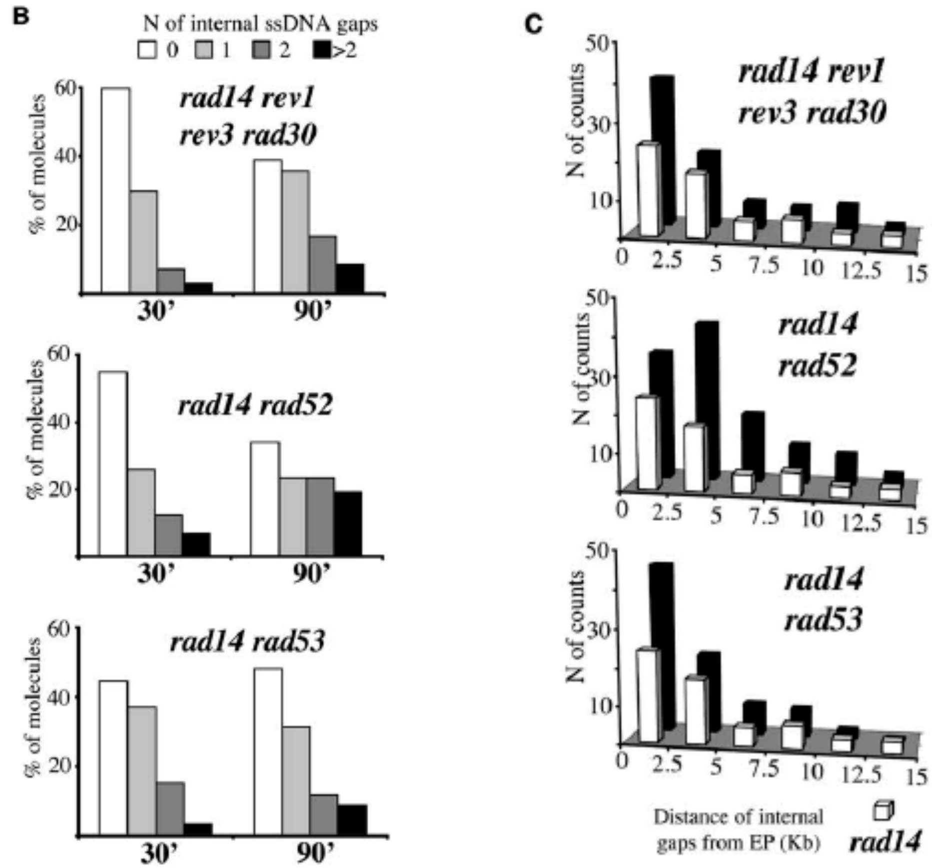


Fig 2C

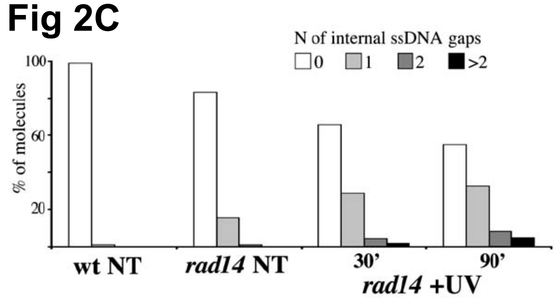


FIGURE 3

⇐ Internal Gaps

Fork Progression at UV-Damaged Template

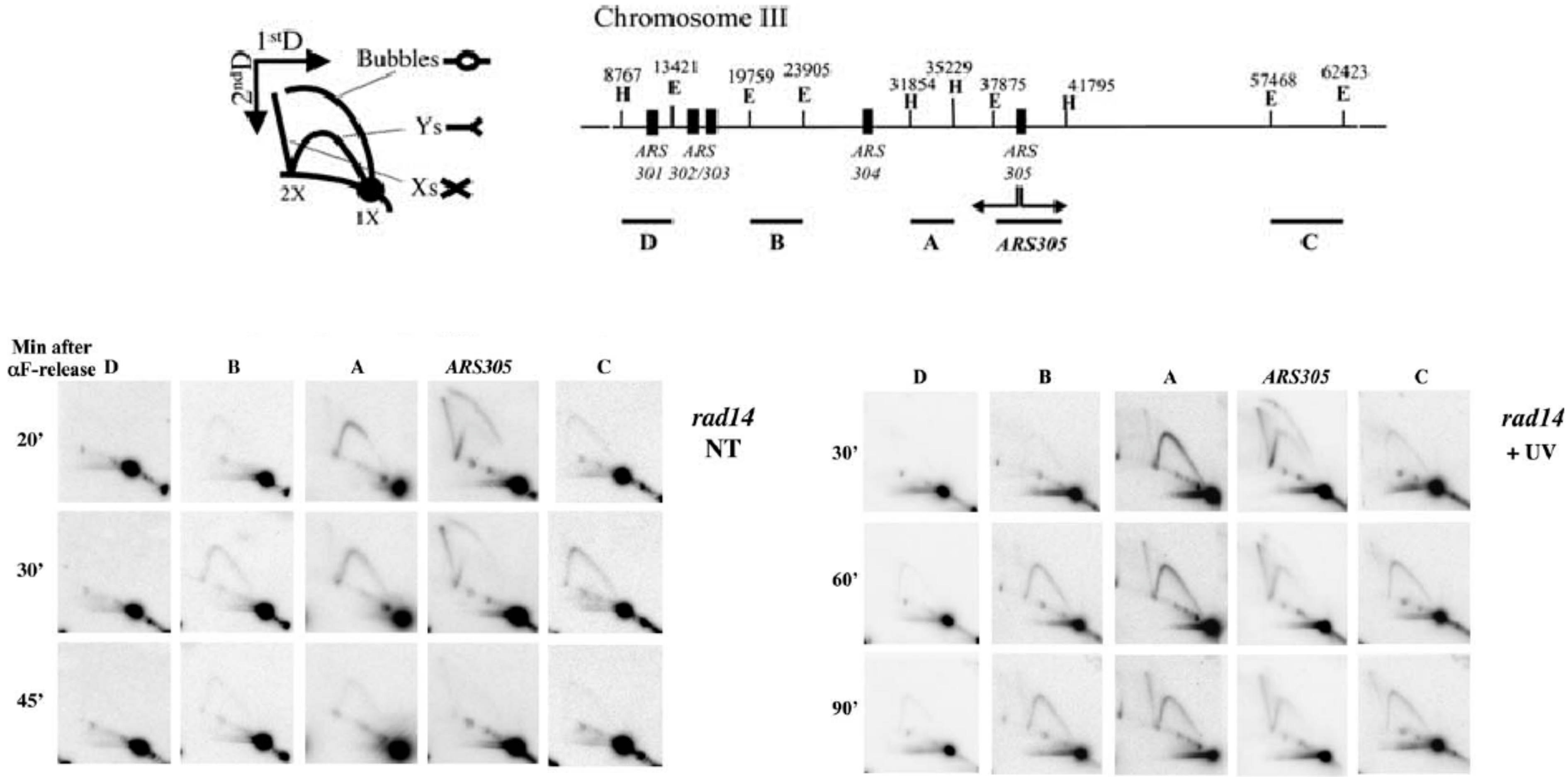
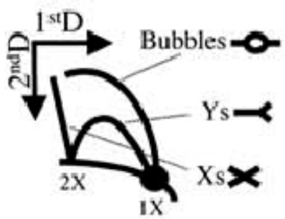
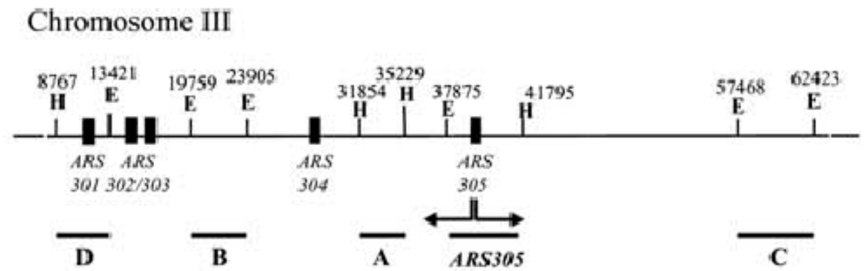


FIGURE 5

Progression and Stability of UV-Damaged Forks:
Contribution of TLS, Recombination, and Checkpoint Factors Above and Beyond Excision Repair Deficiency





- Translesion Synthesis Polymerase
  - No change with replication timing and extent
  - TLS not needed for efficient fork progression through damaged template
  - No change with X molecule
- Recombination Factors
  - Fork movement unaffected
  - Loss of X molecule
- Checkpoint Factors
  - Bubble arc on ARS305 barely detectable – forks originating at this locus may be progressing asymetrically and eventually break
  - Reduction in Y signals far from the origin

UV-Damaged DNA Replication Forks in rad14 Cells

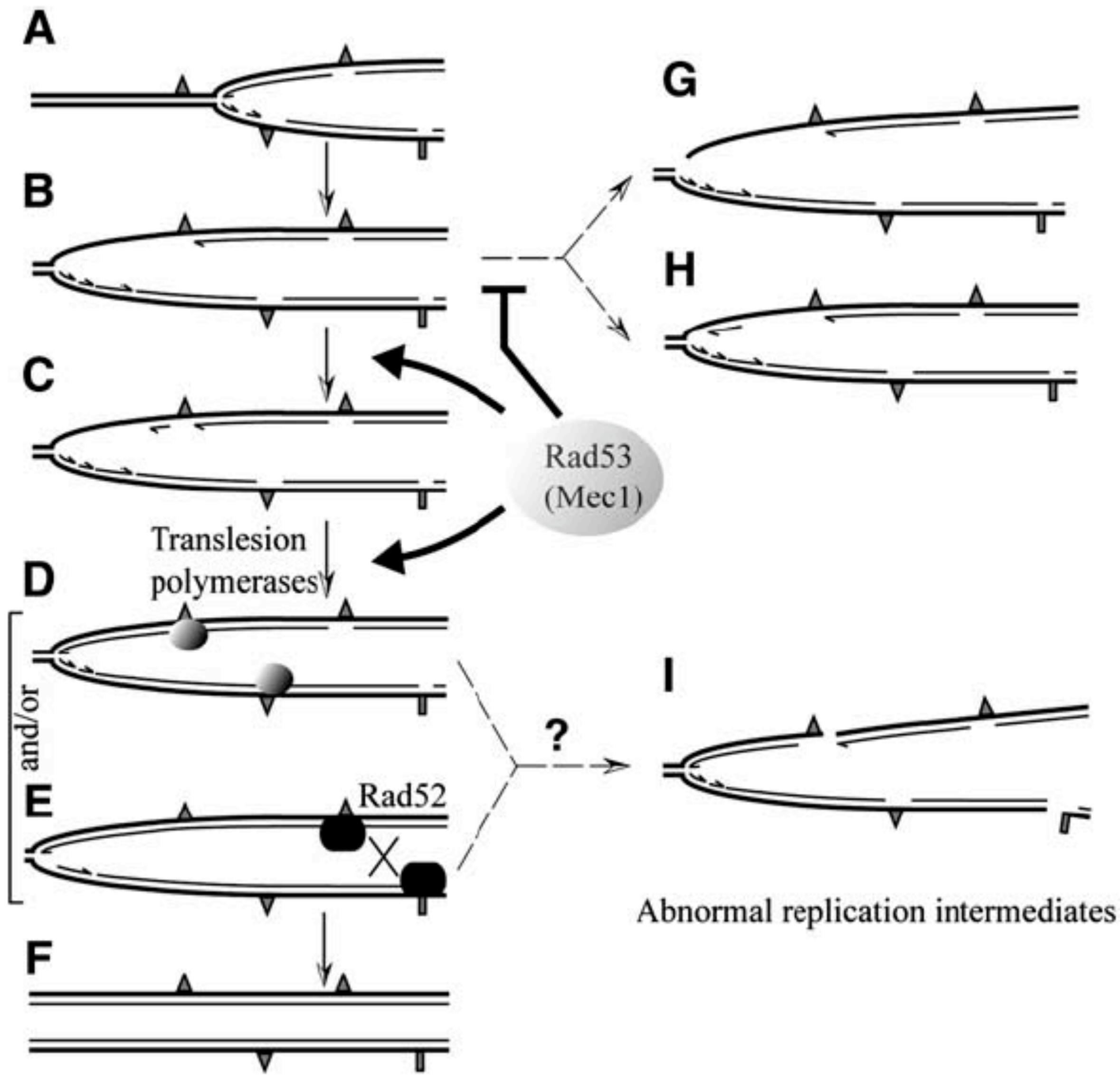


FIGURE 7

Conclusions – Lopes et. al.

- Uncoupled DNA synthesis is detectable *in vivo* when yeast cells are forced to replicate irreparable lesions on chromososmes
    - Long ssDNA regions detected at replication forks restricted to one side (likely the leading strand)
- Internal ssDNA gaps point to repriming events at forks
    - "Easy" fix on lagging strand
    - Replication uncoupled when at leading strand
- Breaks may be occuring *in vivo* at damaged ssDNA regions along the replicated duplexes
- TLS, checkpoint activation, and recombination needed for full replication of a damaged template to protect chromosome from unscheduled processing events