RecBCD

Exonuclease V

330-kDa

RecBCD has very long history
but it's mechanism is unveiled only recently.

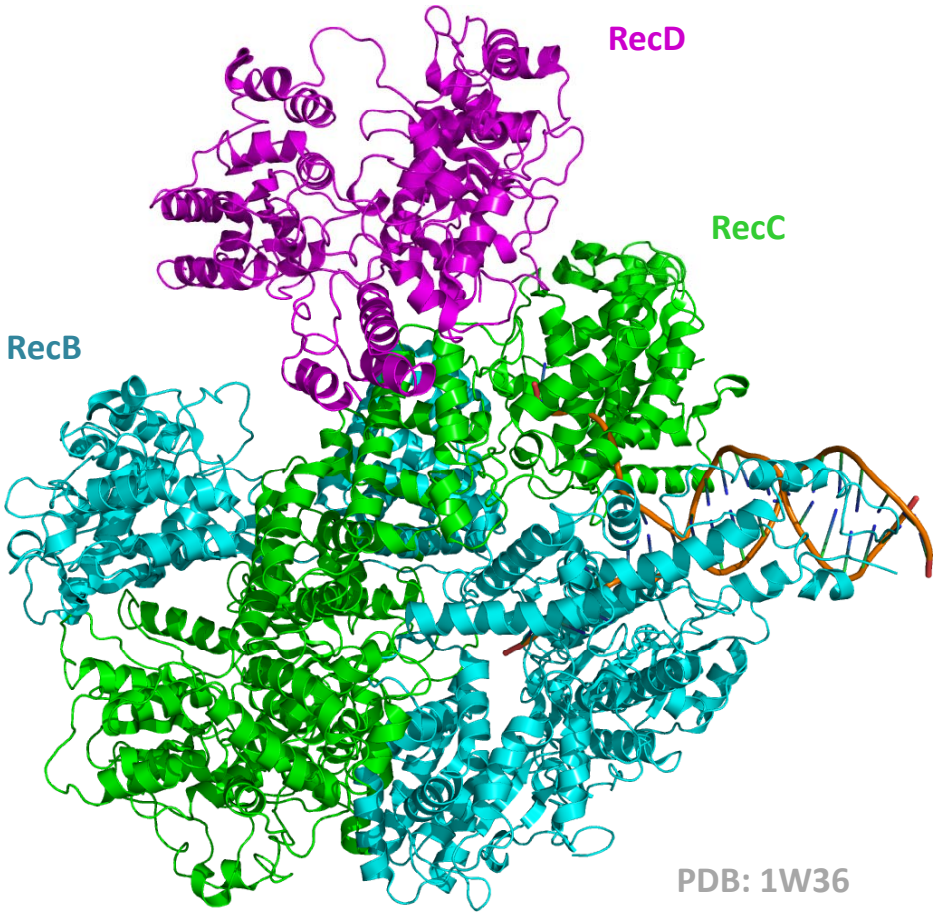
Activities..

1) DNA dependent ATPase
2) Helicase
3) Nuclease

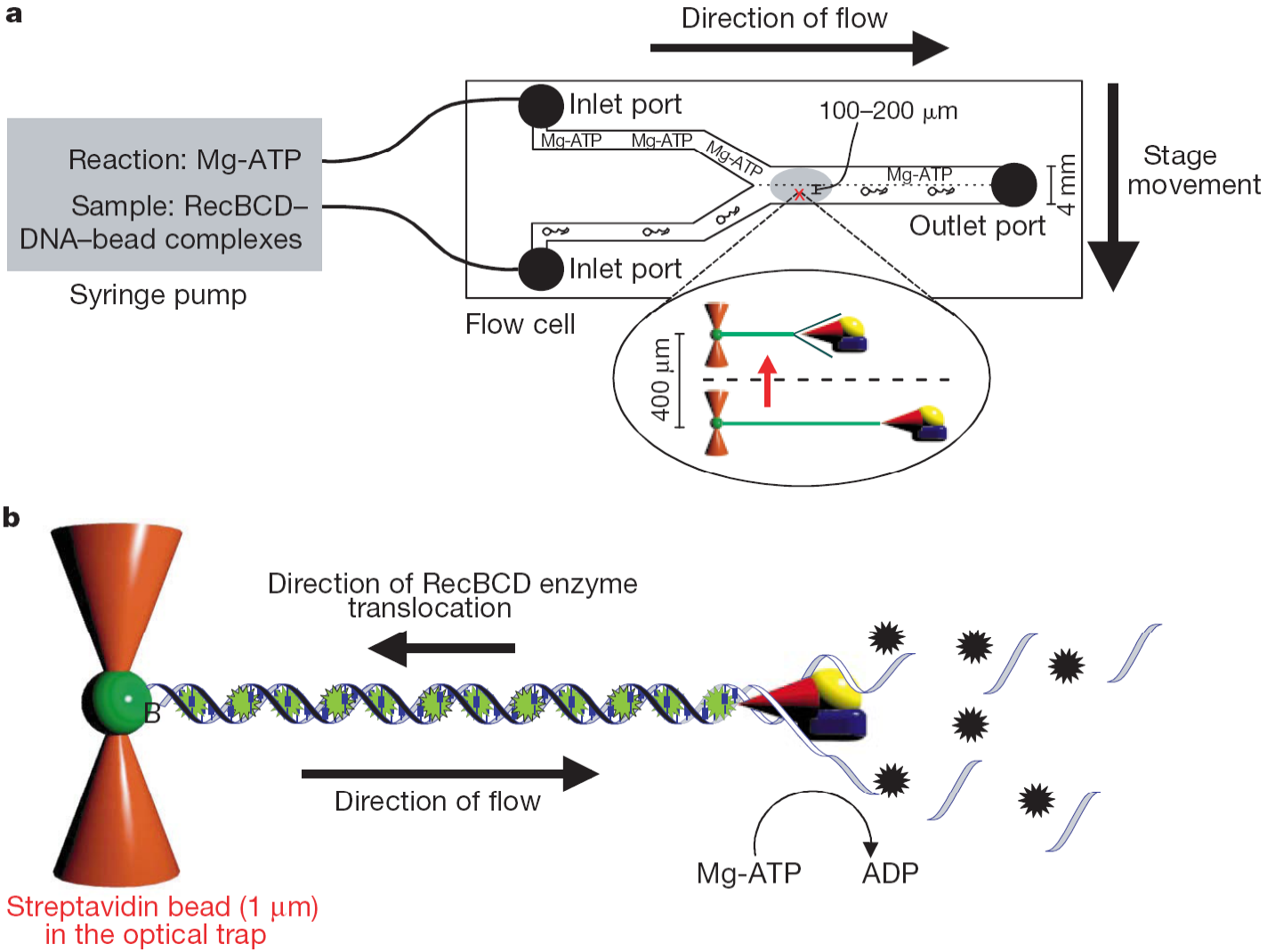
RecB 3'-5' helicase, nuclease

RecC recognizes χ (crossover hotspot instigator)

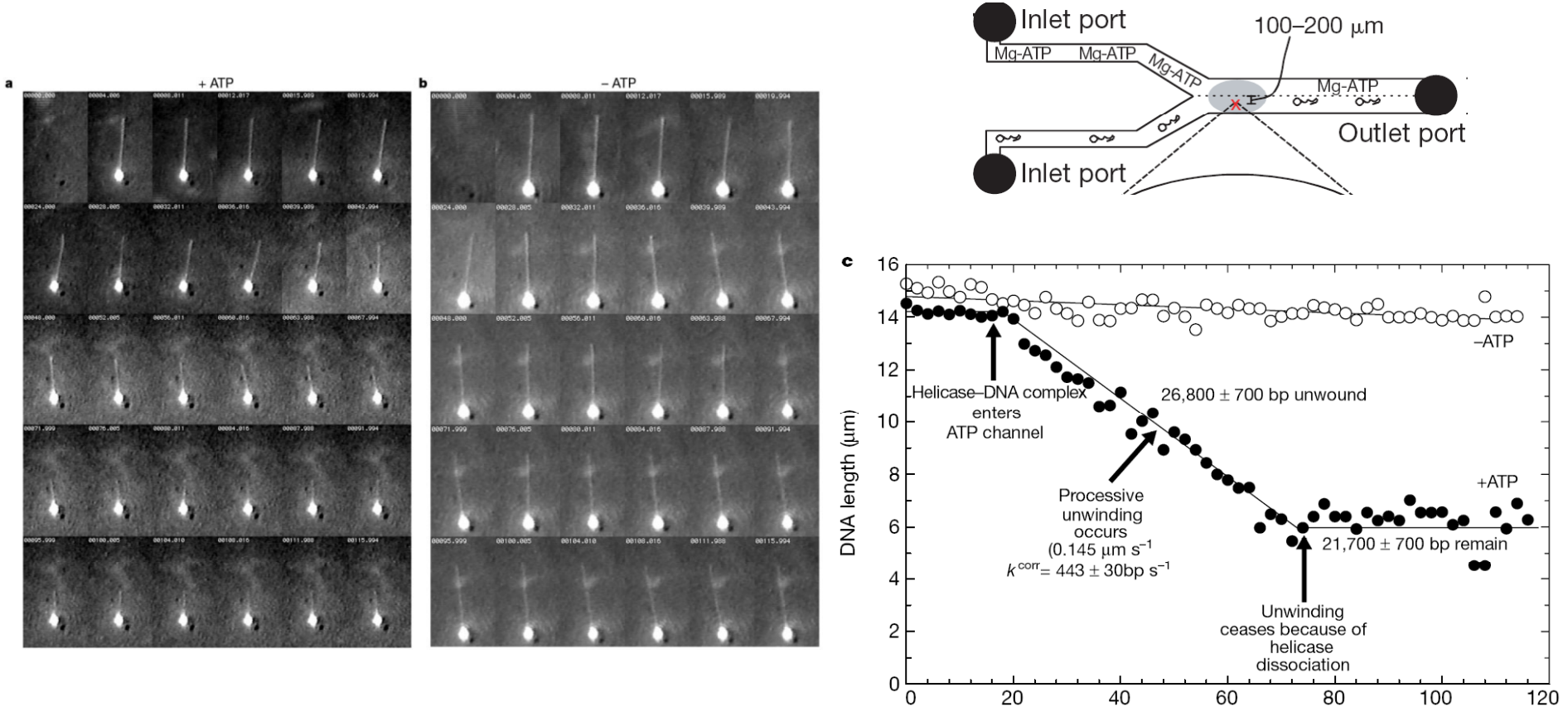
RecD 5'-3' helicase



Bianco et. al, Nature 2001



Bianco et. al, Nature 2001

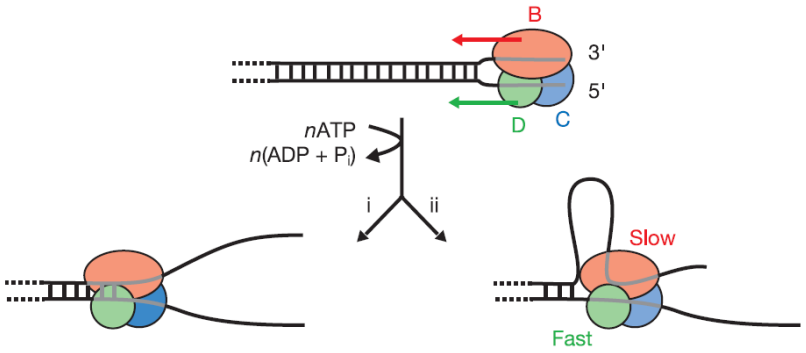


First real-time observation of an individual DNA helicase movement on dsDNA

Dillingham et. al, Nature 2003

RecBCD enzyme is a bipolar DNA helicase

Mark S. Dillingham*†, Maria Spies* & Stephen C. Kowalczykowski*



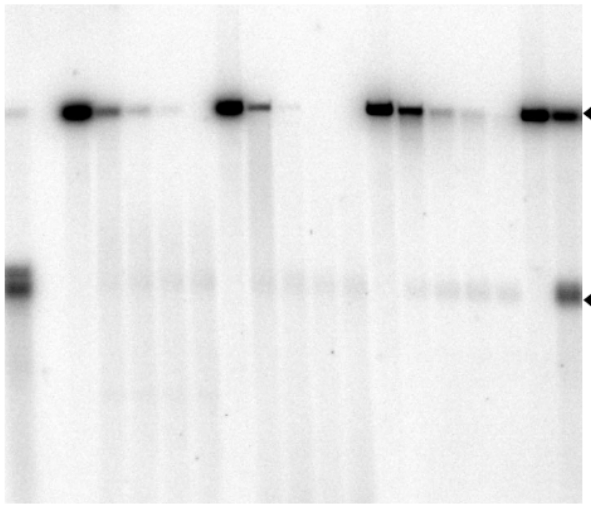
'ssDNA loop-tails' intermediate

RecB inactive

RecD inactive

with out RecD

Enzyme		WT RecBCD					$RecBCD^{K177Q}$					$RecB^{K29Q}CD$					RecBC	
Conc. (nM)		0.2					0.2					1					5	
Time (min)	A	0	1	3	5	10	0	1	3	5	10	0	1	3	5	10	0	10

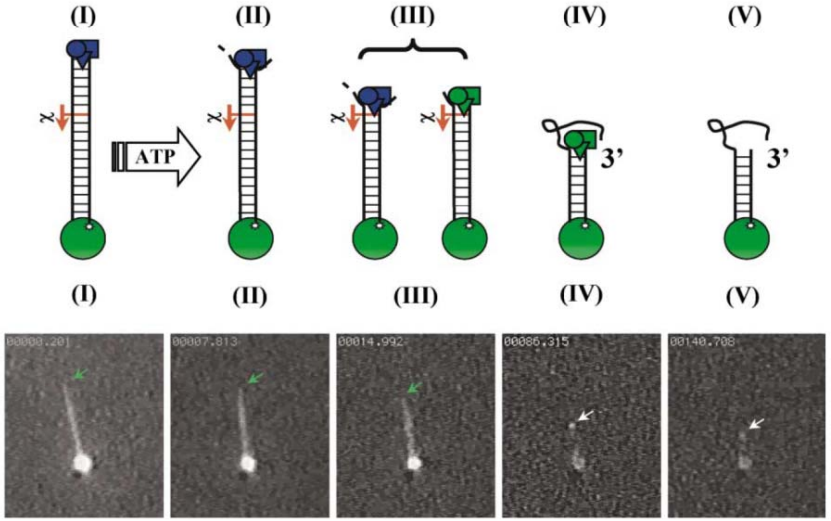


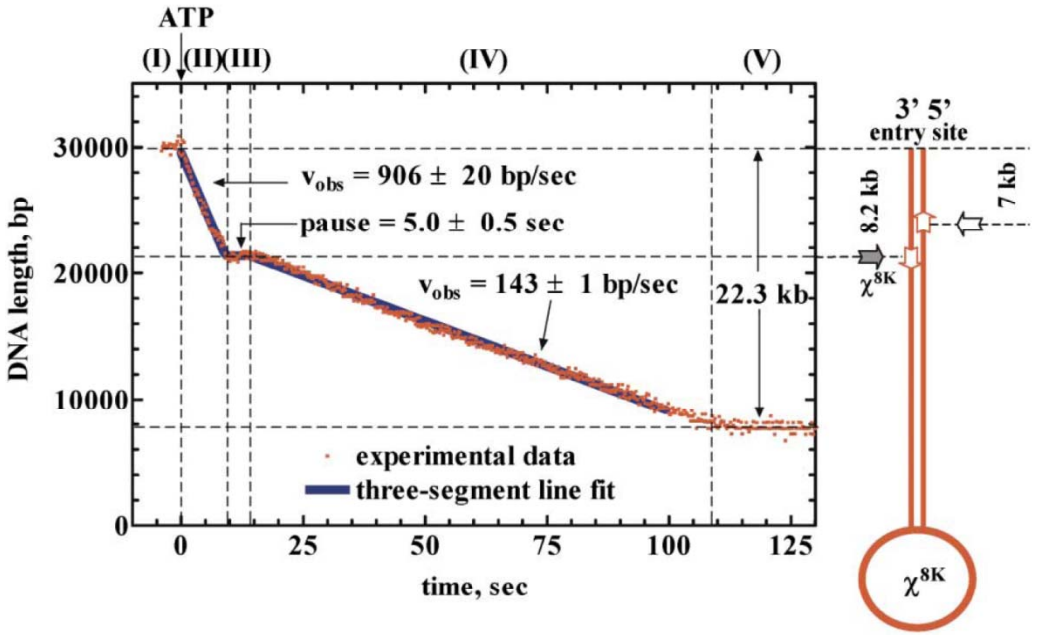
The two DNA helicases travel together with opposite polarities.

Spies et. al, Cell 2003

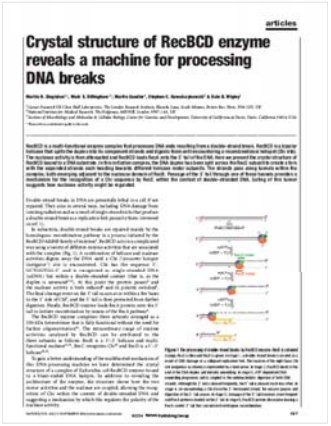
A Molecular Throttle: The Recombination Hotspot χ Controls DNA Translocation by the RecBCD Helicase

Maria Spies,[1,2] Piero R. Bianco,[1,2,3]
Mark S. Dillingham,[1,2,4] Naofumi Handa,[1,2]
Ronald J. Baskin,[2]
and Stephen C. Kowalczykowski[1,2,*]
[1]Section of Microbiology
[2]Section of Molecular and Cellular Biology
Center for Genetics and Development
University of California, Davis
Davis, California 95616



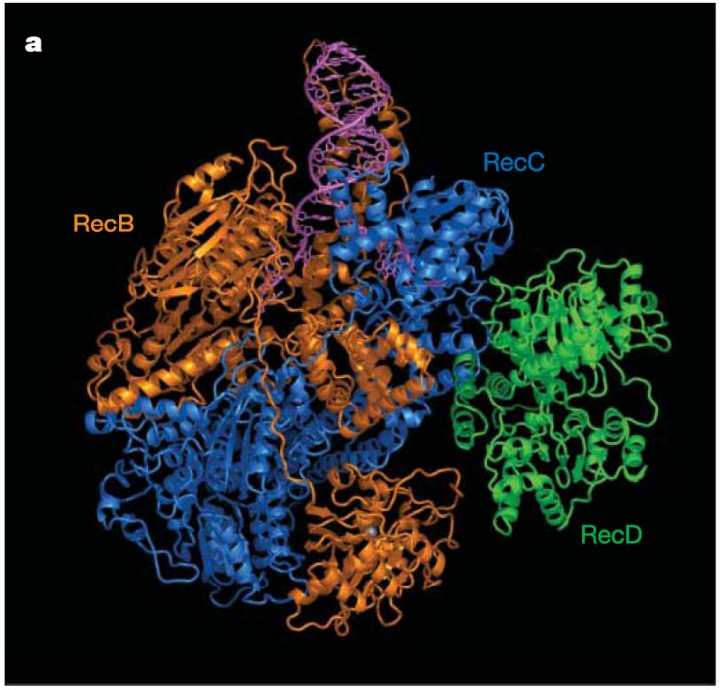


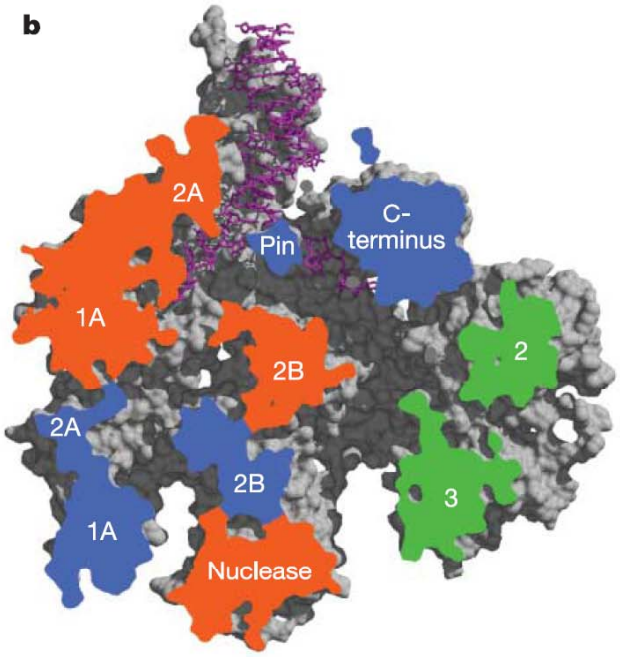
Finally, Xtal is out!!


articles

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

Singleton et. al, Nature 2004

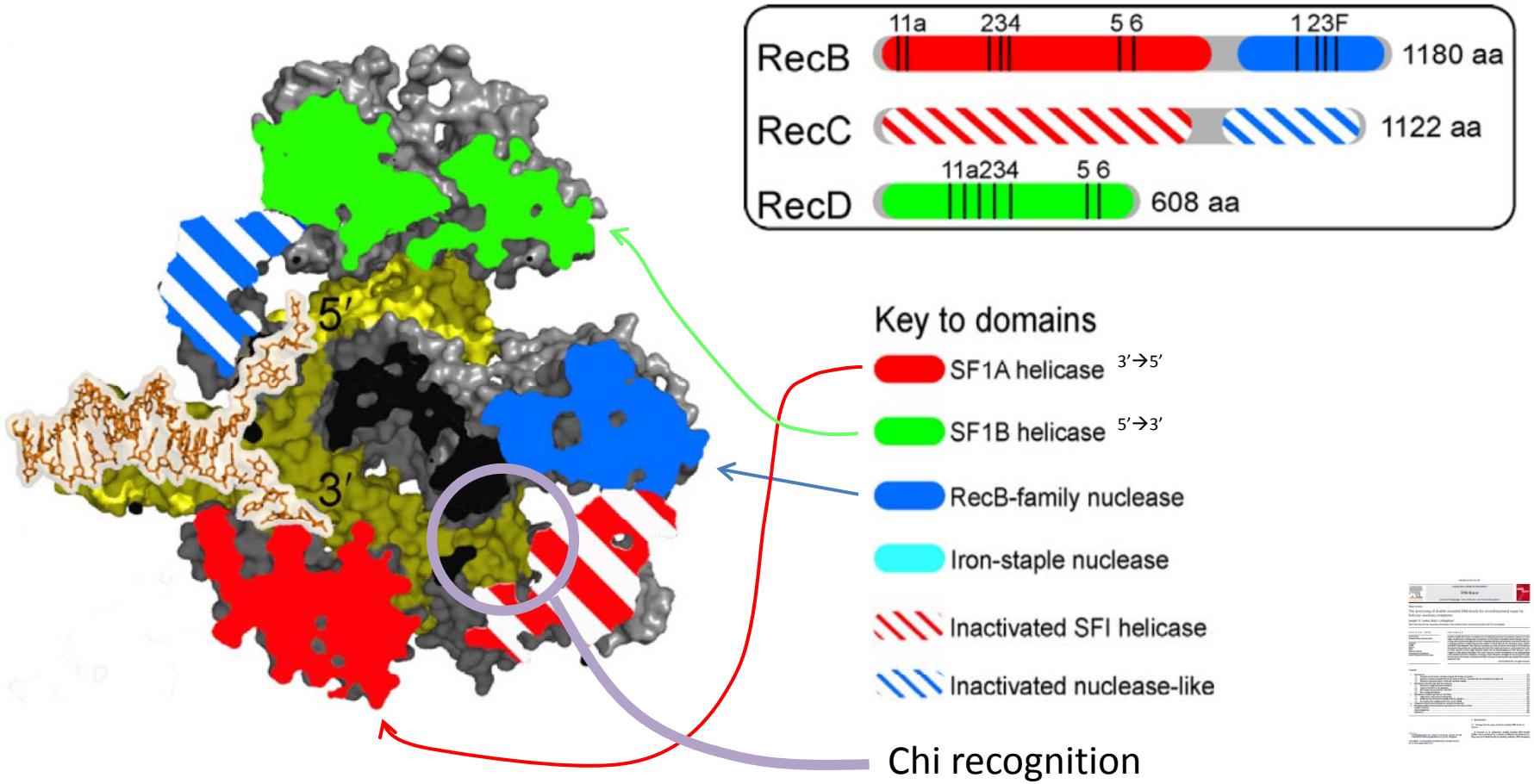




Two exit tunnels for each strands

RecB nuclease domain is tethered by long flexible linker

Geometry of the domains and function



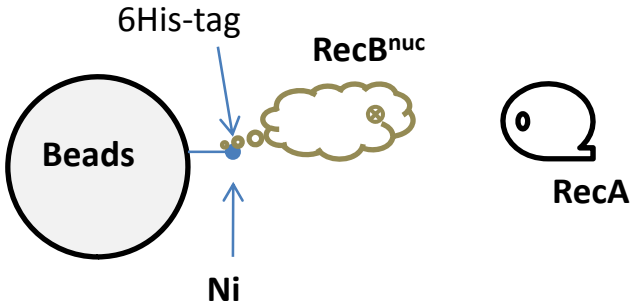
Joseph T.P. Yeeles, Mark S. Dillingham (2010)

Loading of RecA by RecB nuclease domain

The RecA Binding Locus of RecBCD Is a General Domain for Recruitment of DNA Strand Exchange Proteins

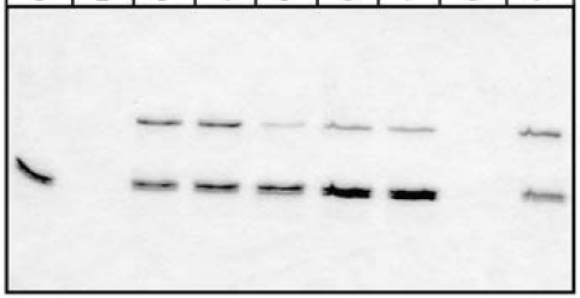
Short Article

Speis et. al, Mol. Cell 2006



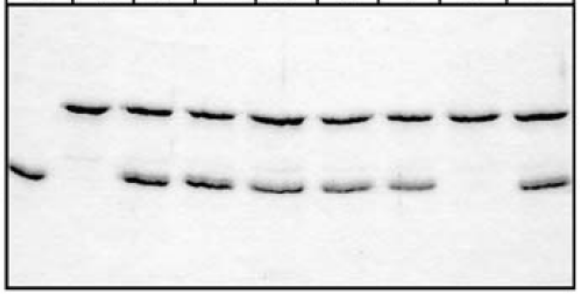
RecB^{nuc}	+	-	+	+	+	+	+	-	+
*Eco*RecA	-	+	+	+	+	+	+	+	+
magnesium	-	-	-	+	+	+	+	+	+
ATP	-	-	-	-	+	-	-	-	-
ATP-γ-S	-	-	-	-	-	+	+	+	-
ssDNA	-	-	-	-	-	-	+	+	+
	1	2	3	4	5	6	7	8	9

A: bound proteins



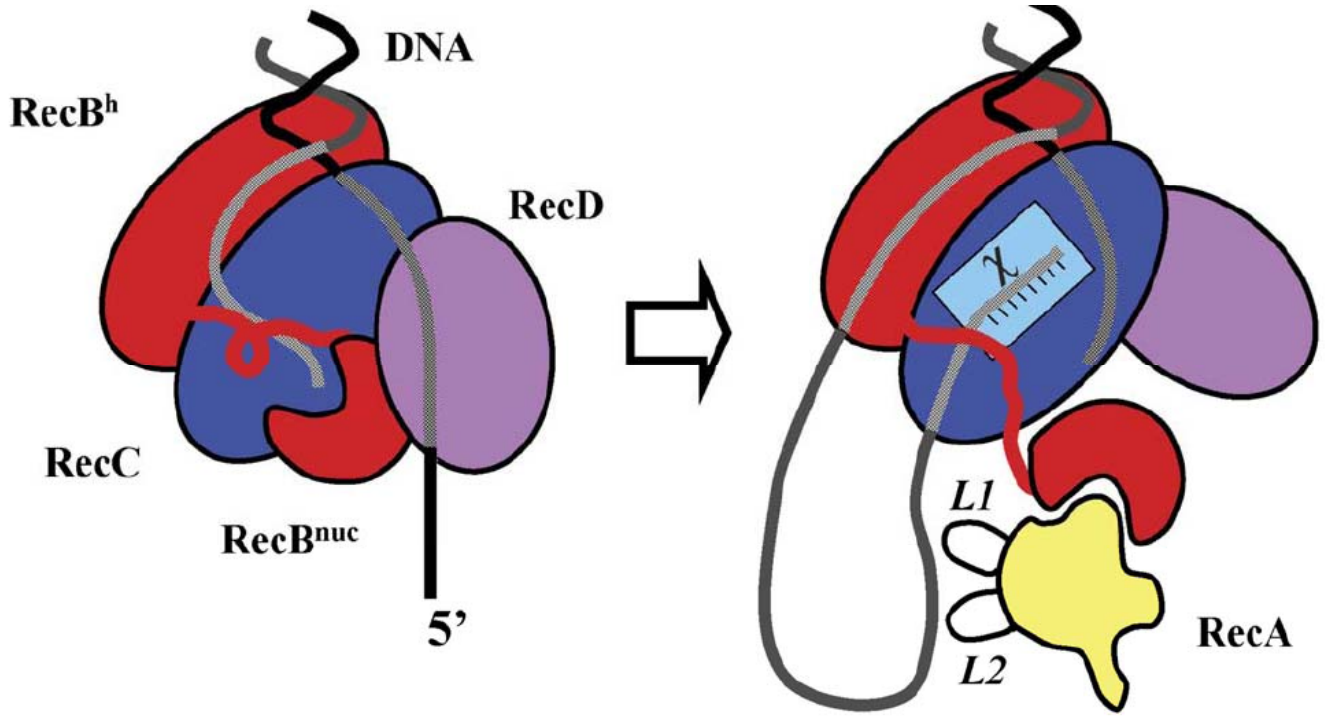
	1	2	3	4	5	6	7	8	9

B: free proteins



Loading of RecA by RecB nuclease domain

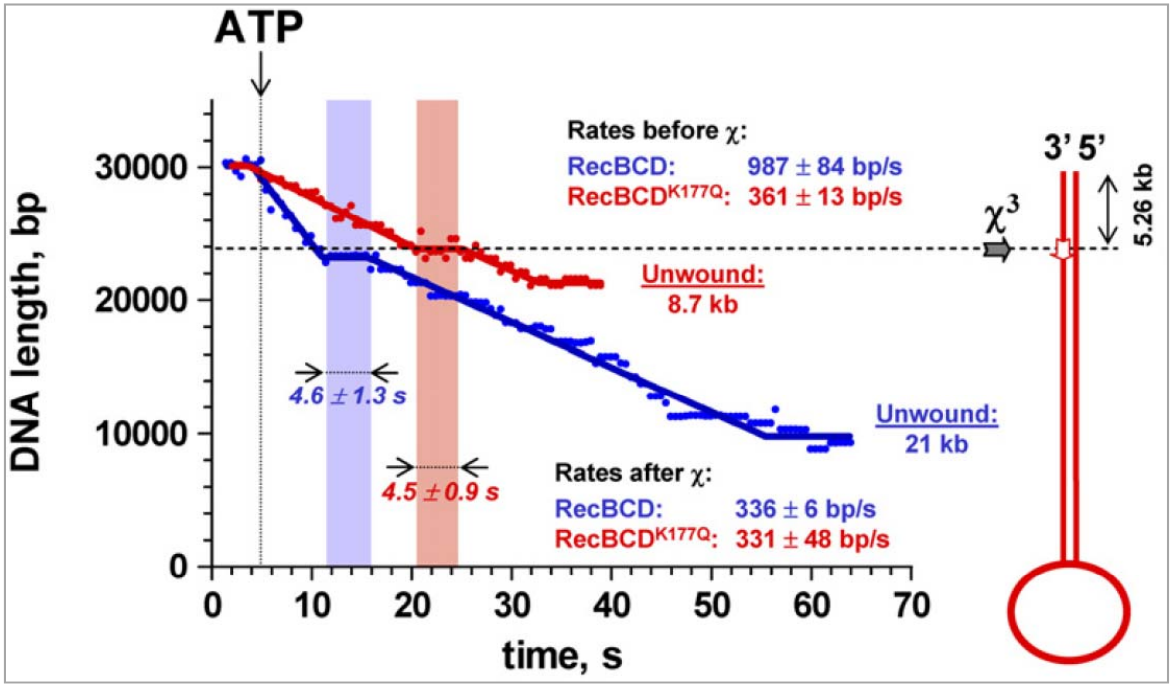
Speis et. al, Mol. Cell 2006



RecBCD Enzyme Switches Lead Motor Subunits in Response to χ Recognition

Speis et. al, Mol. Cell 2007

• RecD drives the holoenzyme before Chi

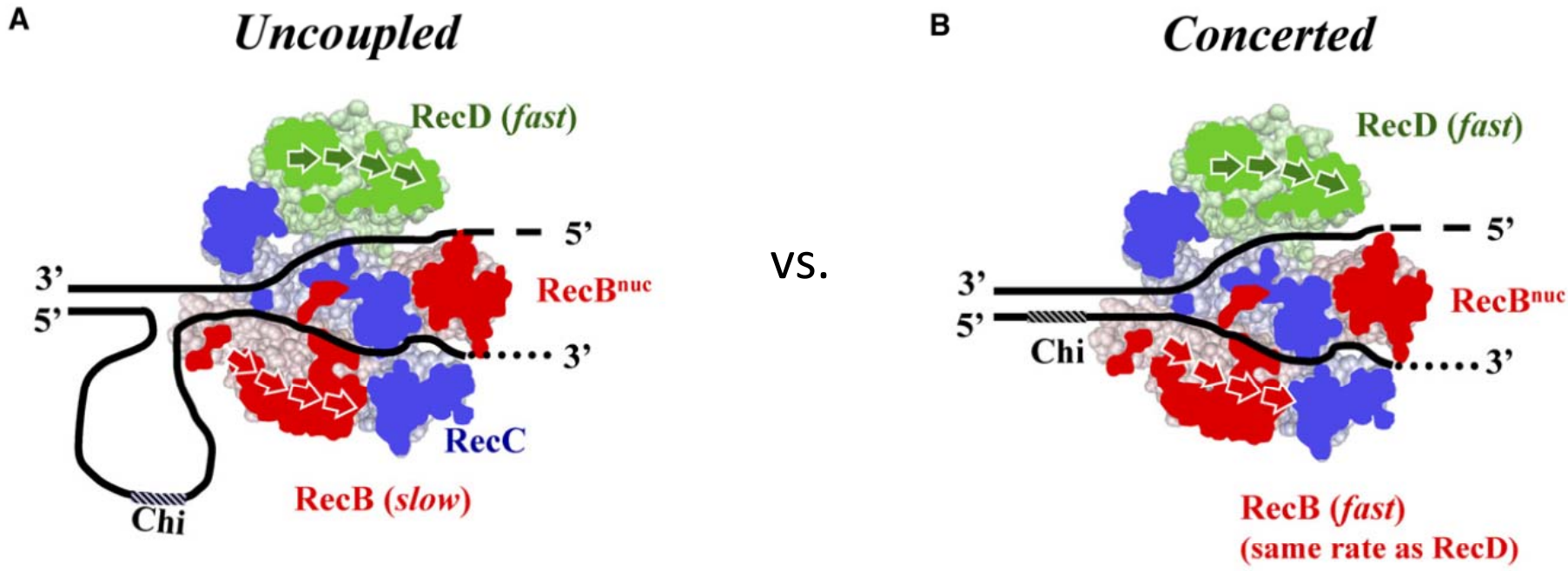


After Chi recognition, RecBCDk177Q shows no speed change.

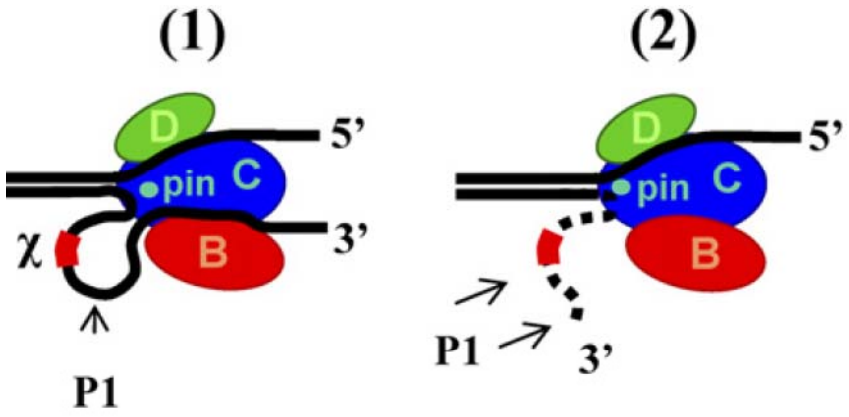
➔ RecB motor drives the Chi-modified enzyme

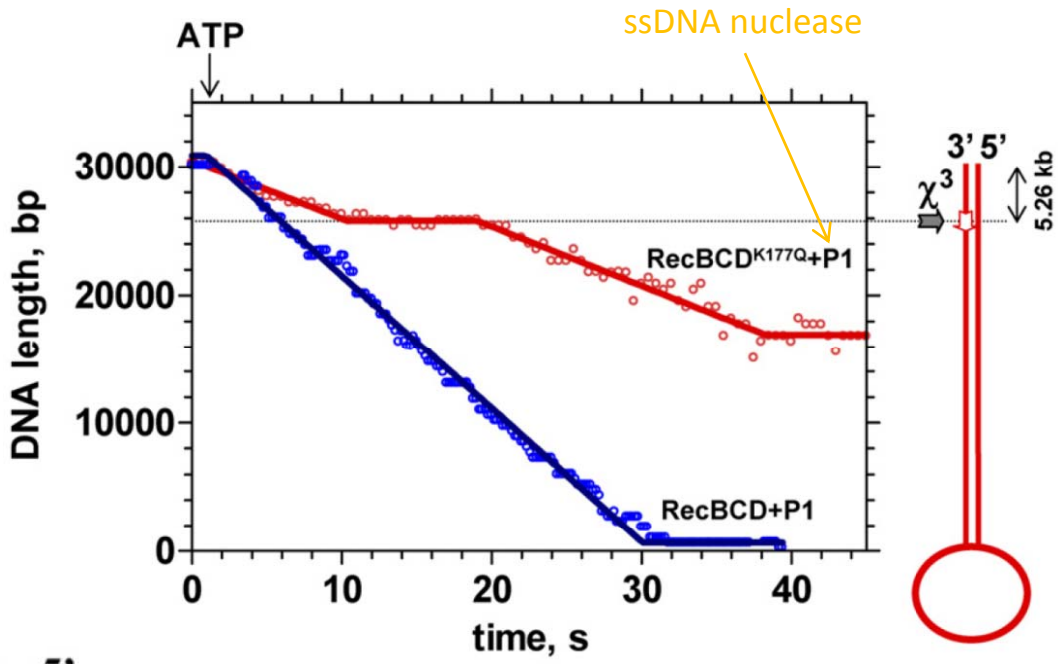
Two models
in translocation of RecBCD

Speis et. al, Mol. Cell 2007



In the uncoupled motors model, ss-loop would be generated.

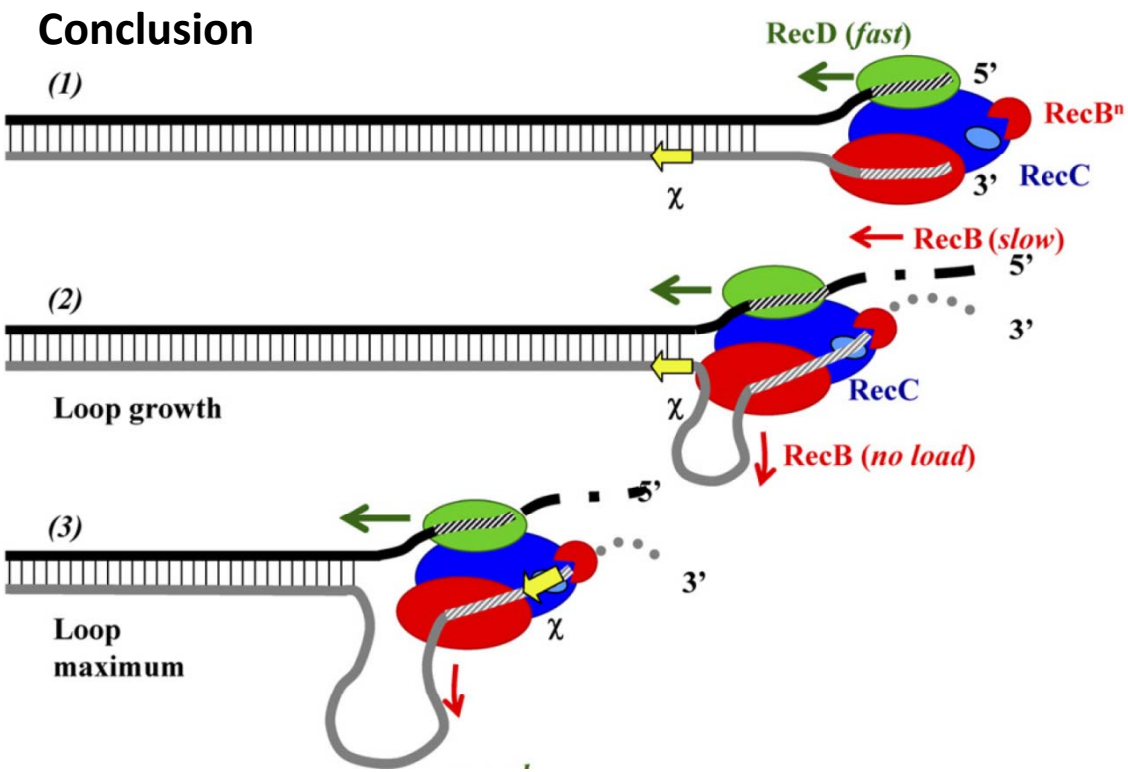


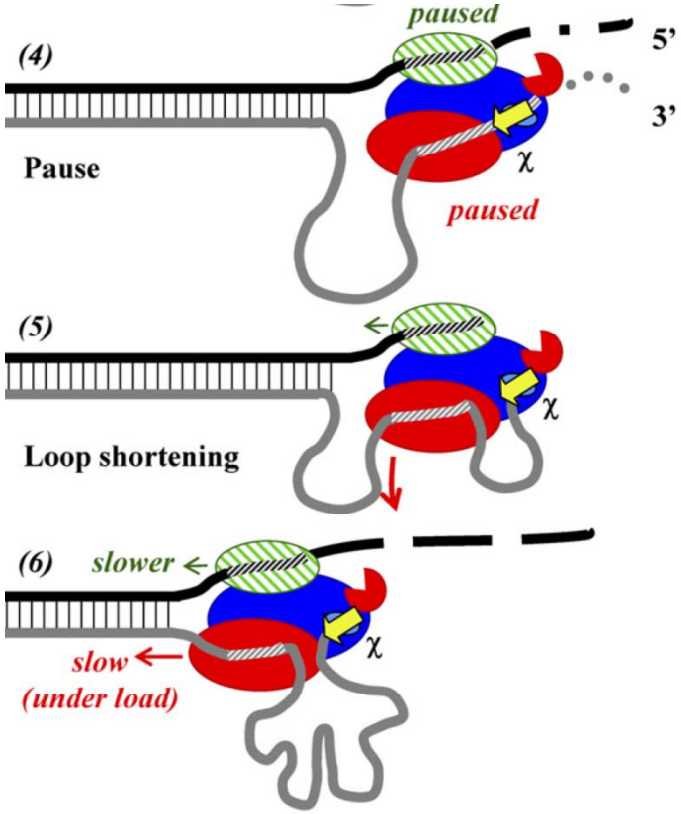


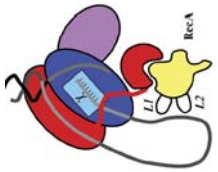
RecD is a leading fast motor prior to Chi.
➔ Supporting "uncorrelated model"

Speis et. al, Mol. Cell 2007

Conclusion







RecA loading

Speis et. al, Mol. Cell 2007