Genomic Rescue:
Restarting failed replication forks

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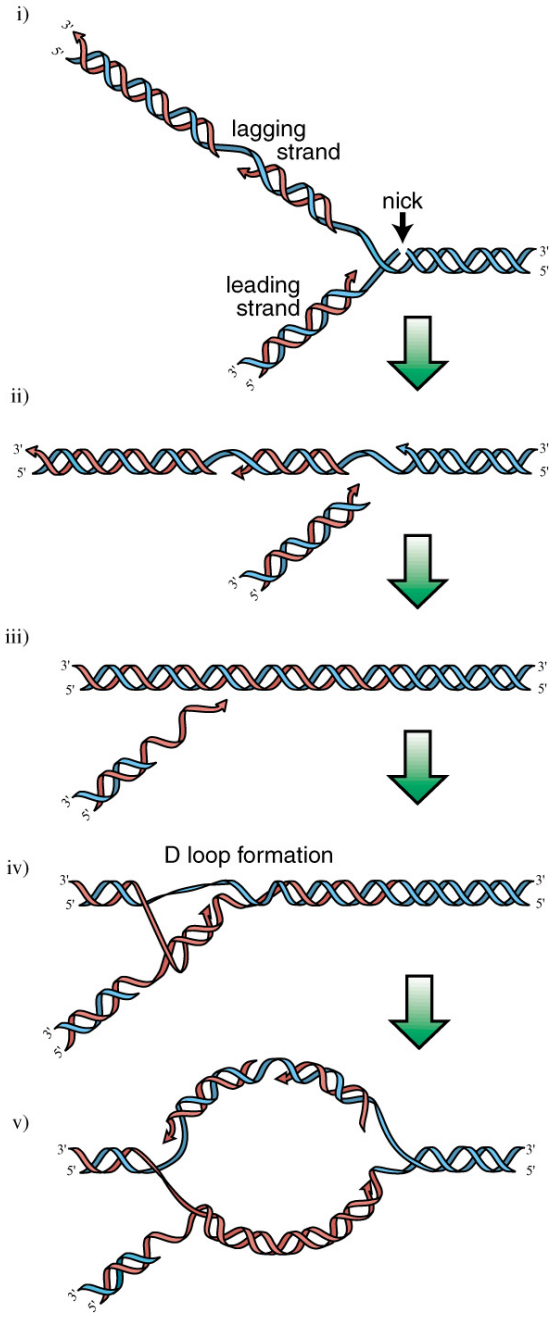
MI/BCH/BIO 615

Why Study *E. coli*?

fundamental metabolic processes generally conserved (at least philosophically) with those in higher organisms

easy to grow: fast and inexpensive
genome completely sequenced
powerful genetic tools

proteins generally well-characterized:
generally one function per protein, one protein per function
many crystallized



PriA

Preferred substrate is a replication fork with a missing **lagging** strand.
Equivalent to a D-loop with an invaded 3'-OH single strand.
Loads the dnaB replicative helicase.

The loading of dnaB is necessary and sufficient for the construction of a new replication fork.

Molecular Cell, Vol 11, 817–826, March 2003
PriA Mediates DNA Replication Pathway Choice at Recombination Intermediates
Liewei Xu and Kenneth J. Marians

RecA

- Binds single-stranded DNA and double-stranded DNA
- Searches for regions of homology
- Exchanges homologous strands

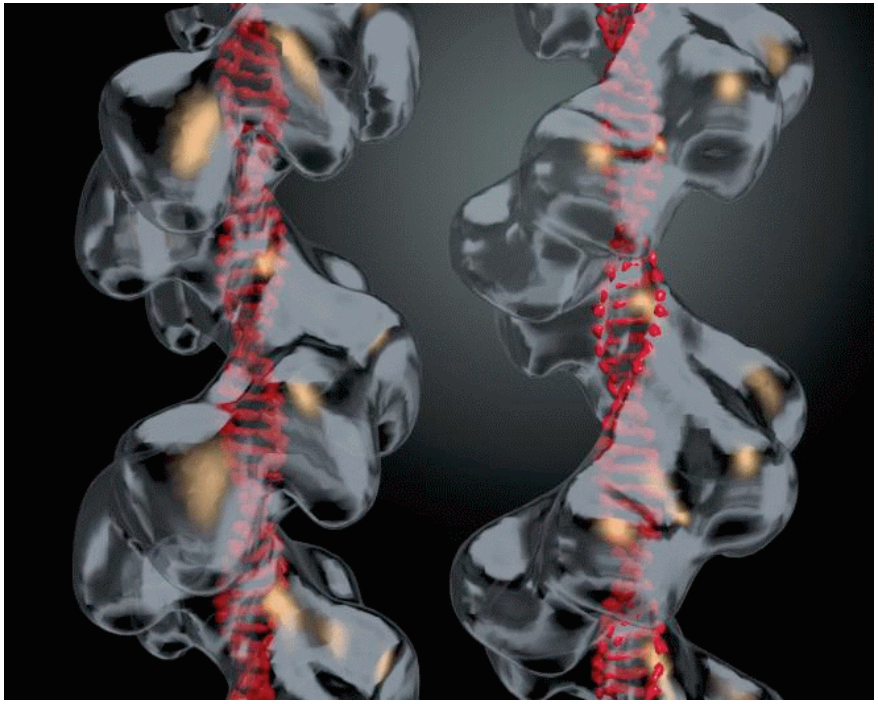
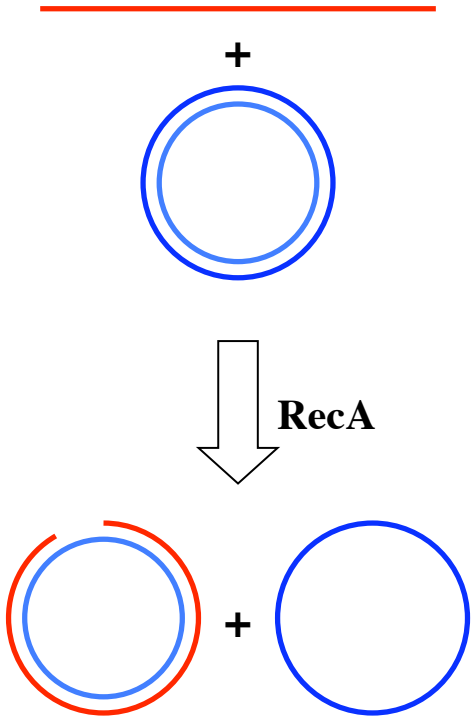
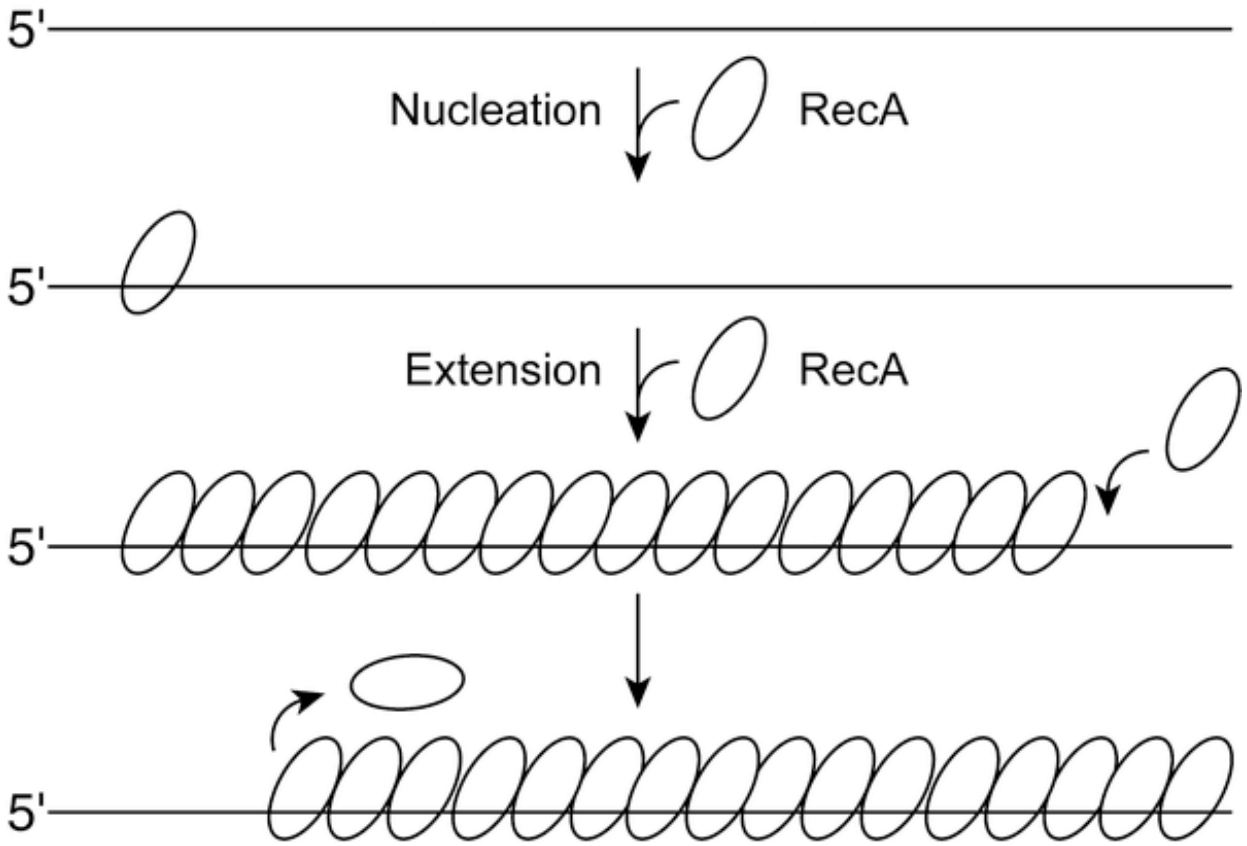


Image is from the cover of the March 26, 1993 issue of *Science*

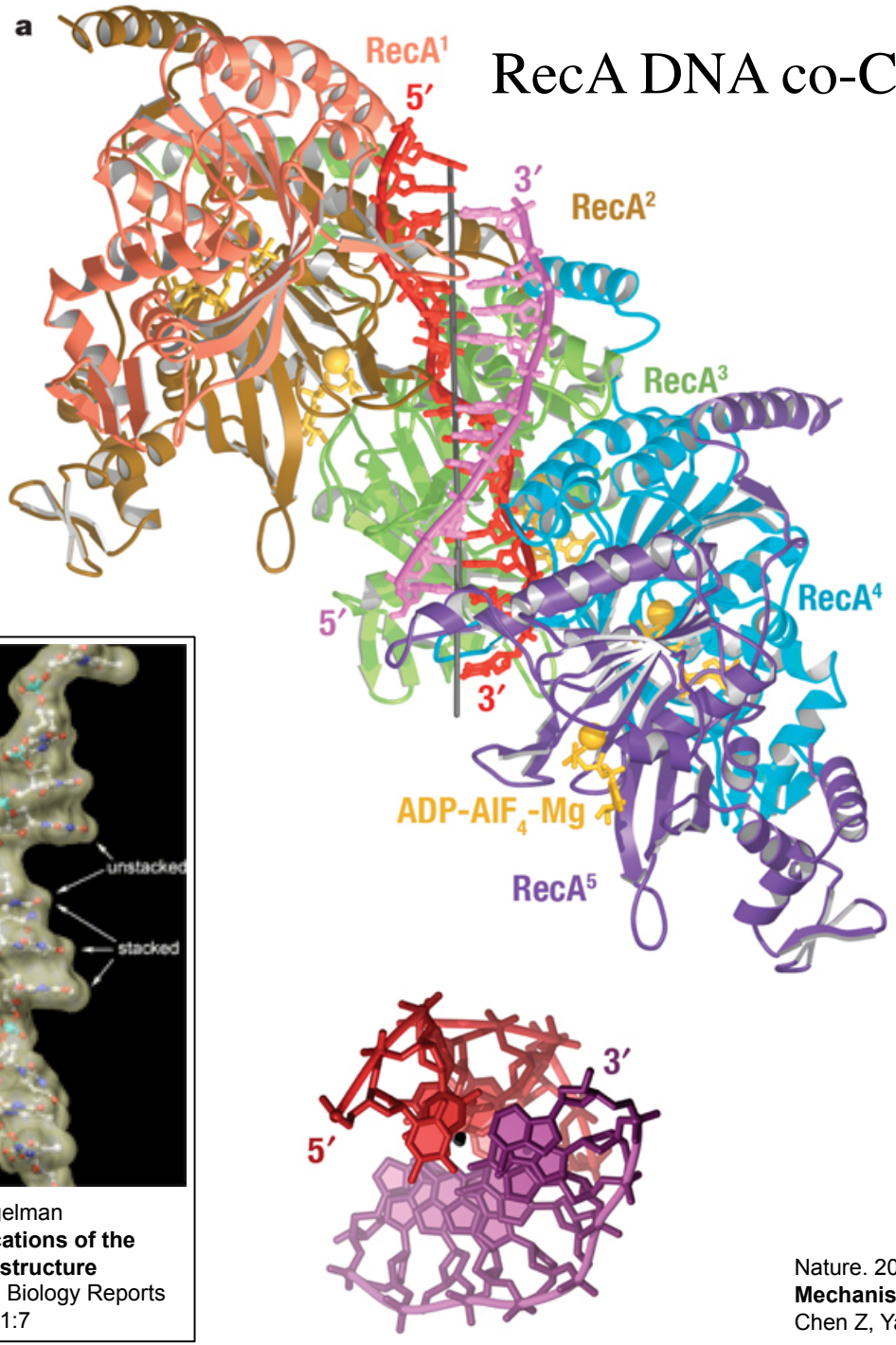


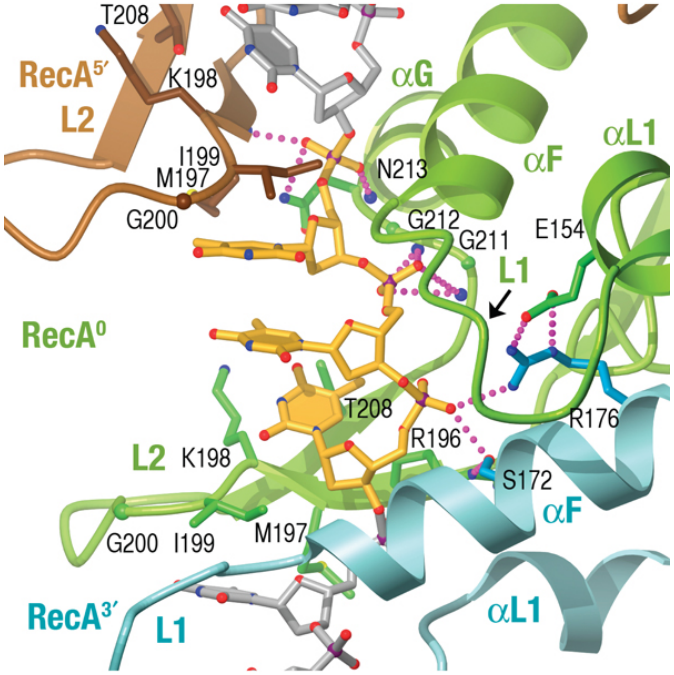
RecA Nucleation and Extension

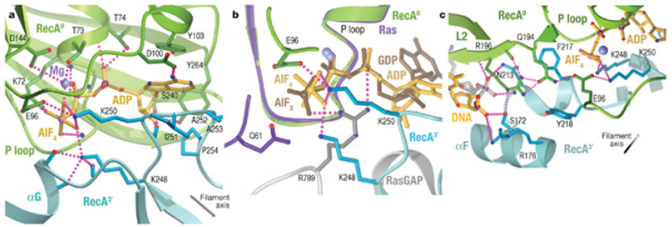


Cox JM, Tsodikov OV, Cox MM.
Organized unidirectional waves of ATP hydrolysis within a RecA filament.
PLoS Biol. 2005 Feb;3(2):e52.

RecA DNA co-Crystal Structure





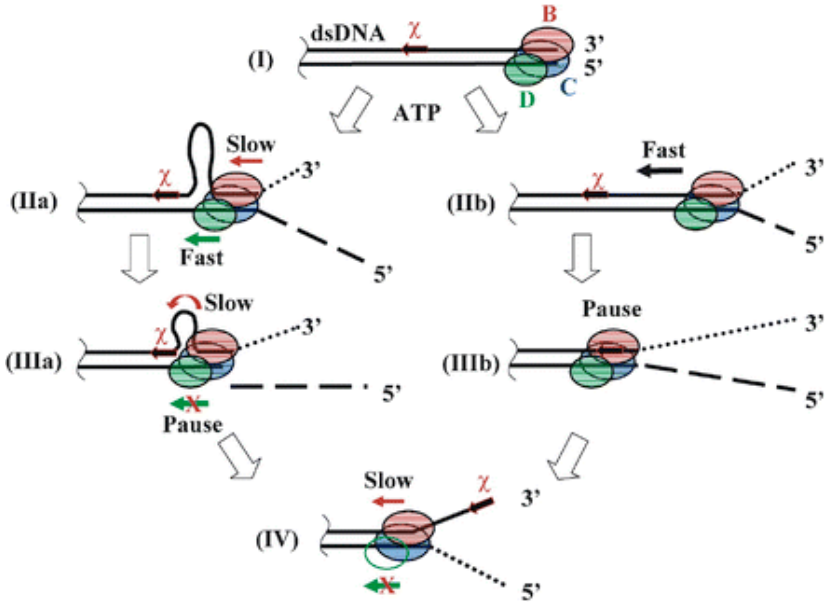


EH Egelman
Implications of the RecA structure
F1000 Biology Reports
2009, 1:7

Nature. 2008 May 22; 453(7194):489-4
Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures.
Chen Z, Yang H, Pavletich NP

RecBCD

- Bind double-stranded DNA ends
- Degrade both stands until a X site (GCTGGTGG) is reached
- Switch to 5'-3' exonuclease generating a 3' single-stranded tail
- Load RecA on the single-stranded tail



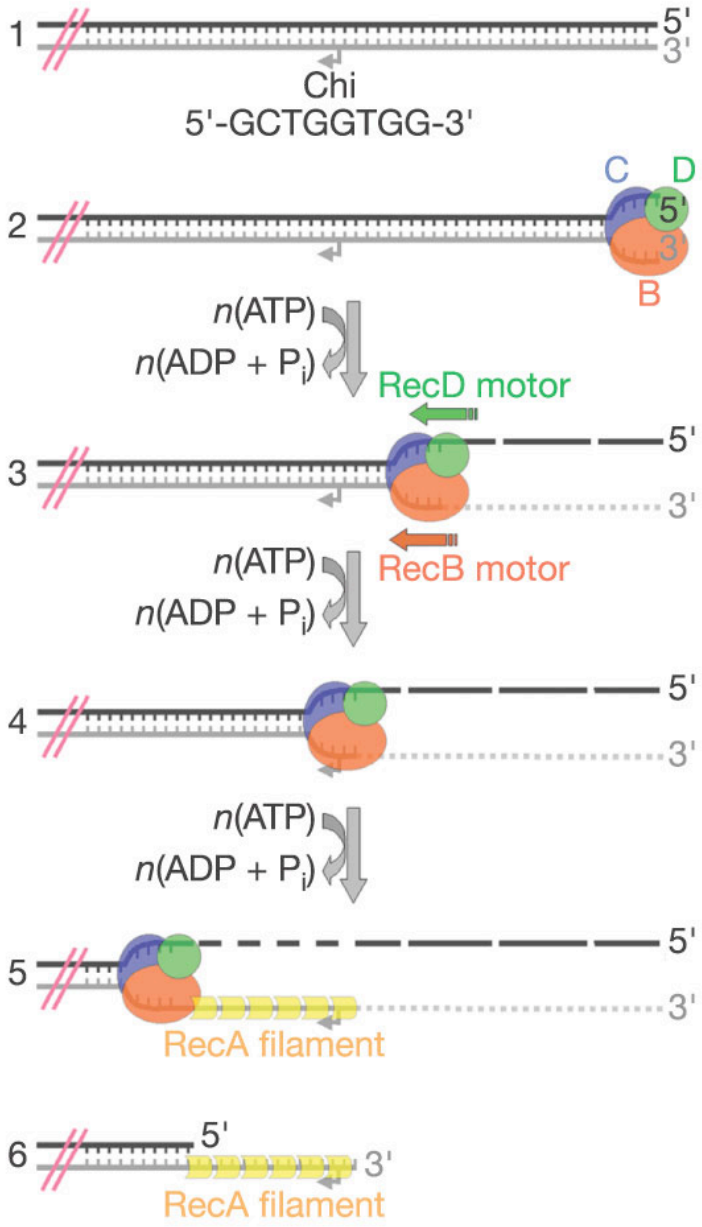
RecB: slow 3' to 5' helicase

RecD: fast 5' to 3' helicase
inhibits loading of RecA

RecBC: loads RecA on constitutively

Cell, Vol 114, 647–654, 5 September 2003
A Molecular Throttle: The Recombination Hotspot χ Controls DNA Translocation by the RecBCD Helicase
Maria Spies, Piero R. Bianco, Mark S. Dillingham, Naofumi Handa, Ronald J. Baskin, and Stephen C. Kowalczykowski

RecBCD



Double-stranded DNA end

RecBCD binds

RecBCD translocates and separates DNA strands
Both strands are degraded

X site is recognized

Degradation of 3' strand stops
RecA is loaded

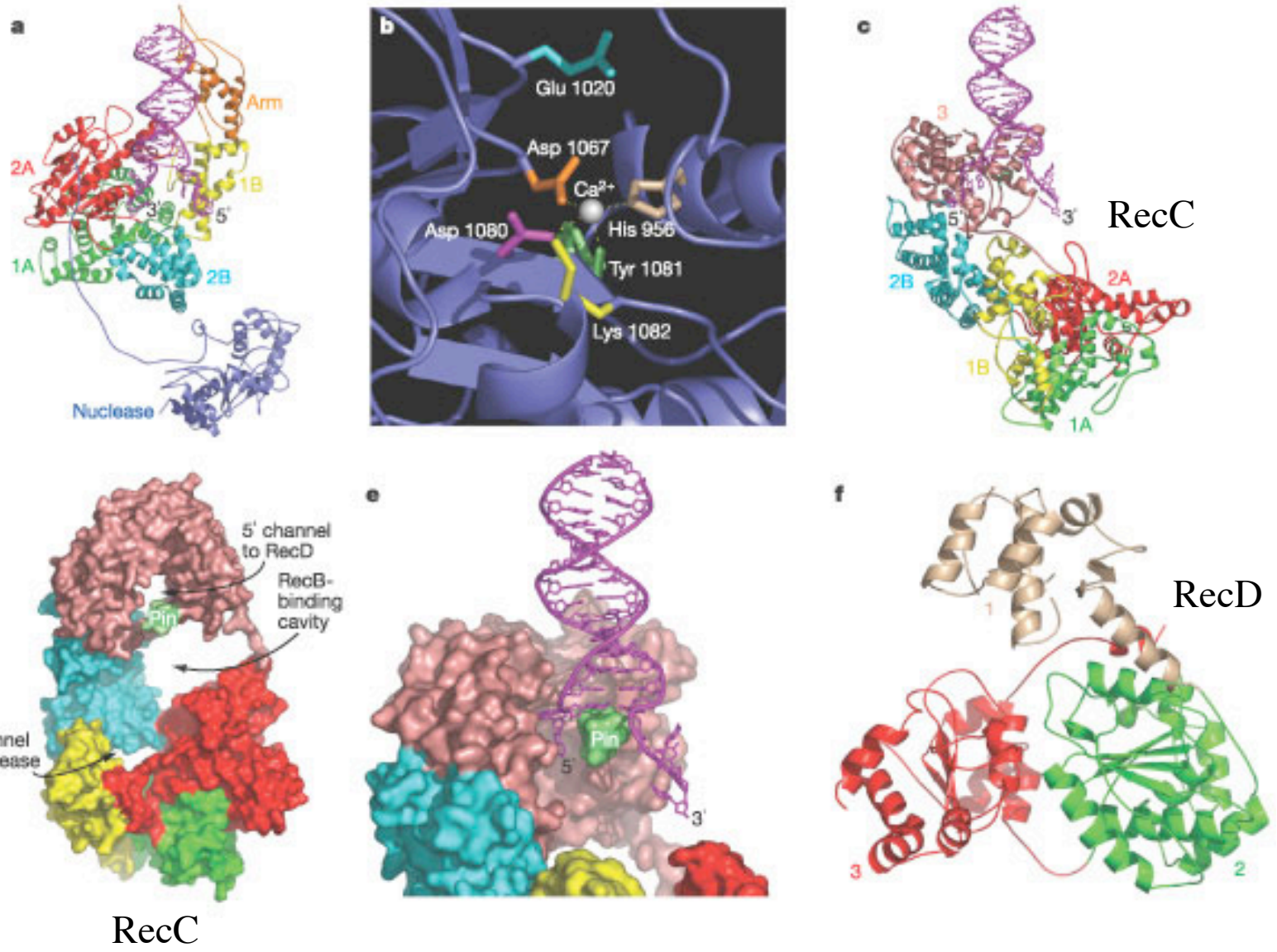
RecBCD dissociates

Nature. 2004 Nov 11;432(7014):187-93.
Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks.
Martin R. Singleton, Mark S. Dillingham, Martin Gaudier, Stephen C. Kowalczykowski and/\Dale B. Wigley

RecB

nuclease active site

RecBCD



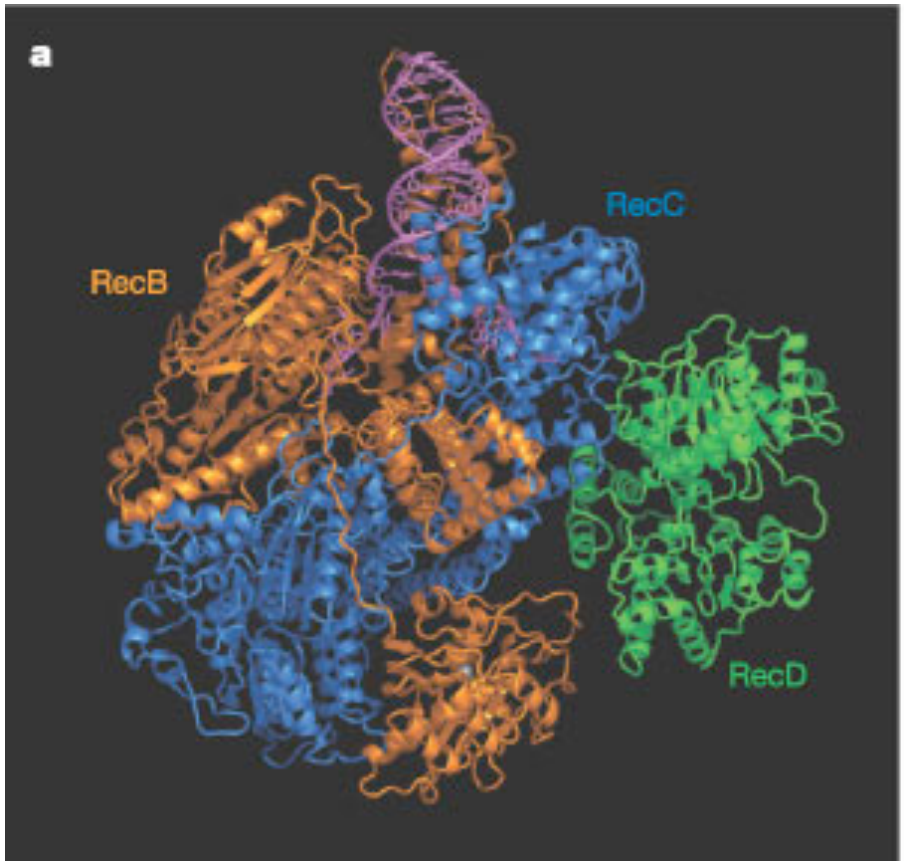
RecC

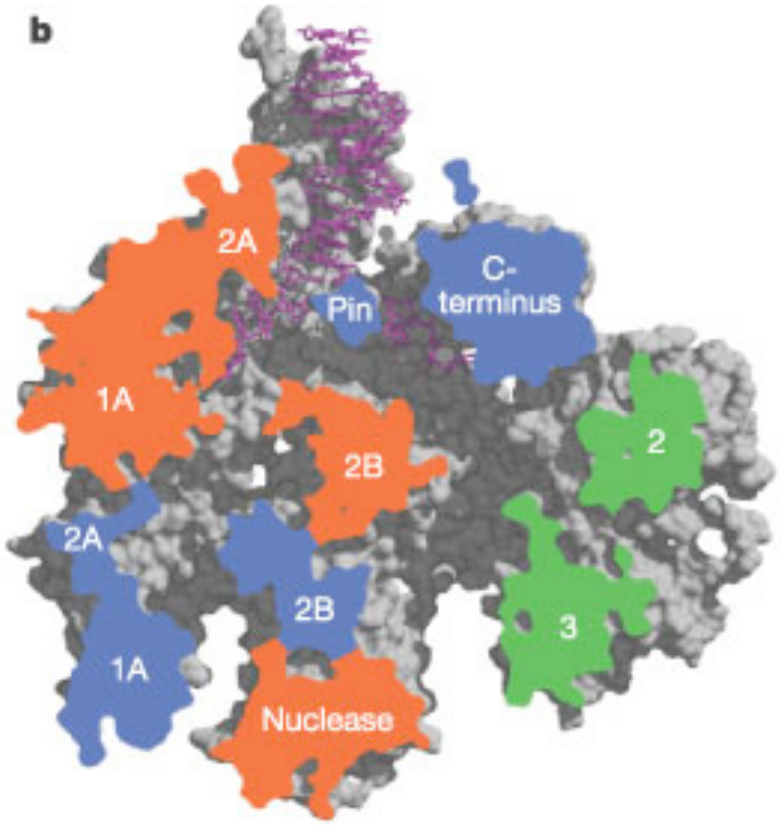
Nature. 2004 Nov 11;432(7014):187-93.

Crystal structure of RecBCD enzyme reveals a machine for processing DNA breaks.

Martin R. Singleton, Mark S. Dillingham, Martin Gaudier, Stephen C. Kowalczykowski and/\Dale B. Wigley

RecBCD Cutaway Details





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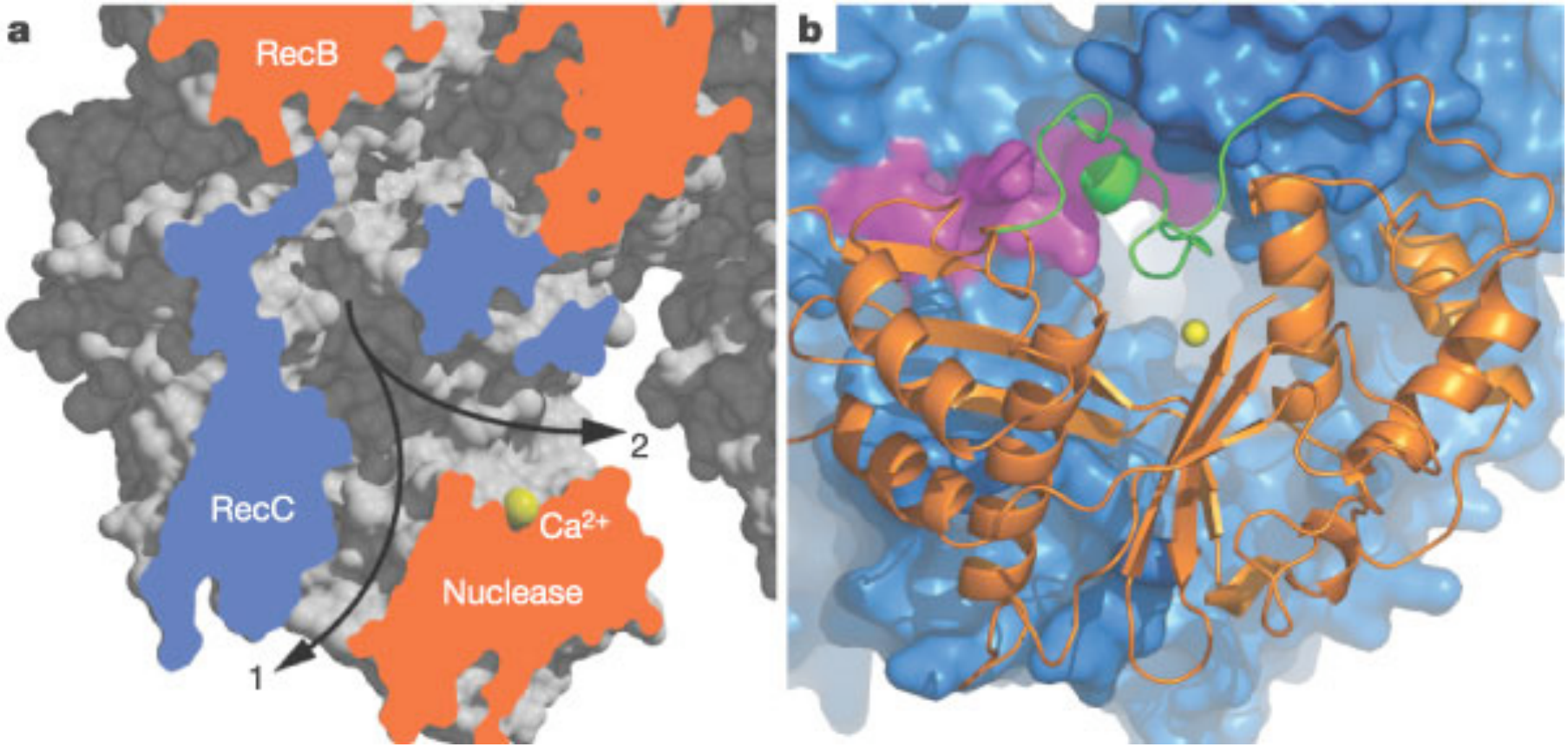

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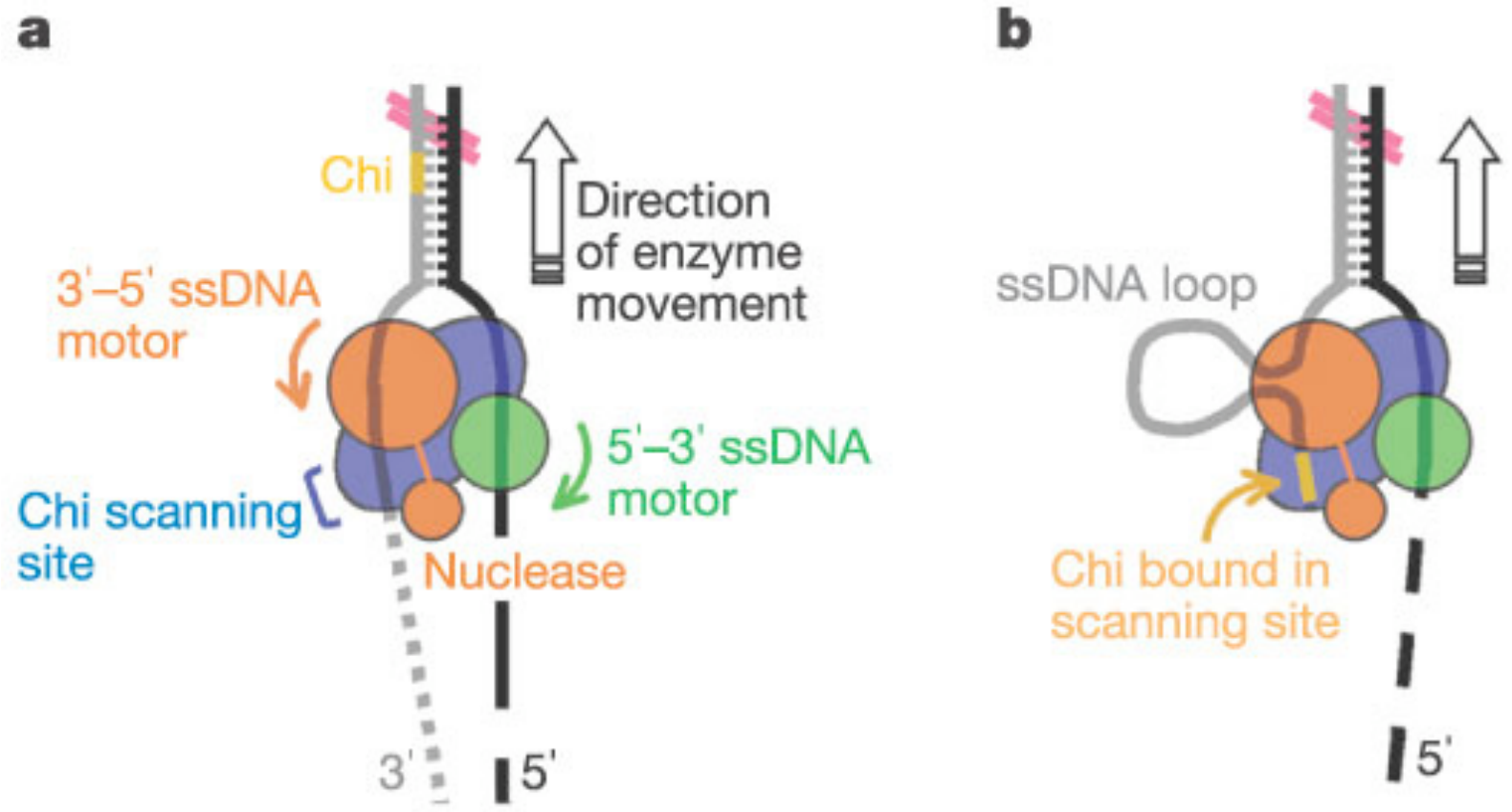
RecBCD -- Activity Modulated by X Recognition



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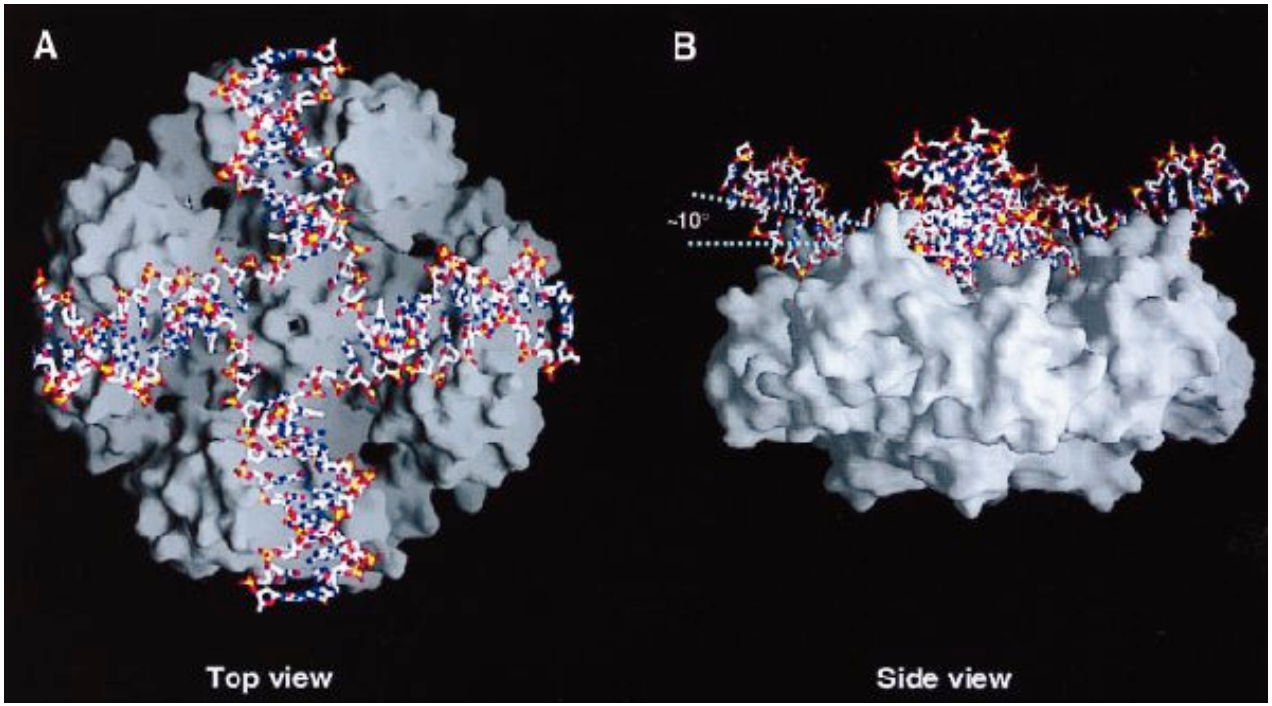
RuvABC

RuvABC branch-migrates and then resolves Holliday junctions

RuvA binds a Holliday junction and maintains a square-planar open orientation

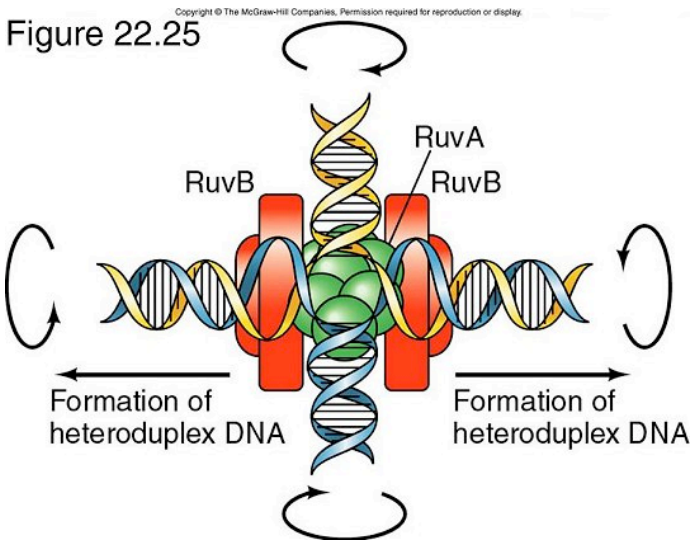


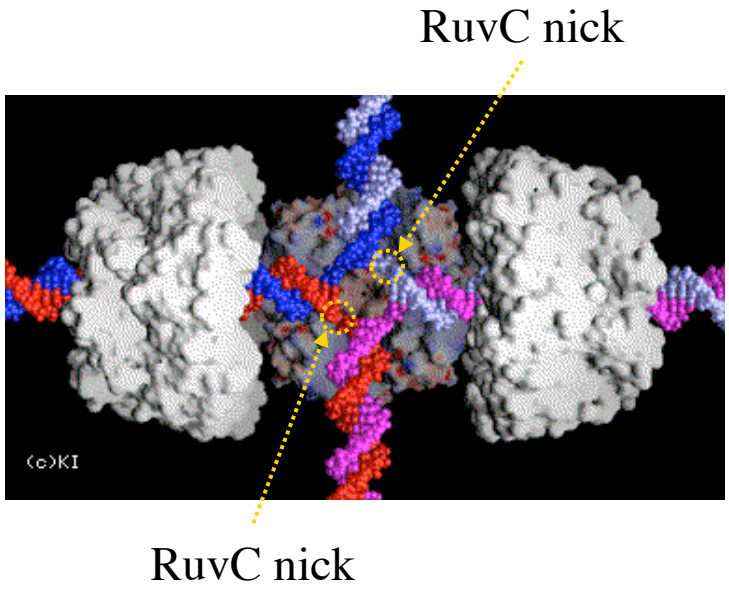
Mariko Ariyoshi, Tatsuya Nishino, Hiroshi Iwasaki, Hideo Shinagawa, and Kosuke Morikaw
Crystal structure of the Holliday junction DNA in complex with a single RuvA tetramer
PNAS 2000 97: 8257-8262

RuvABC

RuvB is a helicase motor that causes the Holliday junction to branch migrate

RuvC is a Holliday junction resolvase that nicks DNA on opposite sides of the square-planar ring





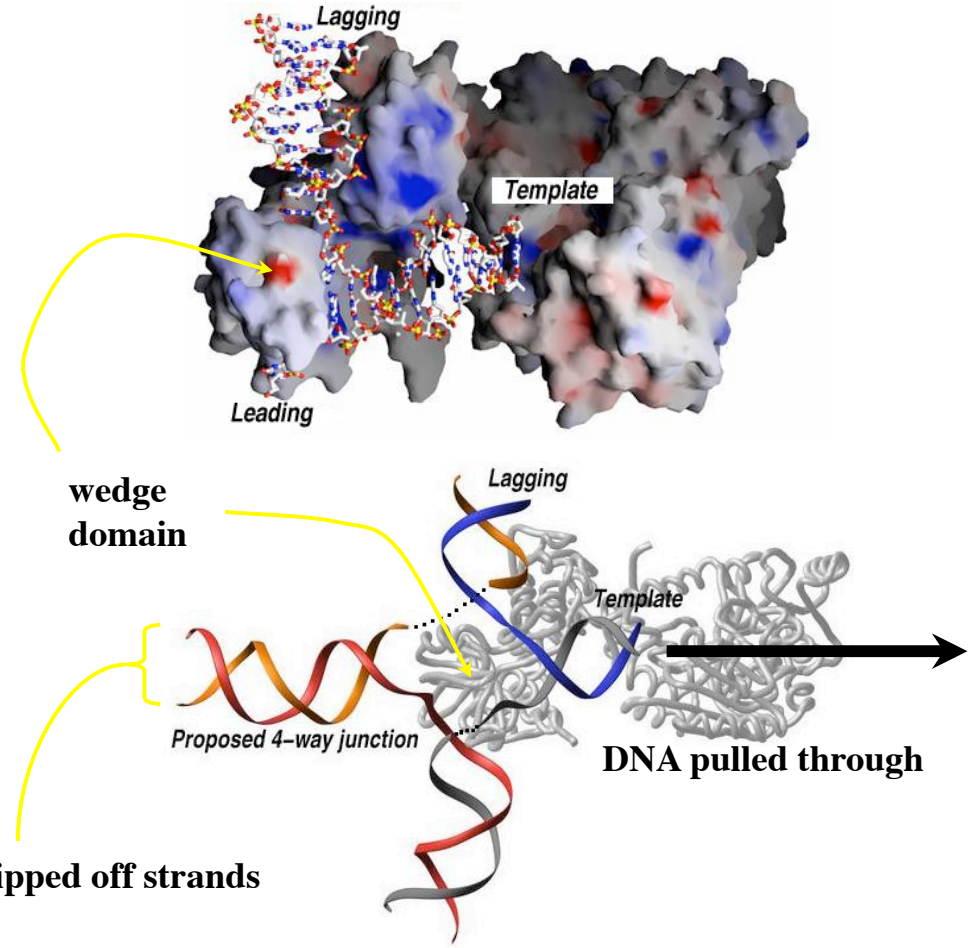
Structure of the Recombination Protein RuvA and a model for its Binding to Holliday Junction
J.B.Rafferty, S.E.Sedelnikova, D.Hargreaves, P.J.Artymiuk, P.J.Baker, G.J.Sharples, A.A.Mahdi, R.G.Lloyd and D.W.Rice
Science 274, (1996)

RecG

Binds replication forks with a missing **leading** strand Equivalent to a D-loop with an invaded 5'-PO_4 single strand.

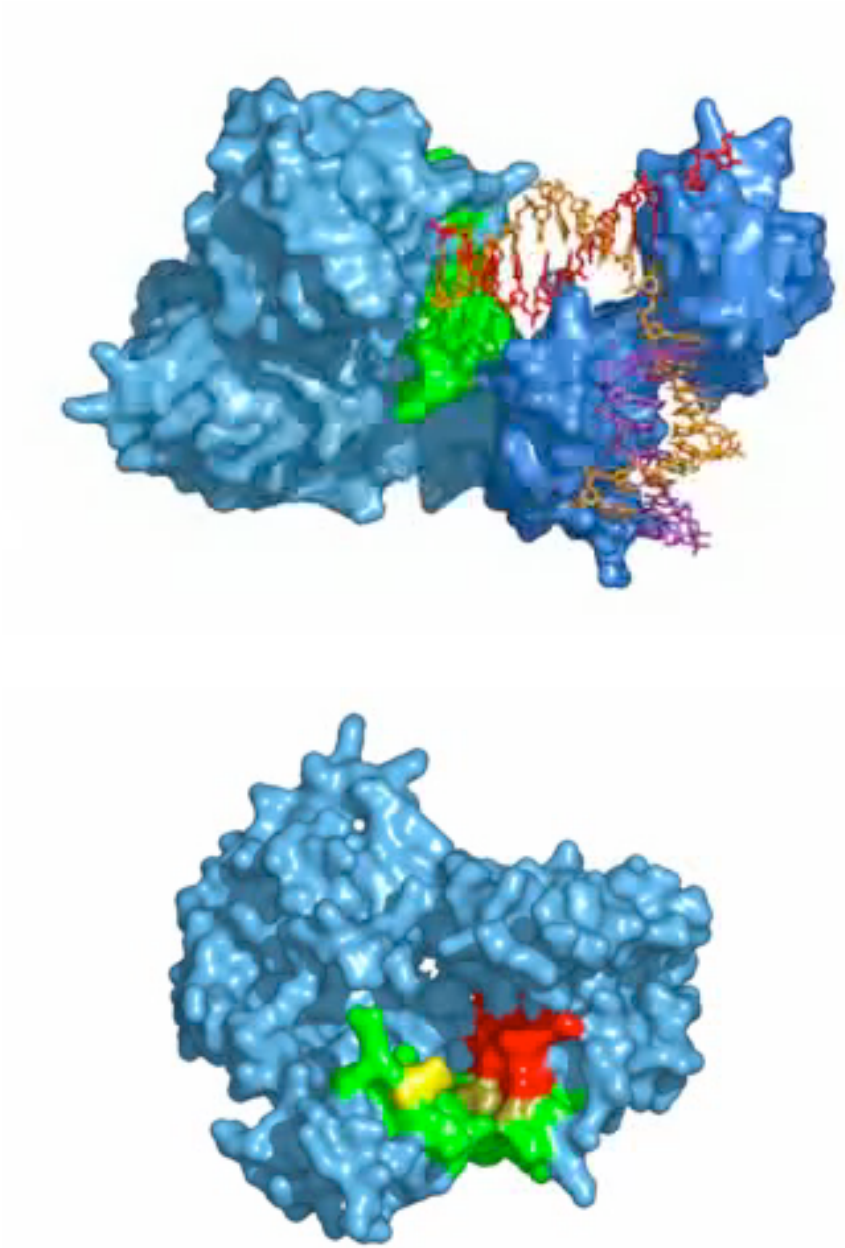
Translocates DNA through the protein using "wedge domain" to strip off any annealed strands

Stripped off strands can anneal to each other to form a Holliday junction



Cell, Vol 107, 79–89, 5 October 2001
Structural Analysis of DNA Replication Fork Reversal by RecG
Martin R. Singleton , Sarah Scaife, and Dale B. Wigley

RecG movies



http://www.nottingham.ac.uk/life-env/contact/academics/lloyd/research.phtml