Biochemistry of Recombinational DNA Repair: Common Themes

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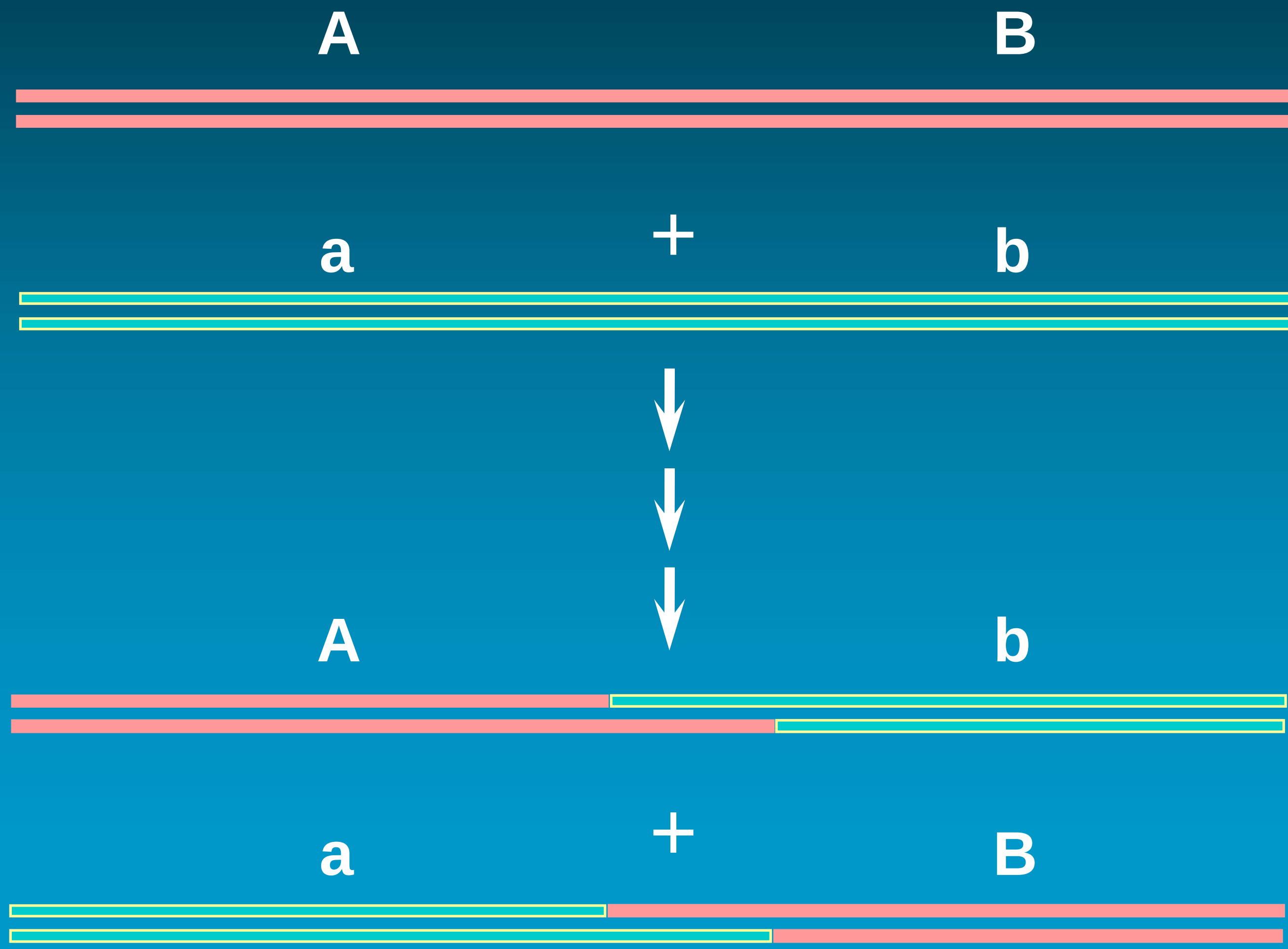
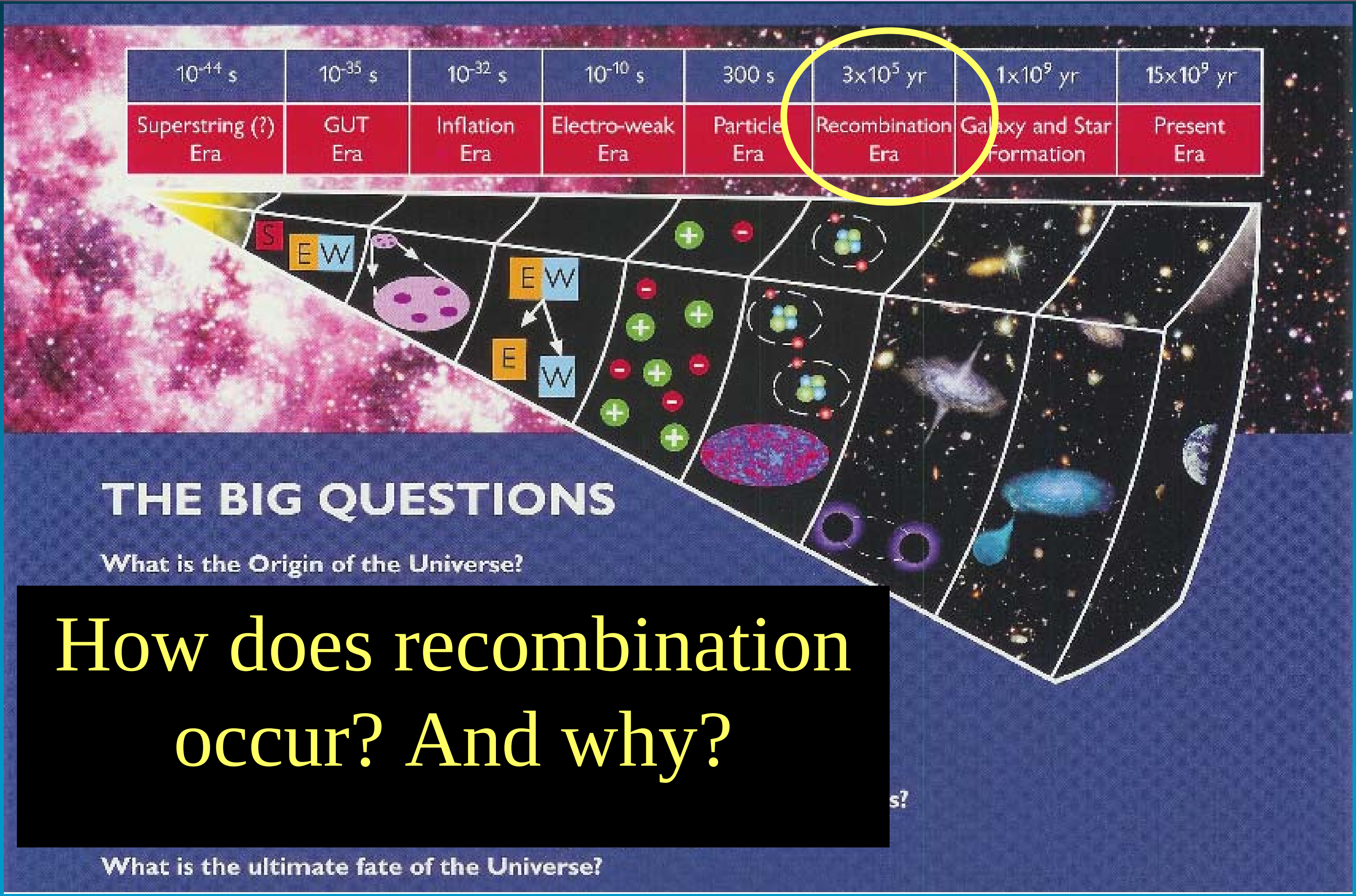
- Overview of genetic recombination and its function.
- Biochemical mechanism of recombination in Eukaryotes.
- Universal features: steps common to all organisms.

Homologous Recombination

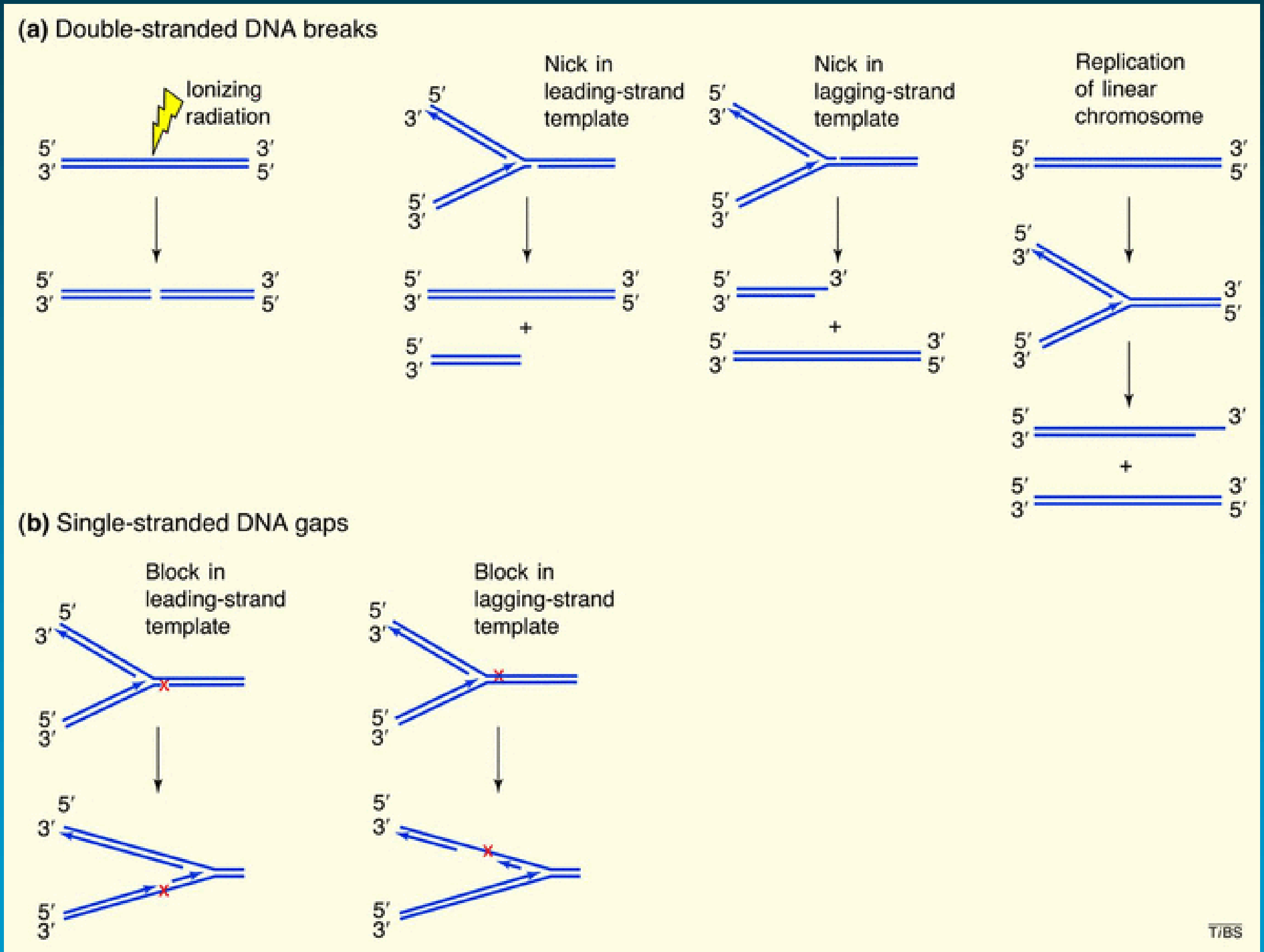


Genesis: Science and the Beginning of Time



How does recombination occur? And why?

DNA Replication Can Produce dsDNA Breaks and ssDNA Gaps



Repair of DNA Breaks

Non-Homologous
End-Joining (NHEJ)
(error-prone)

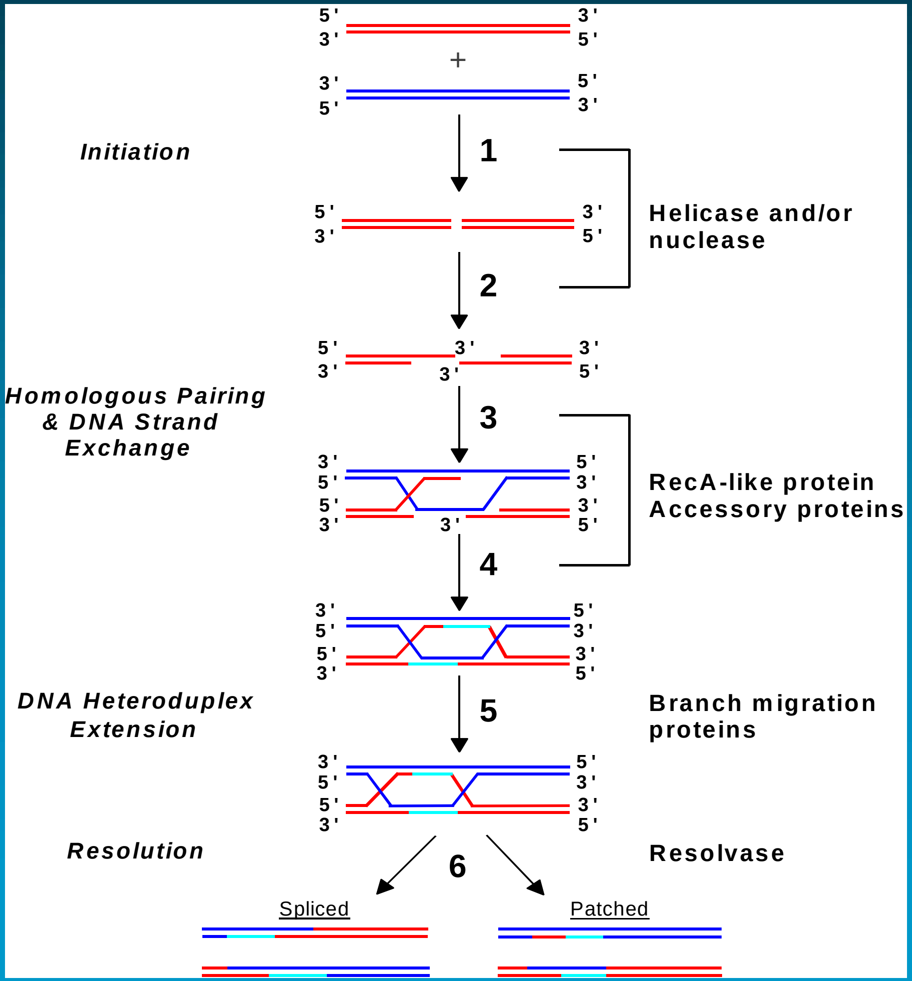
Homologous
Recombination (HR)
(error-free)

dsDNA Break-Repair
(DSBR)

Synthesis-Dependent
Strand-Annealing
(SDSA)

ssDNA Annealing
(SSA)

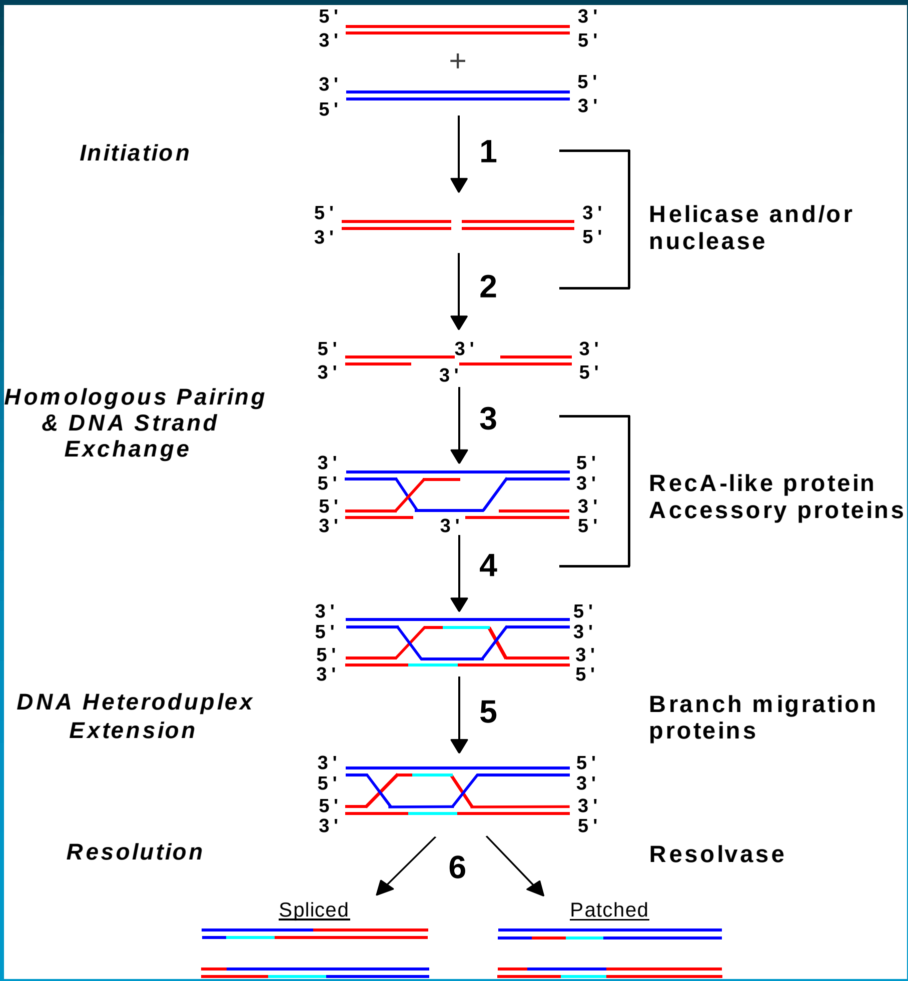
Double-Strand DNA Break Repair



Proteins Involved in Recombinational DNA Repair

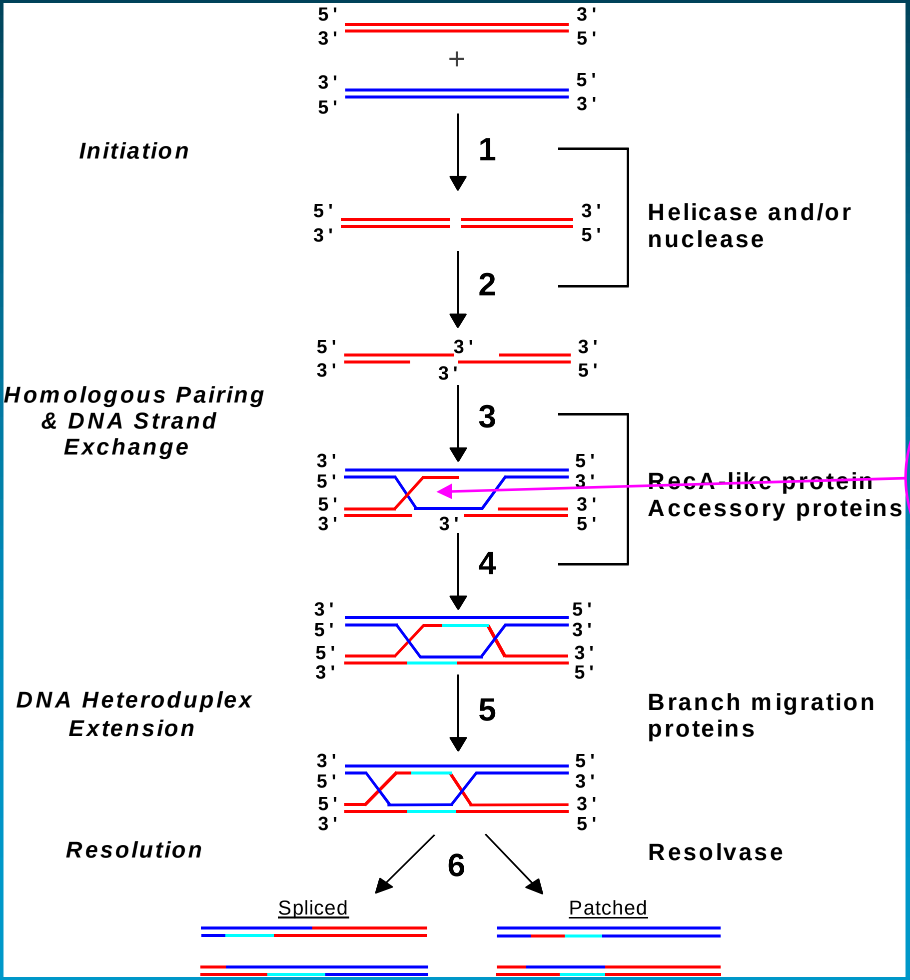
	E. coli	*Archaea*	*S. cerevisiae*	*Human*
Initiation	RecBCD	--	--	--
	SbcCD	Mre11/Rad50	Mre11/Rad50/Xrs2	Mre11/Rad50/Nbs1
	RecQ	Sgs1(?)	Sgs1(?)	RecQ1/4/5 LM/WRN(?)
	RecJ	--	ExoI	ExoI
	UvrD	--	Srs2	--
Homologous Pairing & DNA Strand Exchange	RecA	RadA	Rad51	Rad51
	SSB	SSB/RPA	RPA	RPA
	RecF(R)	RadB/B2/B3(?)	Rad55/57	Rad51B/C/D/Xrcc2/3
	RecO	--	Rad52	Rad52
	--	--	Rad59	--
	--	Rad54	Rad54/Rdh54	Rad54/54B
				Brca2
DNA Heteroduplex Extension	RuvAB	Rad54	Rad54	Rad54
	RecG		--	--
	RecQ		Sgs1(?)	RecQL/4/5 LM/WRN(?)
Resolution	RuvC	Hjc/Hje	--	--
	--	--	Mus81/Mms4	Mus81/Mms4

Proteins Involved in Recombinational DNA Repair



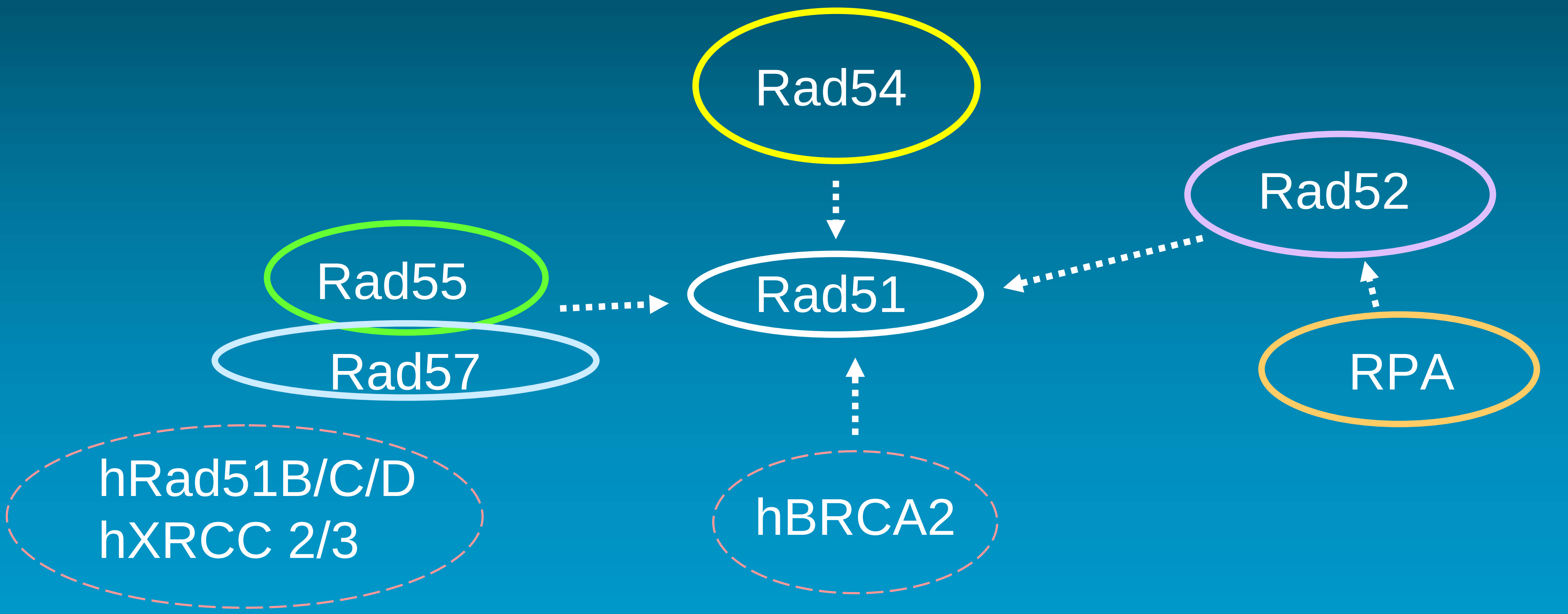
E. coli	*S. cerevisiae*	Human
RecBCD	--	--
SbcCD	Mre11/Rad50/Xrs2	Mre11/Rad50/Nbs1
RecQ	Sgs1(?)	RecQ1/4/5/BLM/WRN(?)
RecJ	ExoI	ExoI
UvrD	Srs2	--
RecA	Rad51	Rad51
SSB	RPA	RPA
RecF(R)	Rad55/57	Rad51B/C/D/Xrcc2/3
RecO	Rad52	Rad52
--	Rad59	--
--	Rad54/Rdh54	Rad54/54B
--	--	Brca2
RuvAB	Rad54	Rad54
RecG	--	--
RecQ	Sgs1(?)	RecQL/4/5/BLM/WRN(?)
RuvC	--	--
--	Mus81/Mms4(?)	Mus81/Mms4(?)

Proteins Involved in Recombinational DNA Repair

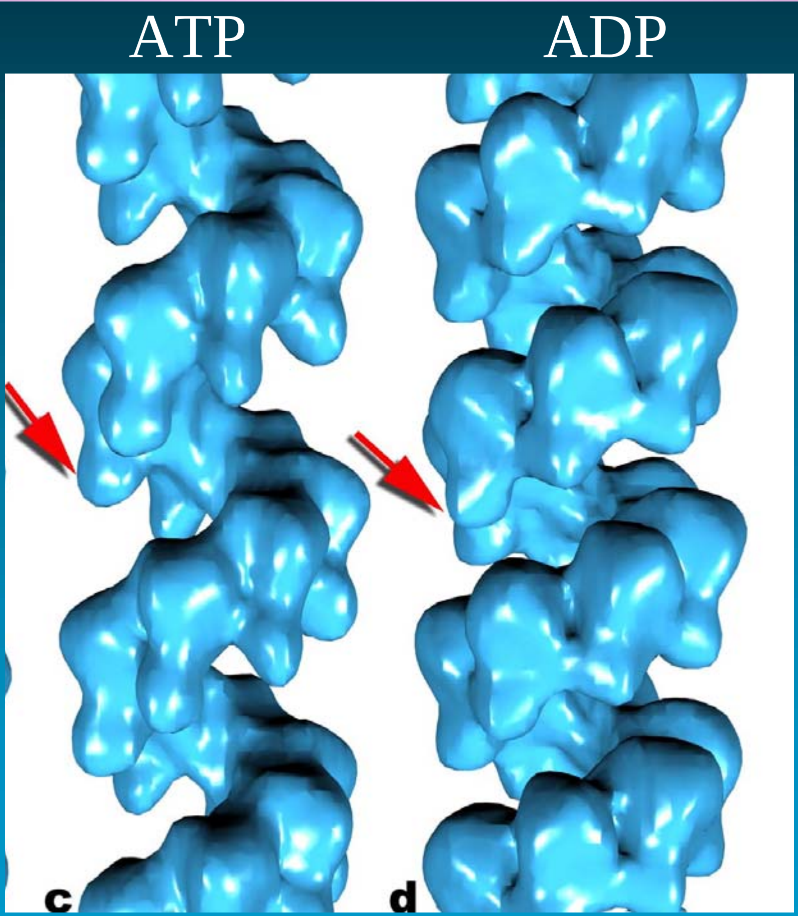


E. coli	*S. cerevisiae*	Human
RecBCD SbcCD RecQ RecJ UvrD	-- Mre11/Rad50/Xrs2 Sgs1(?) ExoI Srs2	-- Mre11/Rad50/Nbs1 RecQ1/4/5/BLM/WRN(?) ExoI --
RecA SSB RecF(R) RecO -- -- --	Rad51 RPA Rad55/57 Rad52 Rad59 Rad54/Rdh54 --	Rad51 RPA Rad51B/C/D/Xrcc2/3 Rad52 -- Rad54/54B Brca2
RuvAB RecG RecQ	Rad54 -- Sgs1(?)	Rad54 -- RecQL/4/5/BLM/WRN(?)
RuvC --	-- Mus81/Mms4(?)	-- Mus81/Mms4(?)

Rad52, Rad54, Rad55, and Rad57 Proteins Interact with Rad51 Protein and Stimulate DNA Strand Exchange



The Rad51 Nucleoprotein Filament

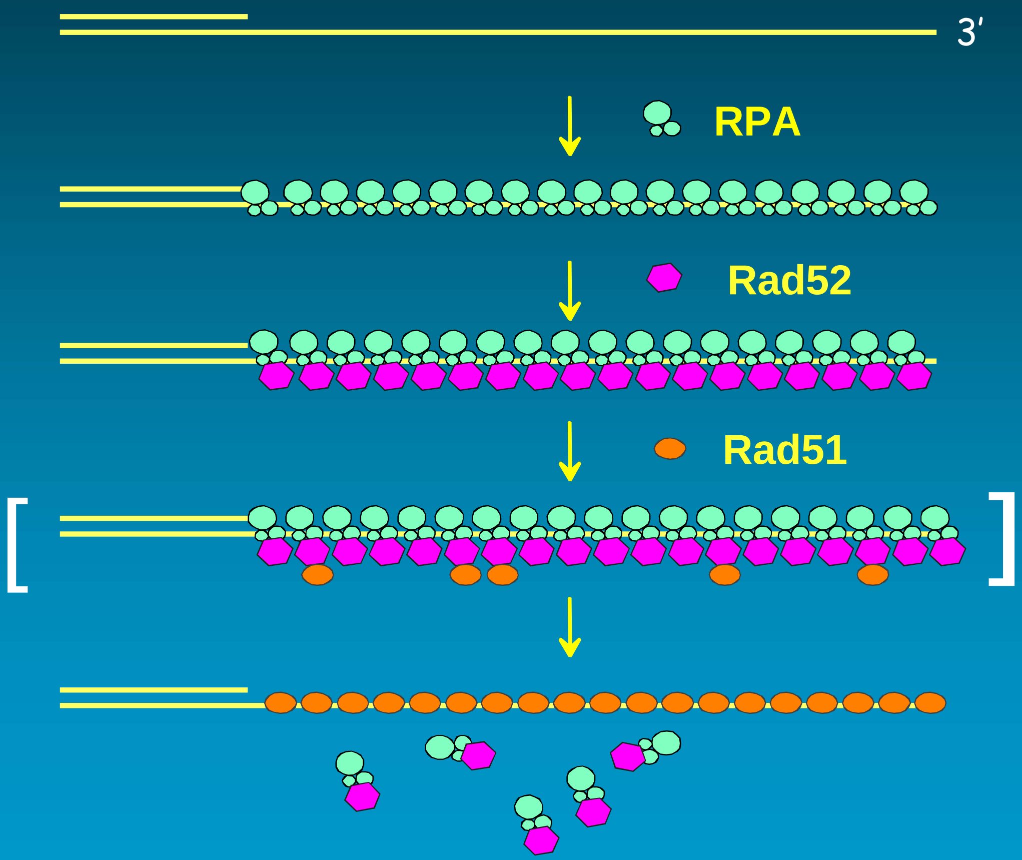


Yang, S., Yu, X., Seitz, E.M., Kowalczykowski, S.C., and Egelman, E.H. (2001) ***J. Mol. Biol., 314,*** 1077-1085

Why Are So Many Proteins Required to Make a RecA/Rad51Nucleoprotein Filament?

Answer: ssDNA Binding Protein (SSB/RPA)

The Temporal Order of Presynaptic Complex Formation



Processed dsDNA with 3’-ssDNA overhang

RPA-ssDNA complex

Rad52 binds to the RPA-ssDNA complex

Rad51 is recruited by Rad52

Rad51 uniformly coats ssDNA
to form the presynaptic complex

New, J.H., Sugiyama, T., Zaitseva, E., and Kowalczykowski, S.C. (1998) ***Nature, 391***, 407-410

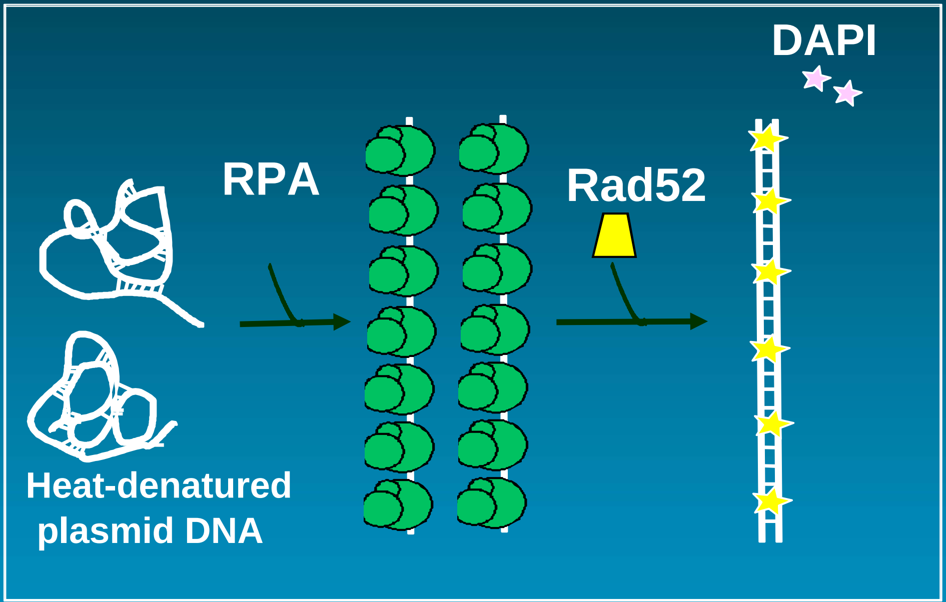
Kowalczykowski, S.C. (2000). Some assembly required…. ***Nature Struct. Biol., 7,*** 1087-1089

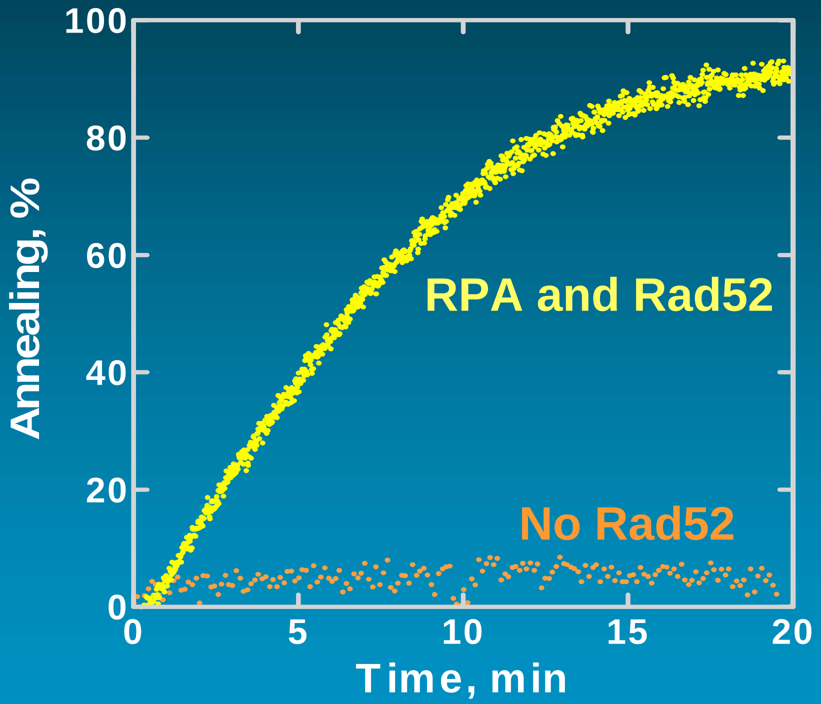
Rad52 Protein Has Two Functions

1) Rad52 is a “recombination mediator” protein: it promotes displacement of RPA by Rad51 to facilitate Rad51 nucleoprotein filament formation.

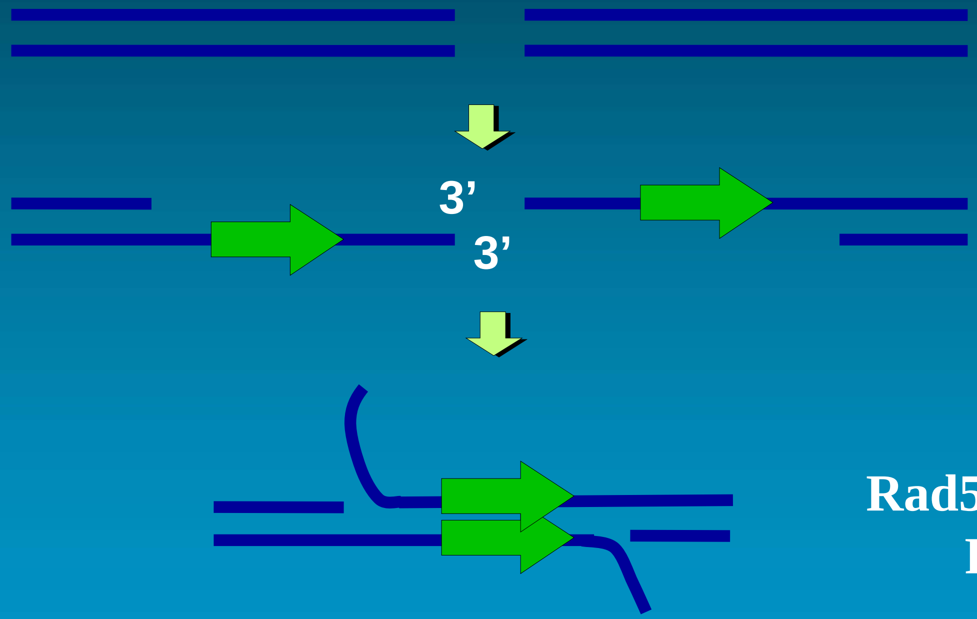
2) Rad52 anneals ssDNA that is complexed with RPA: it promotes ssDNA annealing (SSA) and “second-end” capture in DSBR.

Rad52 Mediates Annealing of RPA-ssDNA Complexes



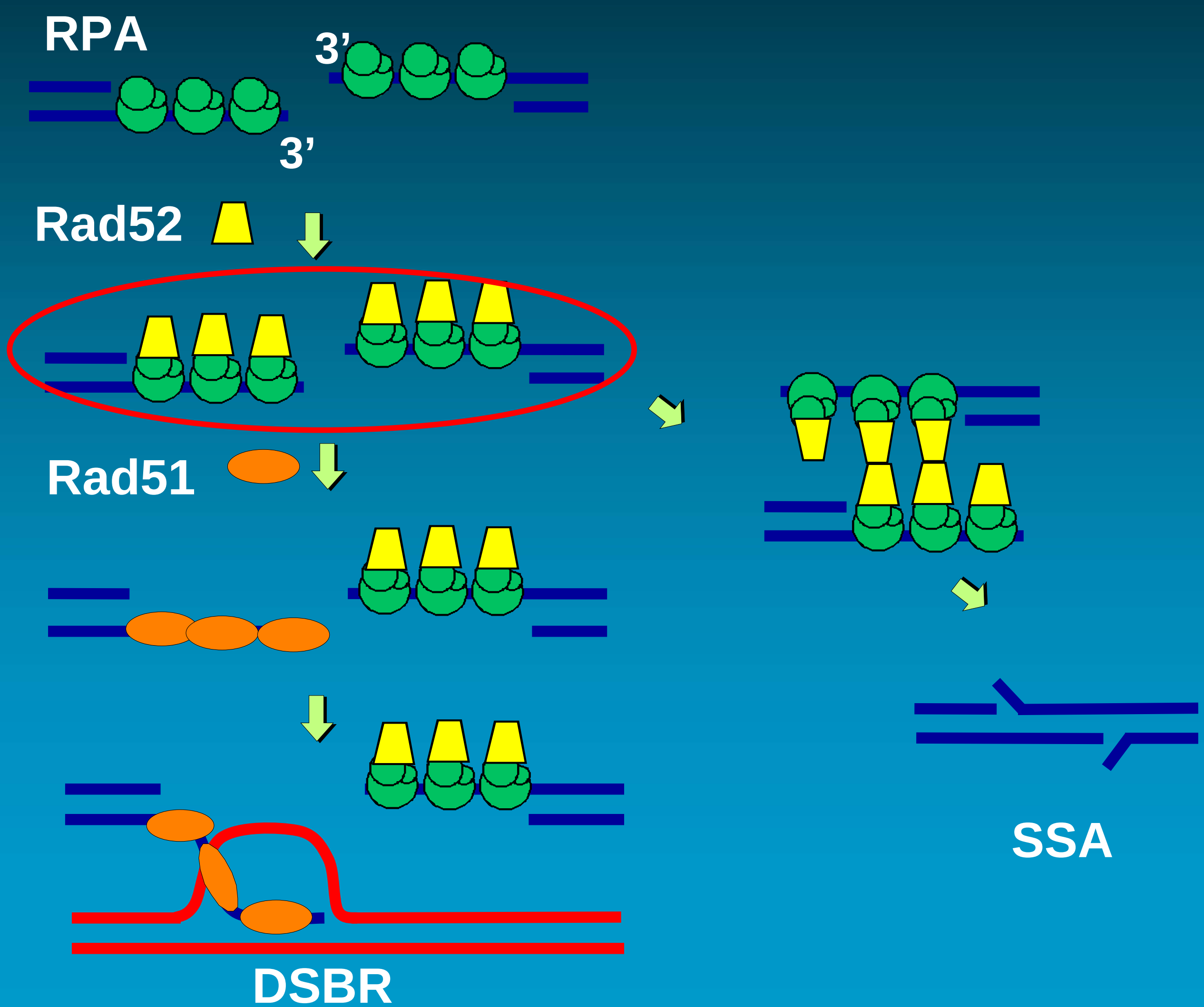


Single-strand DNA Annealing

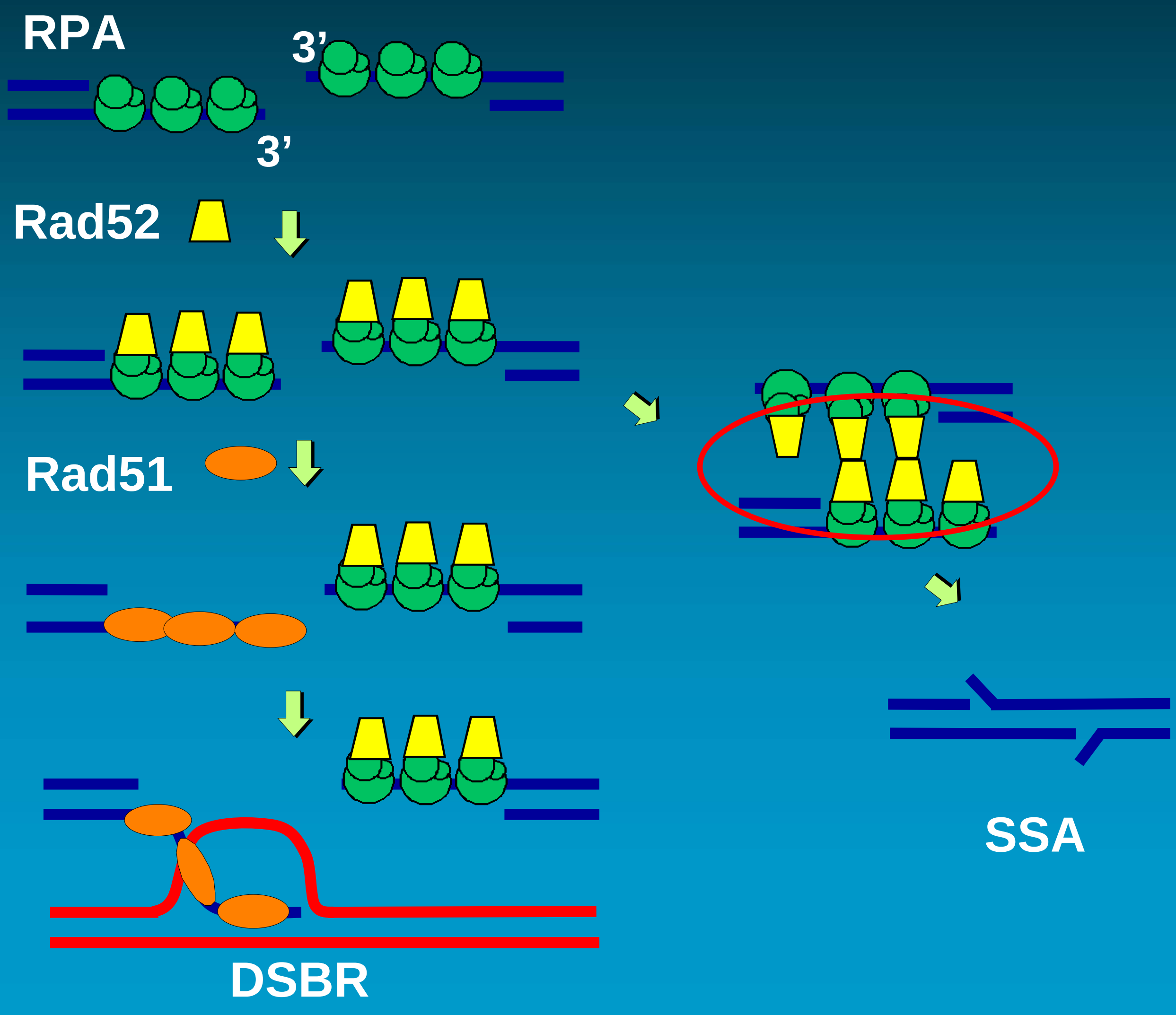


Rad52, RPA, Rad59,
Rad1/Rad10

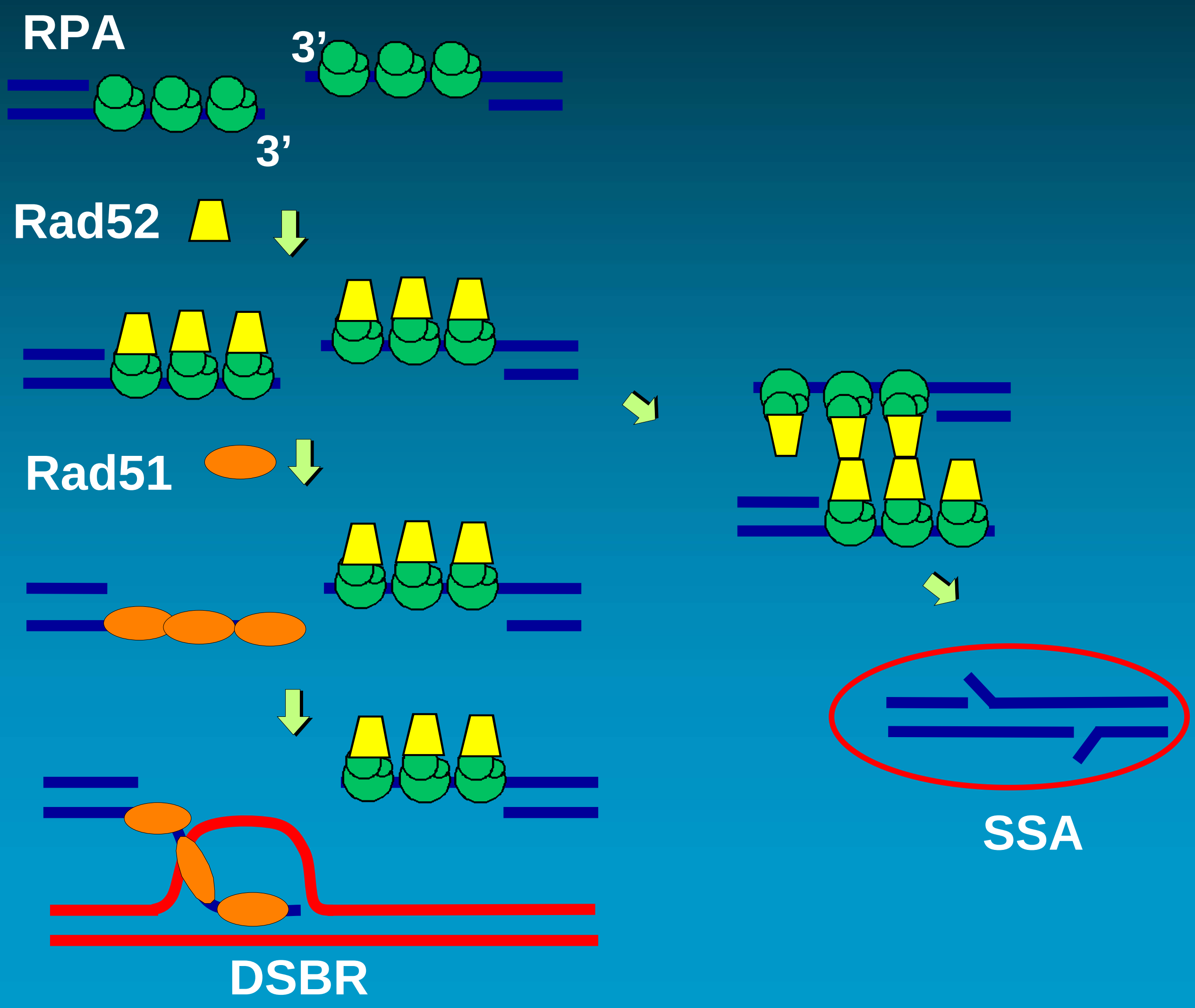
Rad52 Protein Facilitates both DNA Strand Invasion and ssDNA Annealing



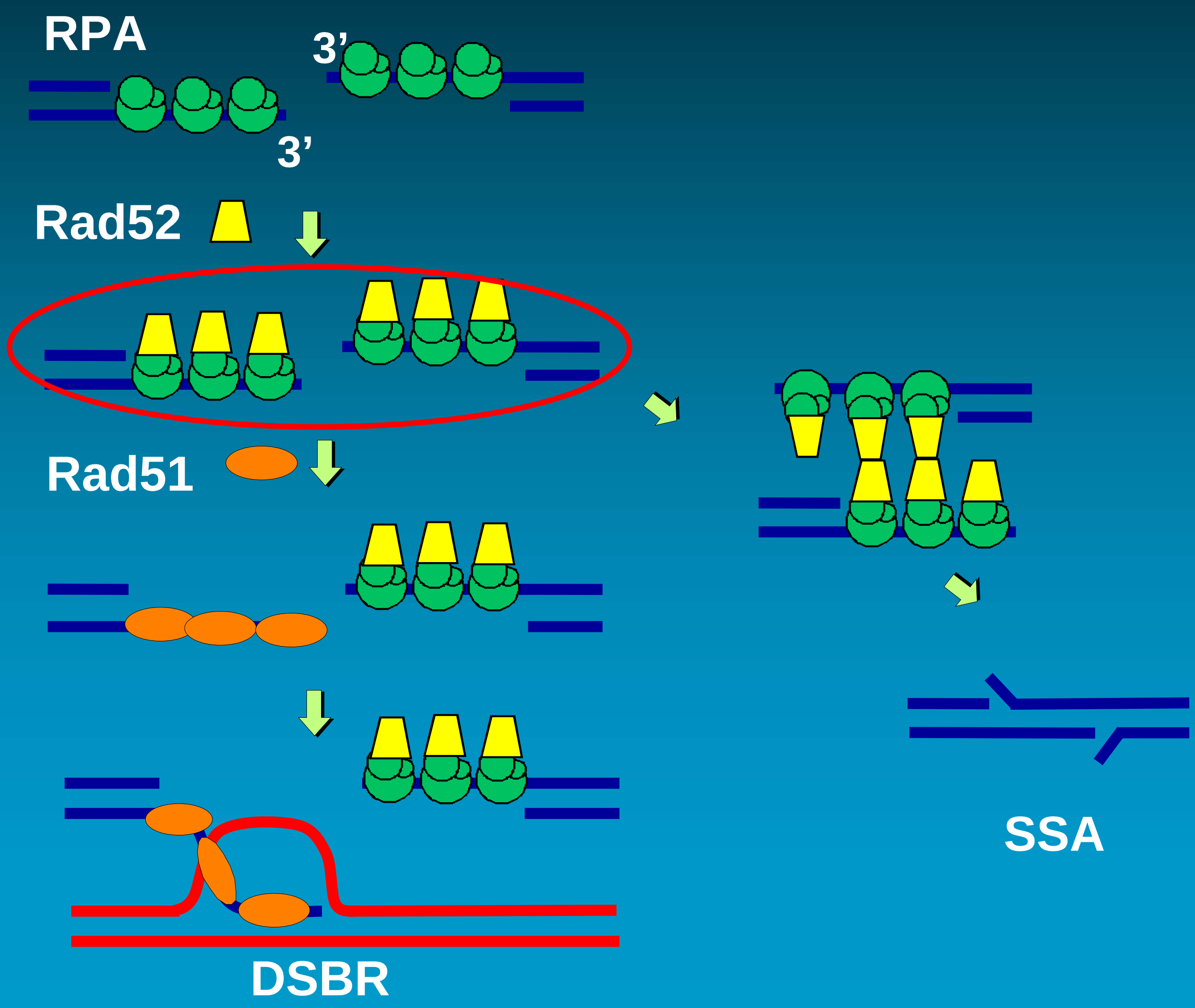
Rad52 Protein Catalyzes Annealing of ssDNA



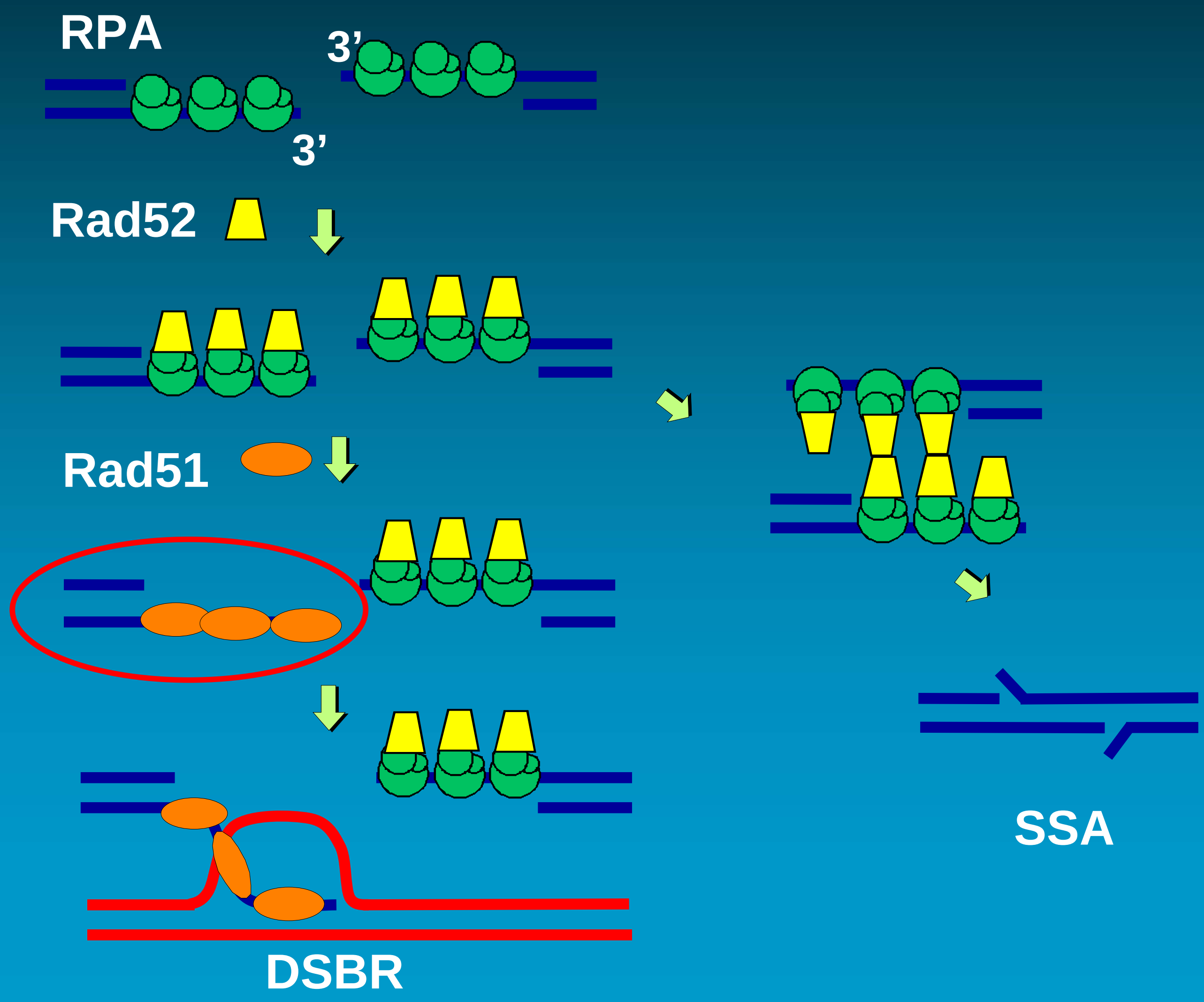
Rad52 Protein Catalyzes Annealing of ssDNA



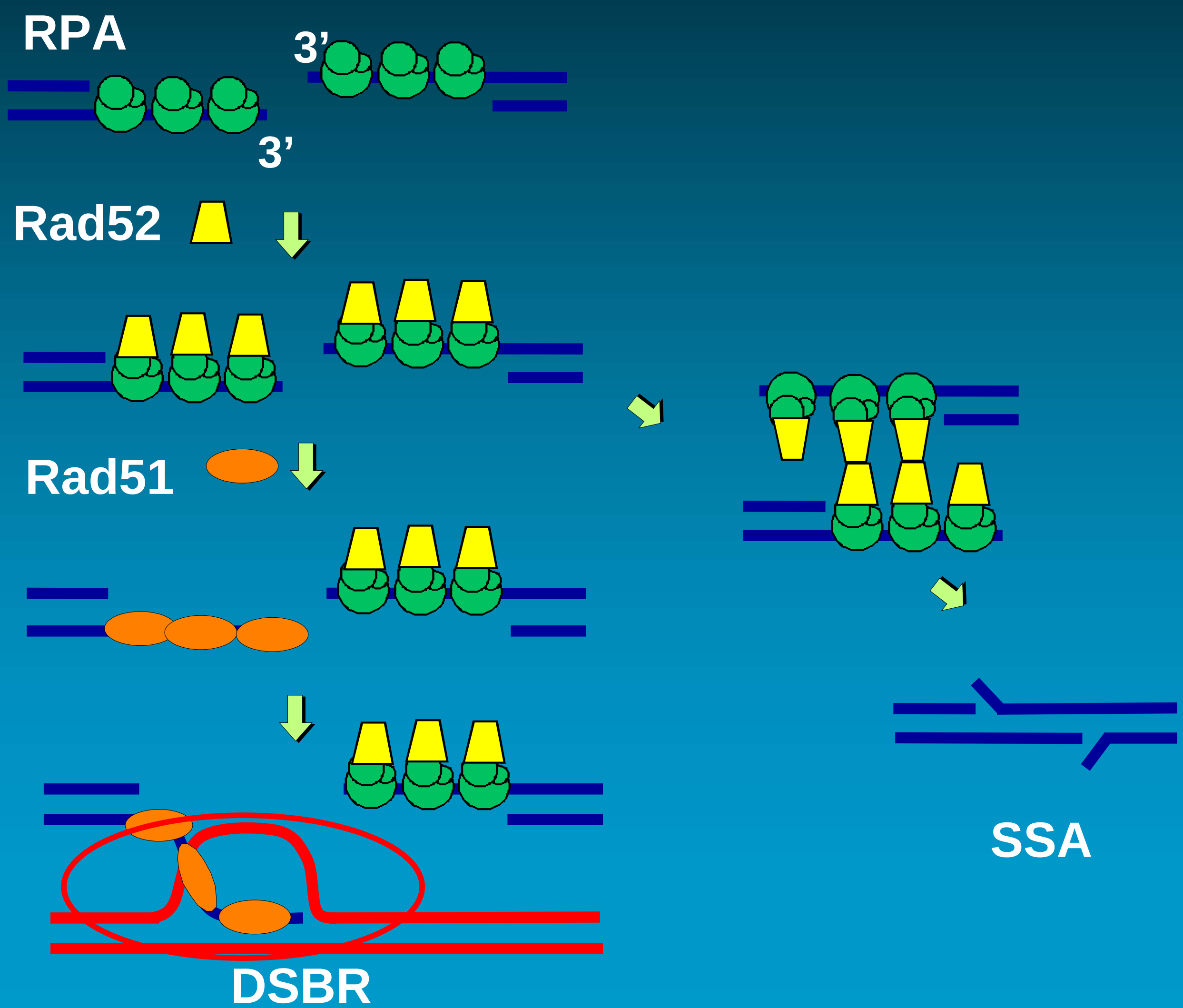
Rad52 Protein Facilitates both DNA Strand Invasion and ssDNA Annealing



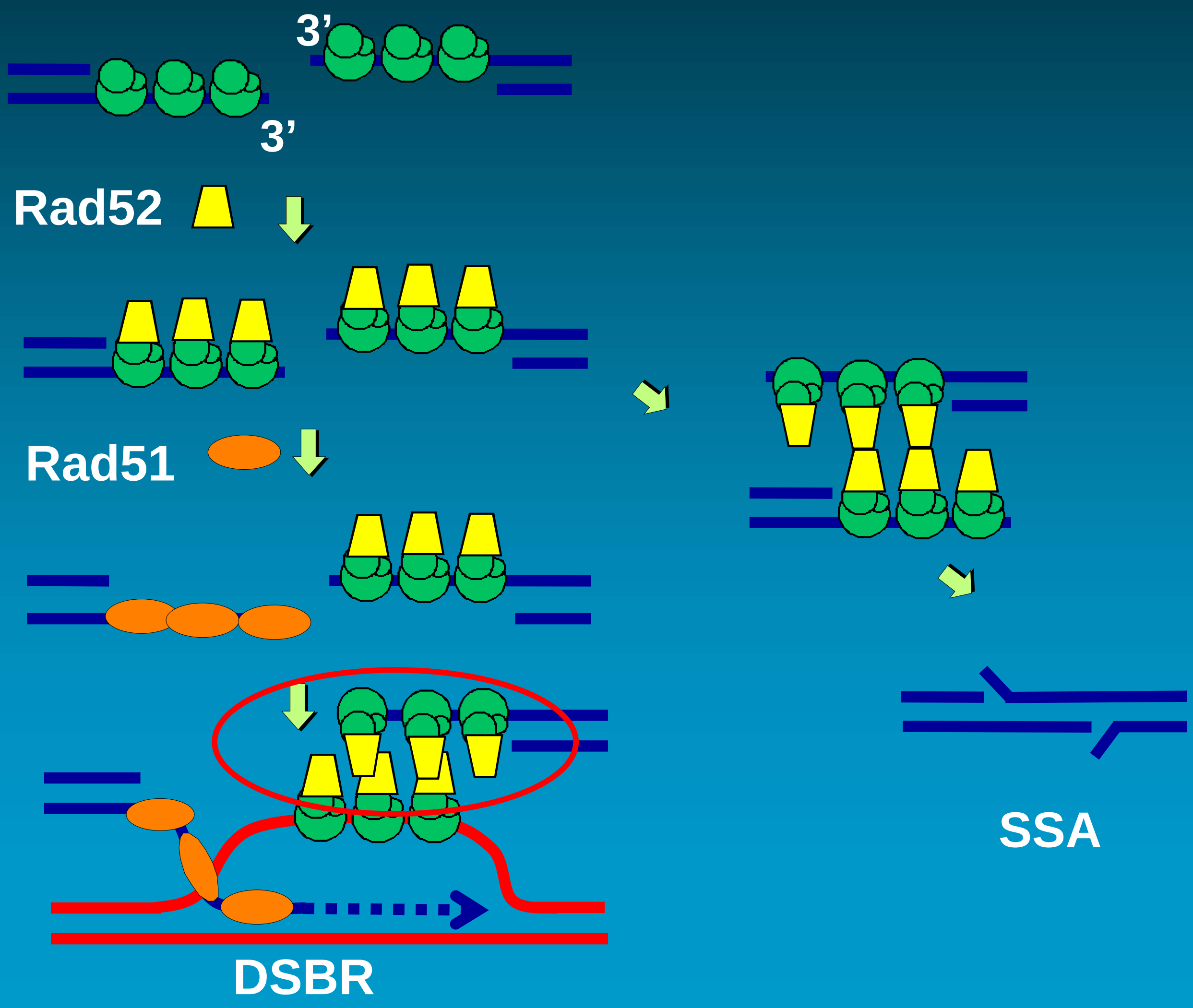
Rad52 Protein Mediates Rad51 Nucleoprotein Filament Formation



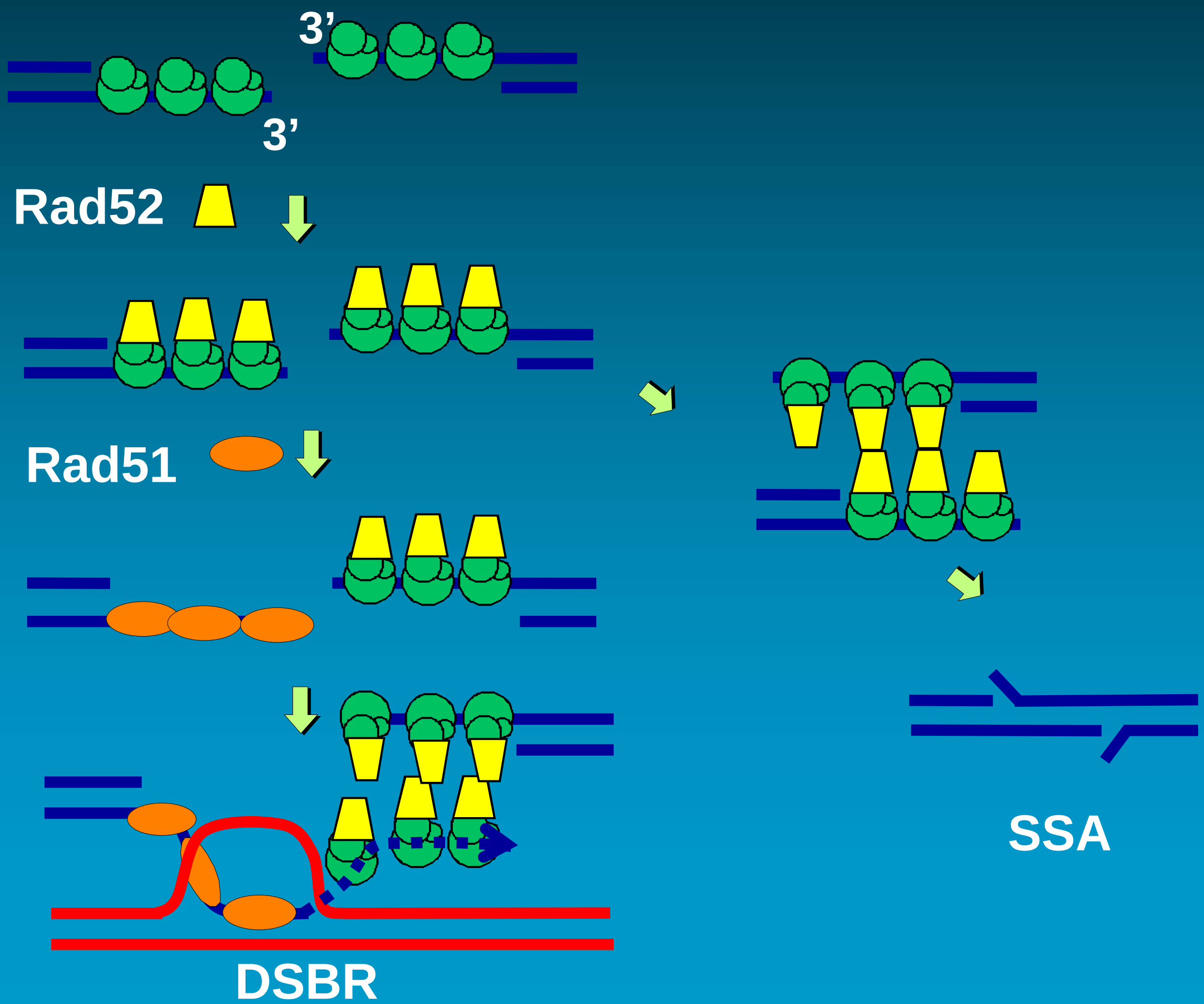
Rad52 Protein Mediates Rad51 Nucleoprotein Filament Formation



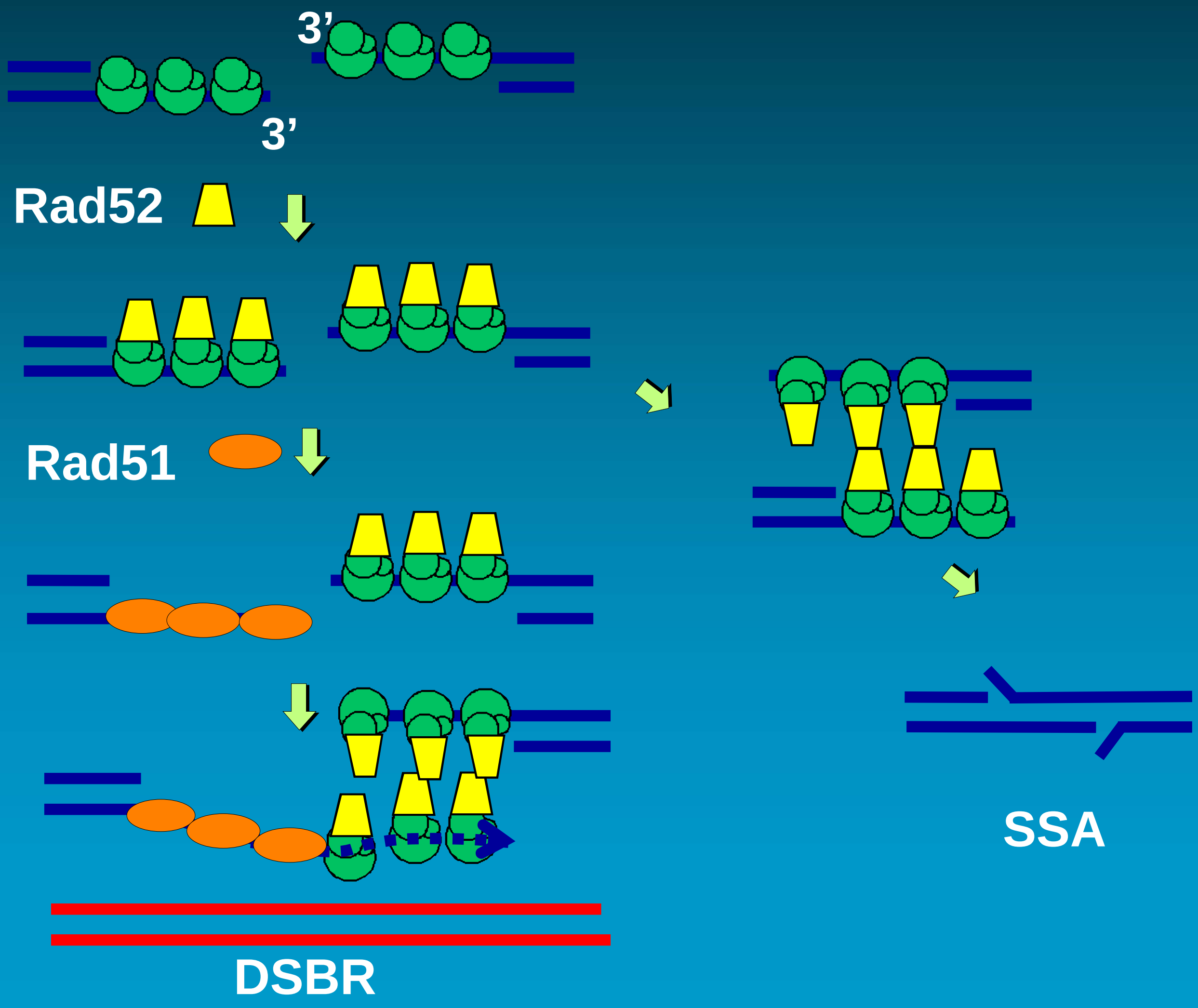
Rad52 Protein Can Promote Second-End Capture by ssDNA Annealing



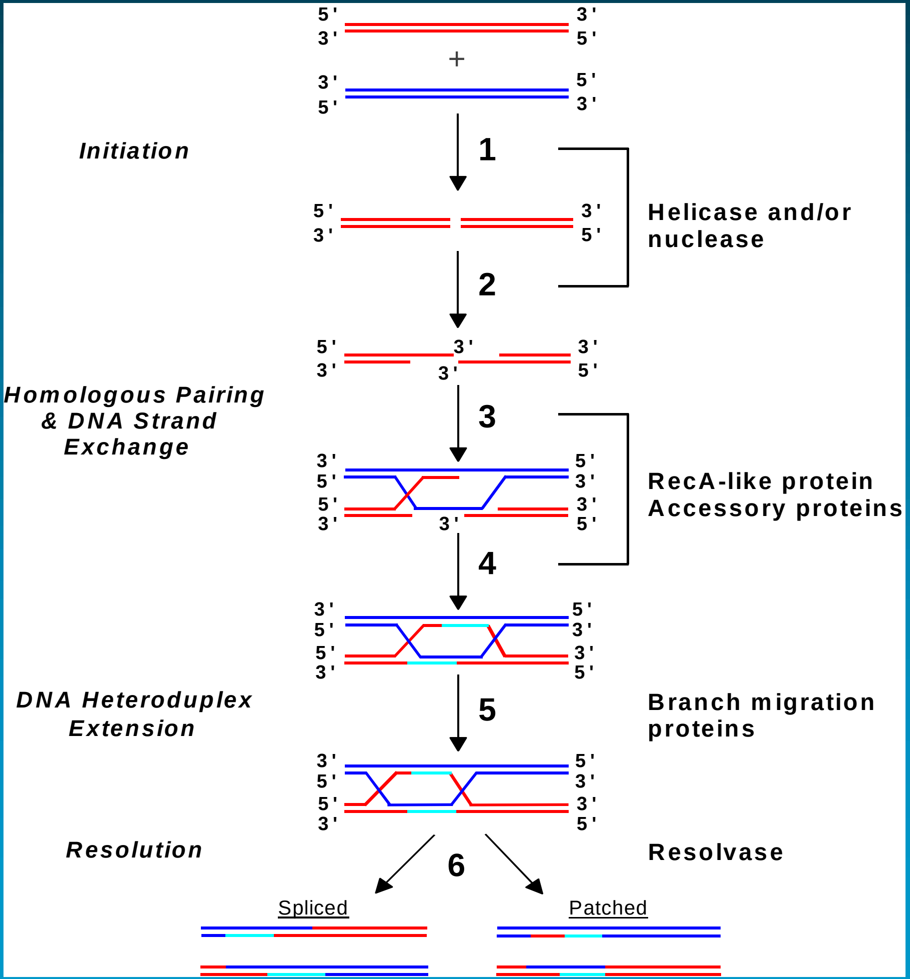
Rad52 Protein Can Promote Synthesis-Dependent Strand Annealing



Rad52 Protein Can Promote Synthesis-Dependent Strand Annealing



Proteins Involved in Recombinational DNA Repair



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RecBCD	--	--
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RecJ	ExoI	ExoI
UvrD	Srs2	--
RecA	Rad51	Rad51
SSB	RPA	RPA
RecF(R)	Rad55/57	Rad51B/C/D/Xrcc2/3
RecO	Rad52	Rad52
--	Rad59	--
--	Rad54/Rdh54	Rad54/54B
--	--	Brca2
RuvAB	Rad54	Rad54
RecG	--	--
RecQ	Sgs1(?)	RecQL/4/5/BLM/WRN(?)
RuvC	--	--
--	Mus81/Mms4(?)	Mus81/Mms4(?)

The Srs2 Helicase Prevents Recombination by Disrupting Rad51 Nucleoprotein Filaments

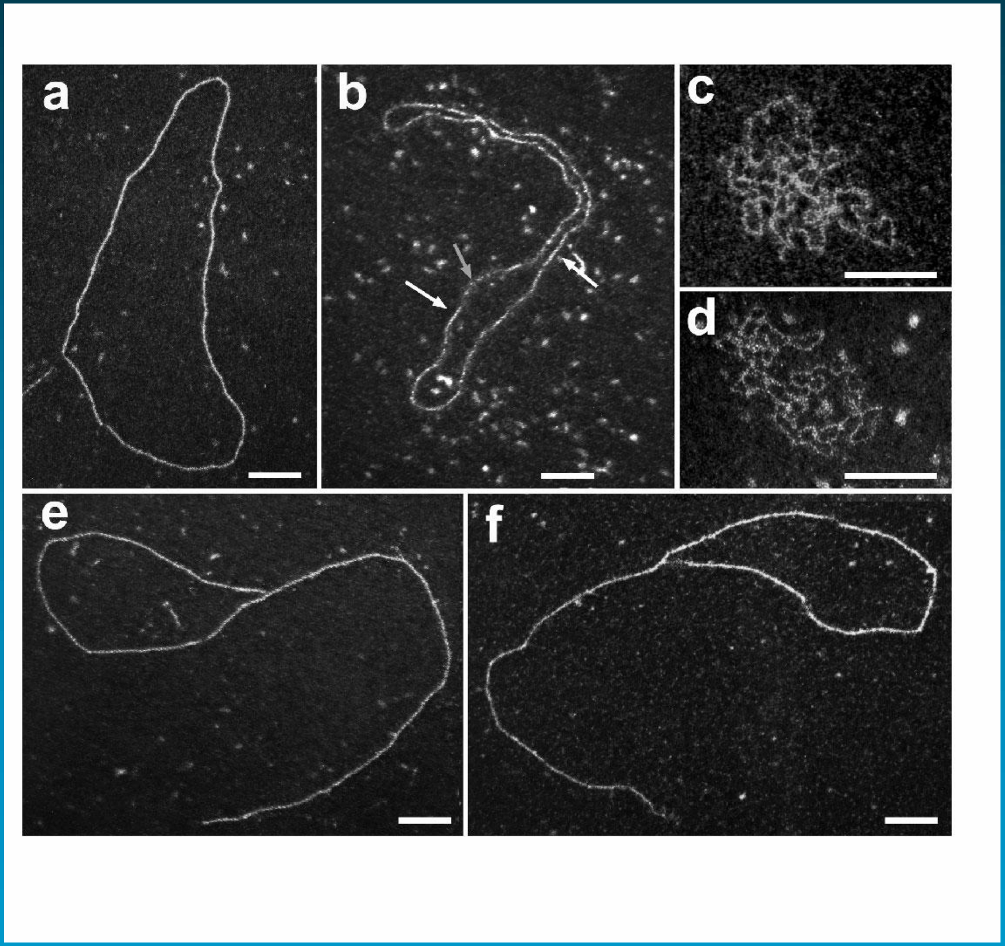
Presynaptic filament formation is also under negative control.

Veaute, X., Jeusset, J., Soustelle, C., Kowalczykowski, S.C., Le Cam, E., and Fabre, F. (2003) ***Nature, 423***, 309-312

Srs2 Helicase Disrupts Rad51 Nucleoprotein Filaments formed on ssDNA, but not on dsDNA

ssDNA:

dsDNA:

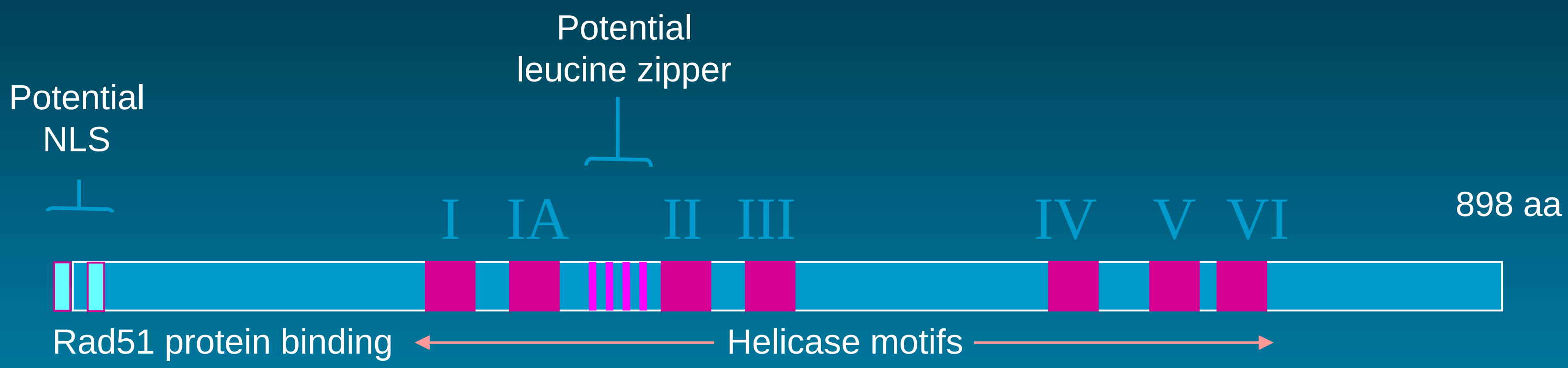


Rad54 Protein Both Stabilizes the Rad51-ssDNA Nucleoprotein Filament and Possesses Rad51-Stimulated Chromatin-Remodeling Activity

Mazin, A.V., Alexeev, A., and Kowalczykowski, S.C. (2003). ***J. Biol. Chem., 278,*** 14029–14036.

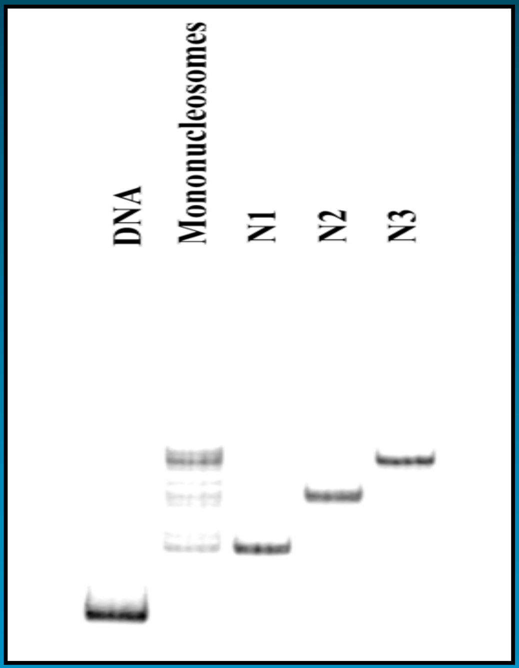
Alexeev, A., Mazin, A., and Kowalczykowski, S.C. (2003) ***Nature Struct. Biol.*** 10, 182-186

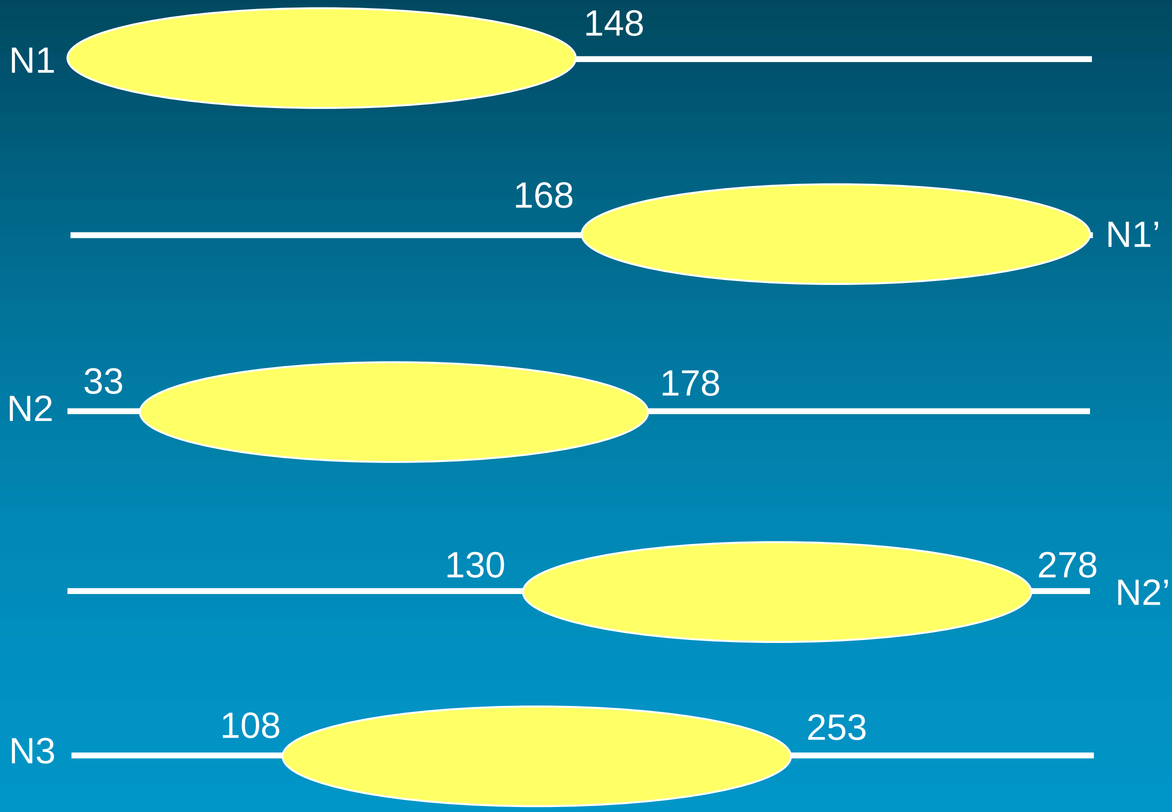
Rad54 Protein Is a Member of Swi2/Snf2 Family of Proteins



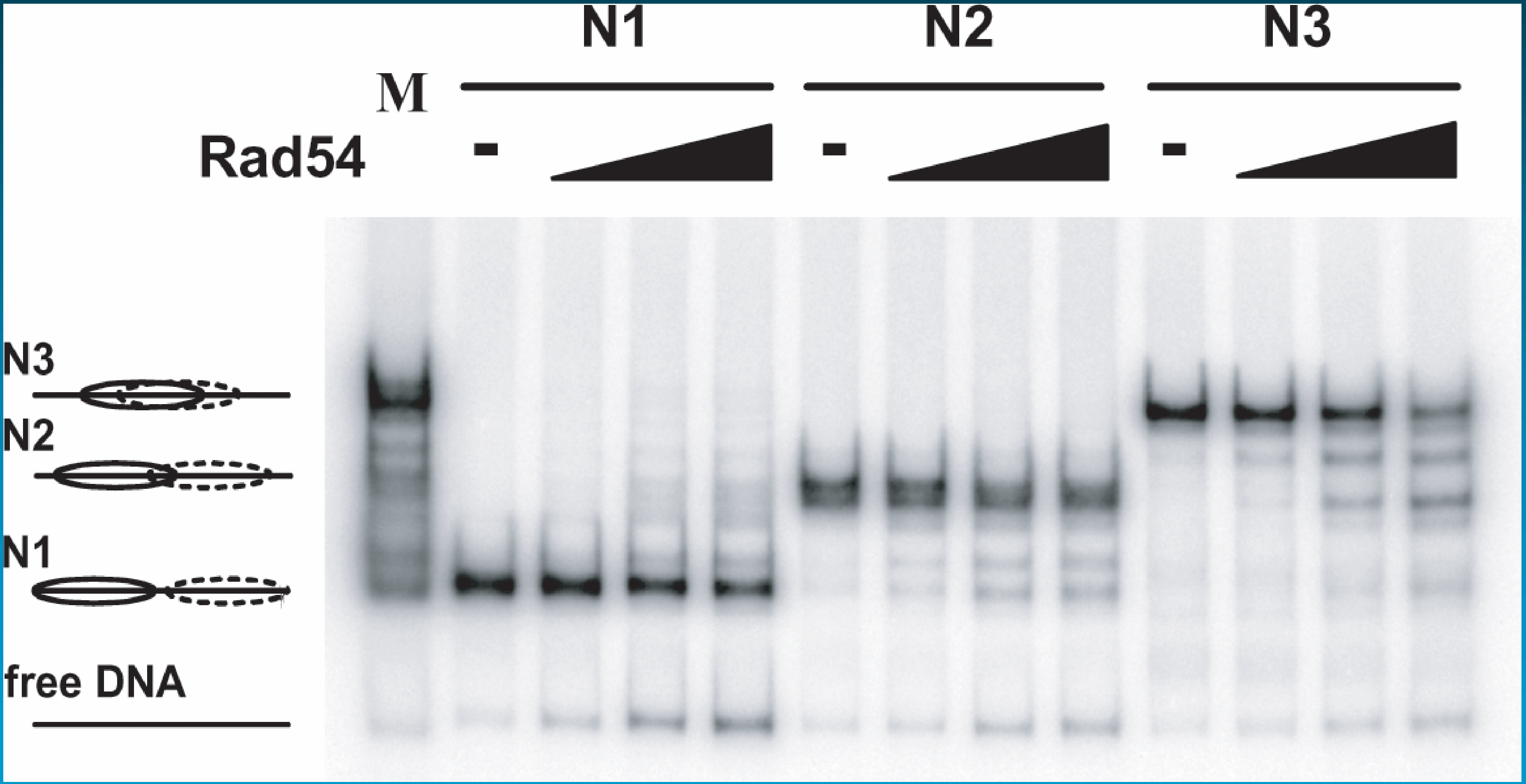
- dsDNA-dependent ATPase activity
- *No* DNA helicase activity
- Topologically unwinds dsDNA
- Interacts with free Rad51 protein
- Stimulates DNA pairing by Rad51 protein

Reconstitution of Mononucleosomes With Defined Positions

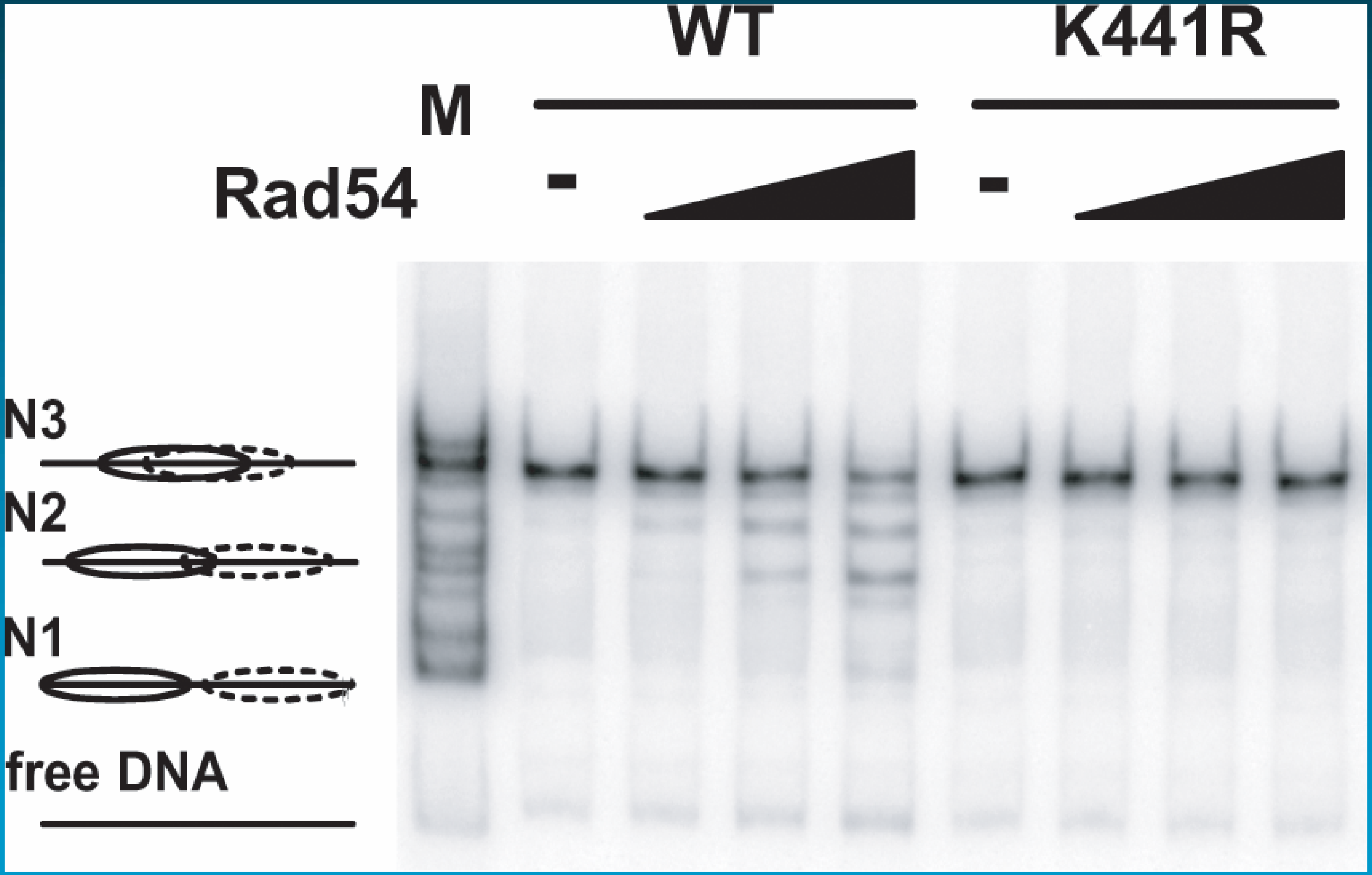




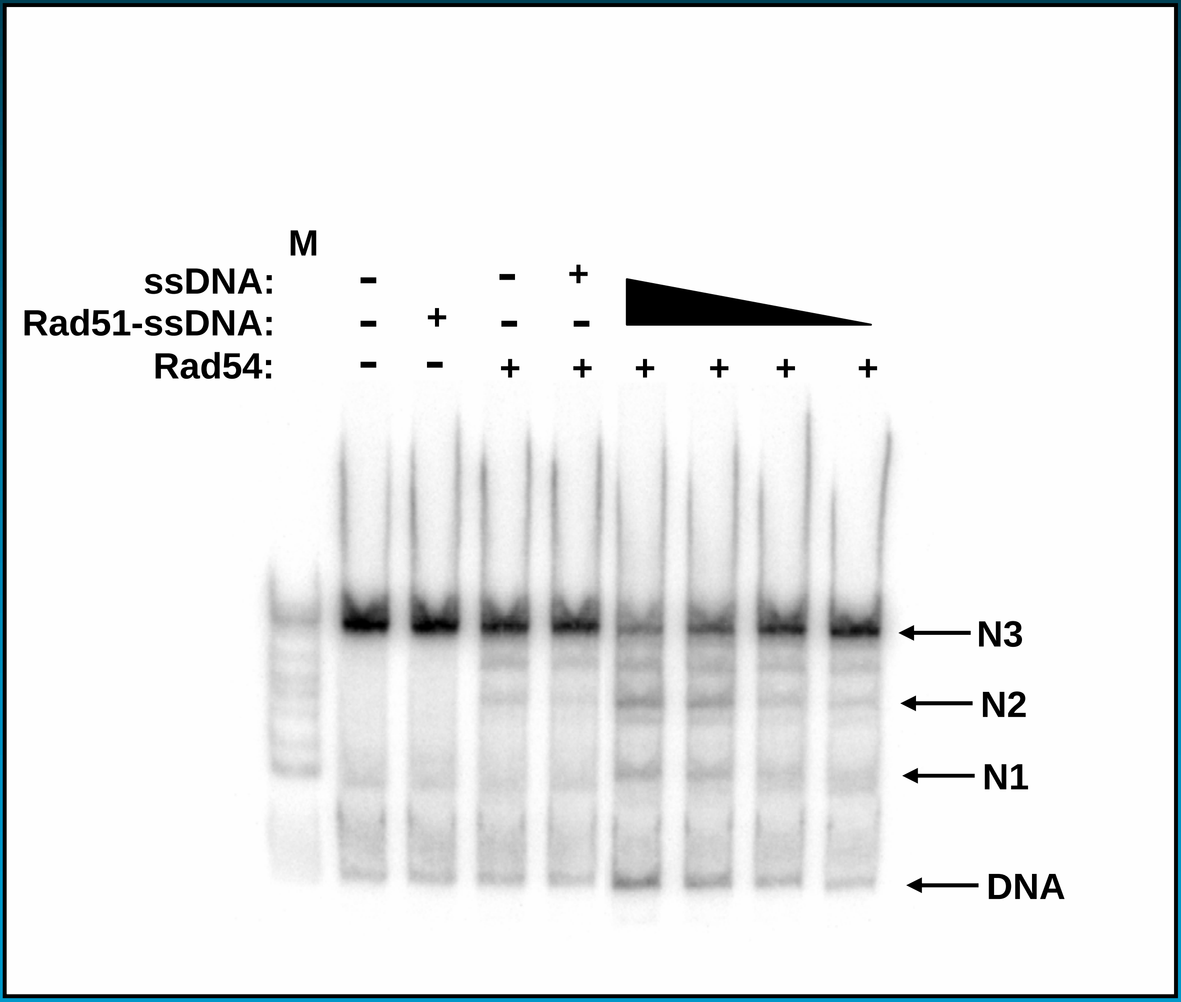
Rad54 Protein Facilitates Nucleosome Mobility



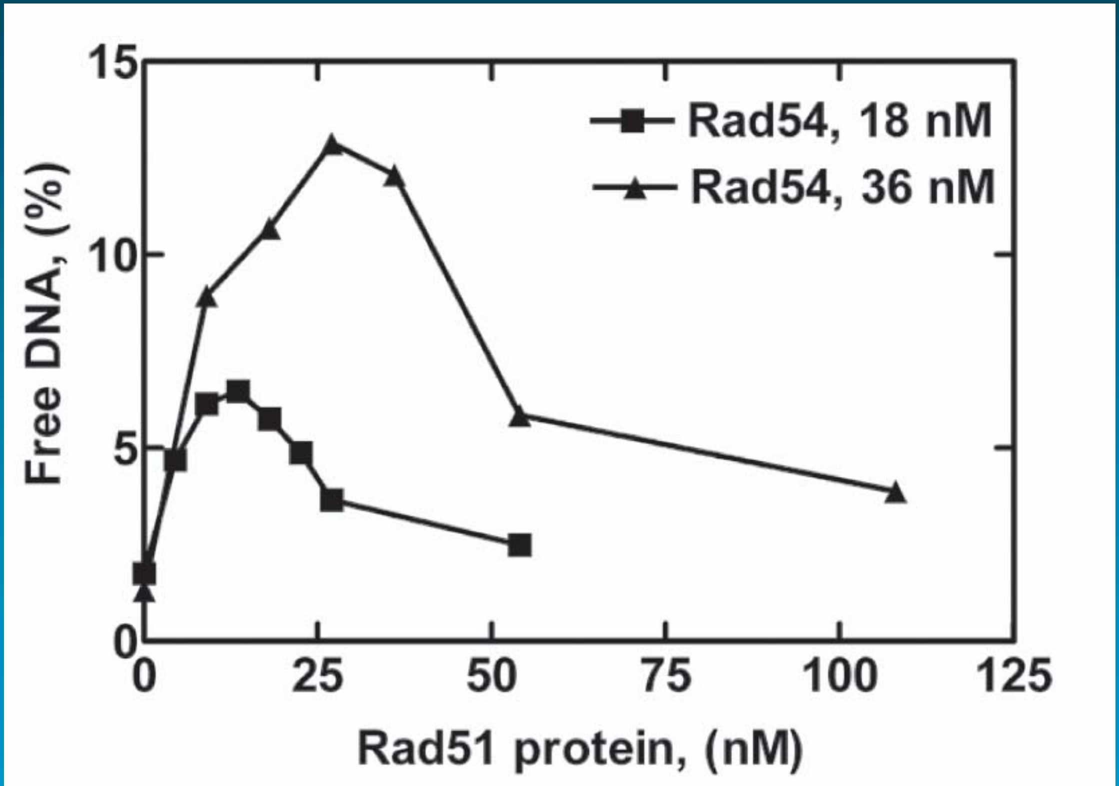
ATP Hydrolysis is Required for Remodeling



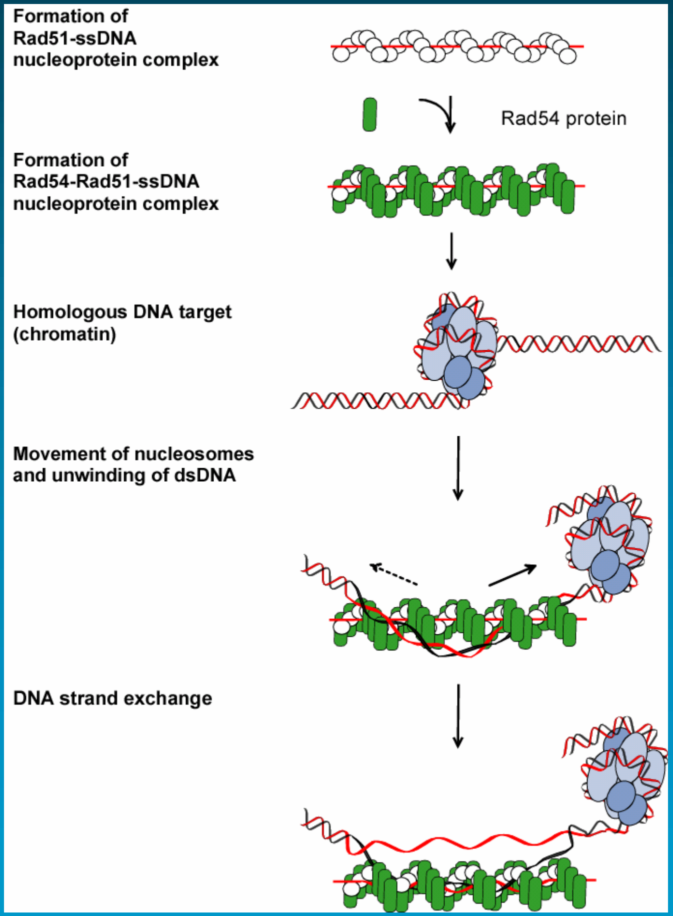
The Rad51-ssDNA Nucleoprotein Filament Enhances Nucleosome Remodeling by Rad54



A Stoichiometric Complex of Rad54 Protein and Rad51-ssDNA Nucleoprotein Filament is Optimal for Nucleosome Remodeling

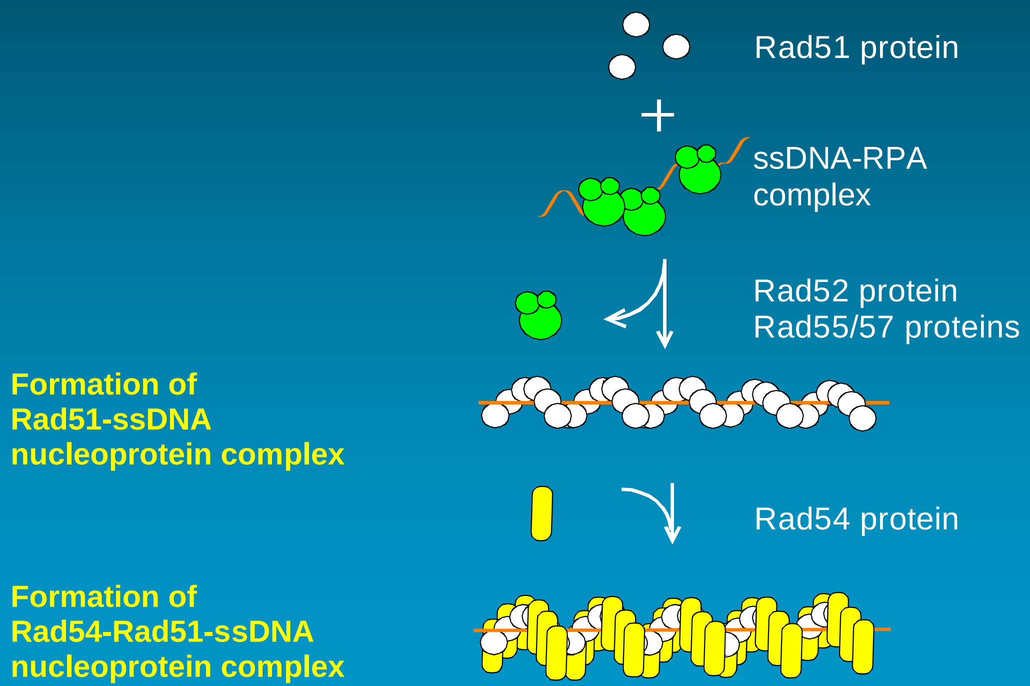


Stimulatory Role of Rad54 Protein in DNA Strand Exchange

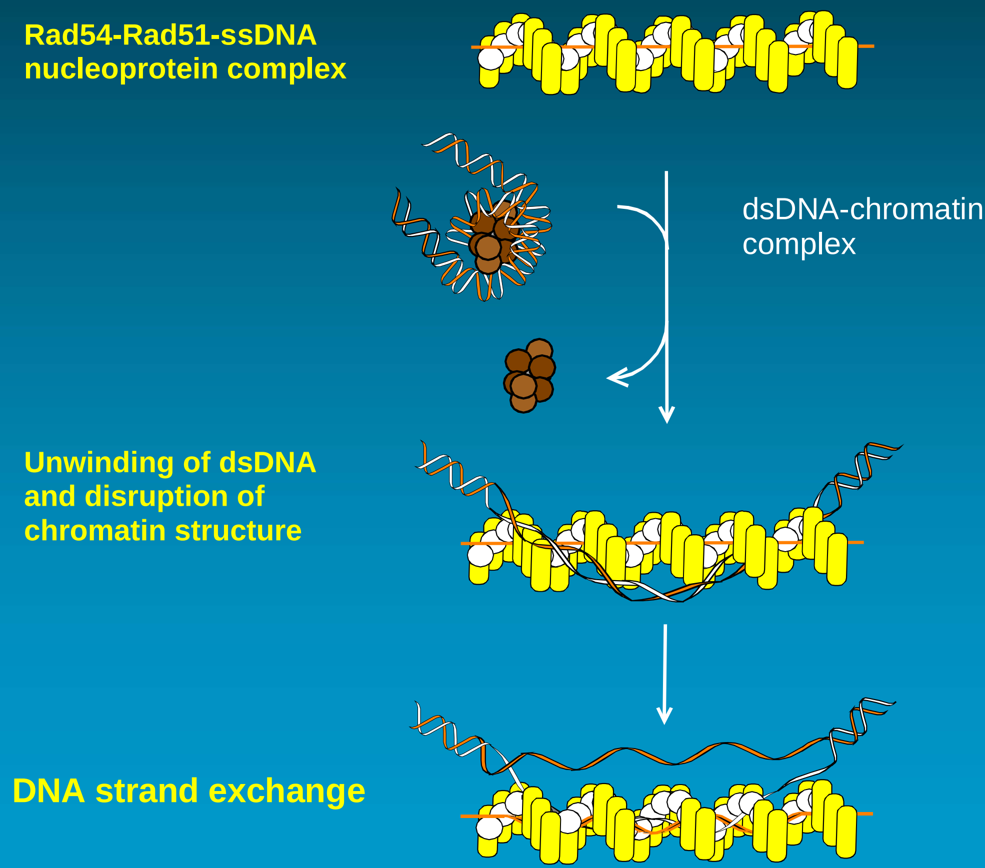


DNA Strand Exchange

Presynapsis

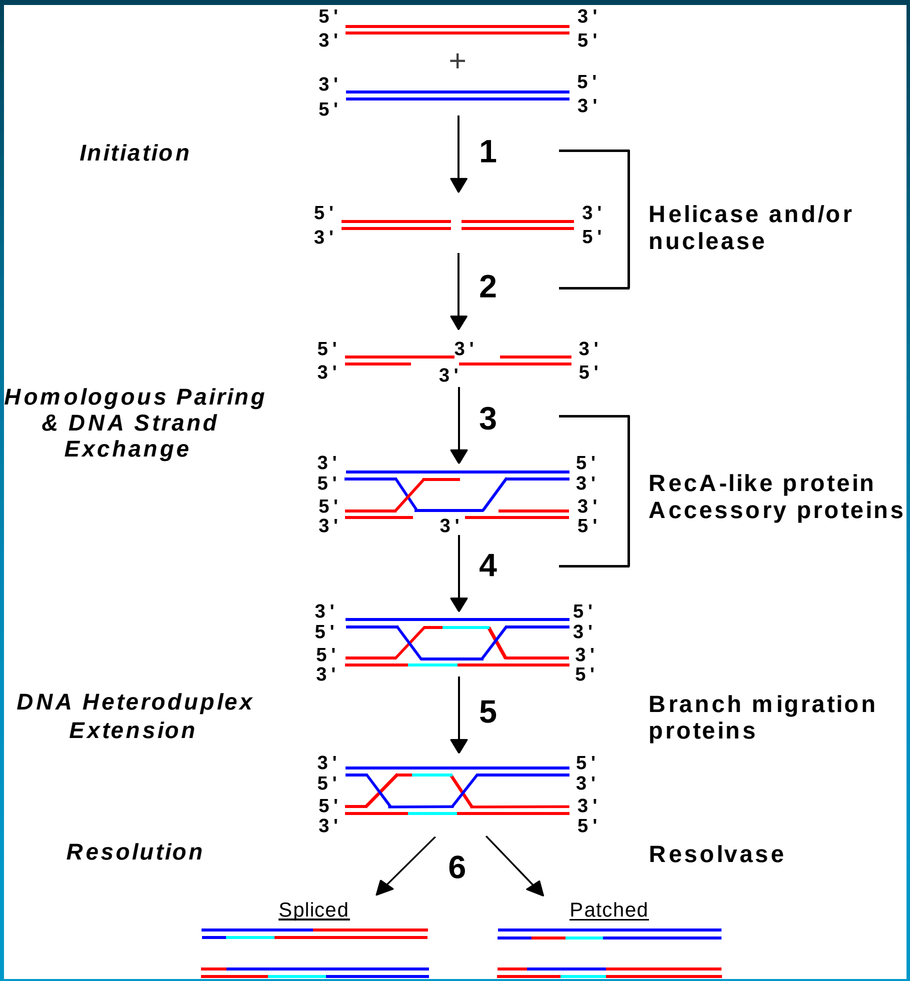


Synapsis



Does Recombination in Prokaryotes Proceed by the Same Mechanism as in Eukaryotes?

Proteins Involved in Recombinational DNA Repair



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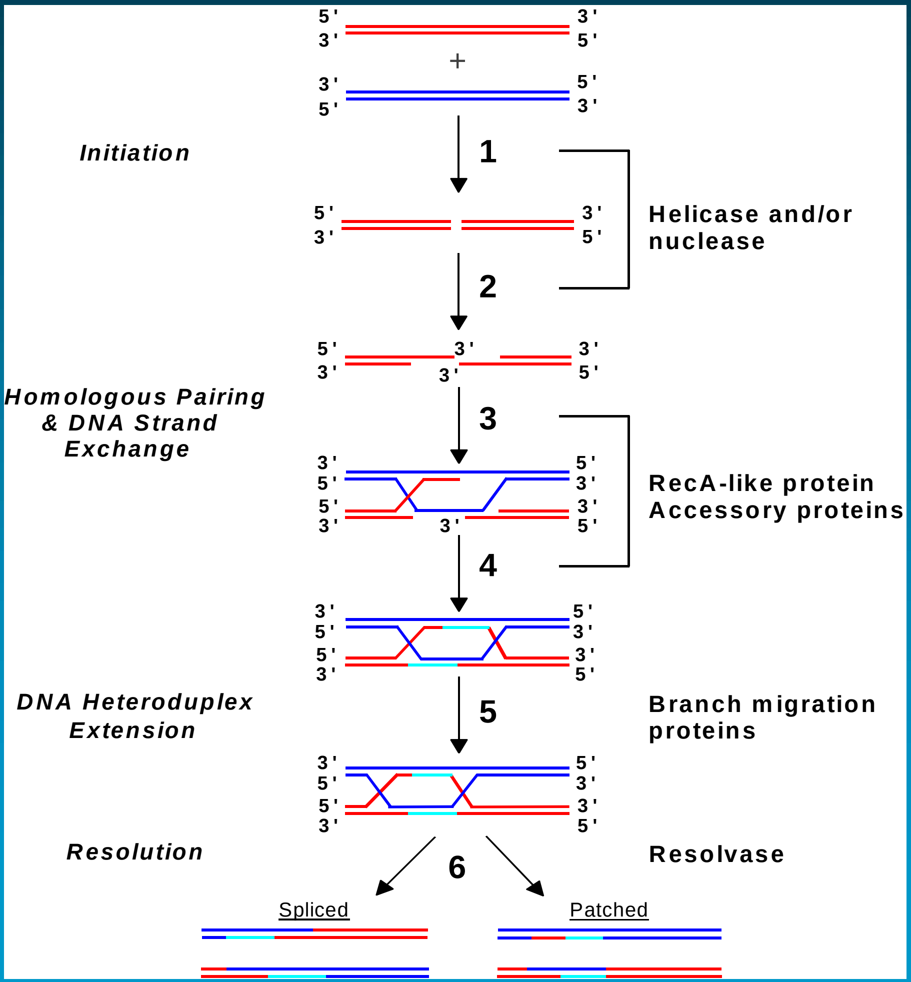
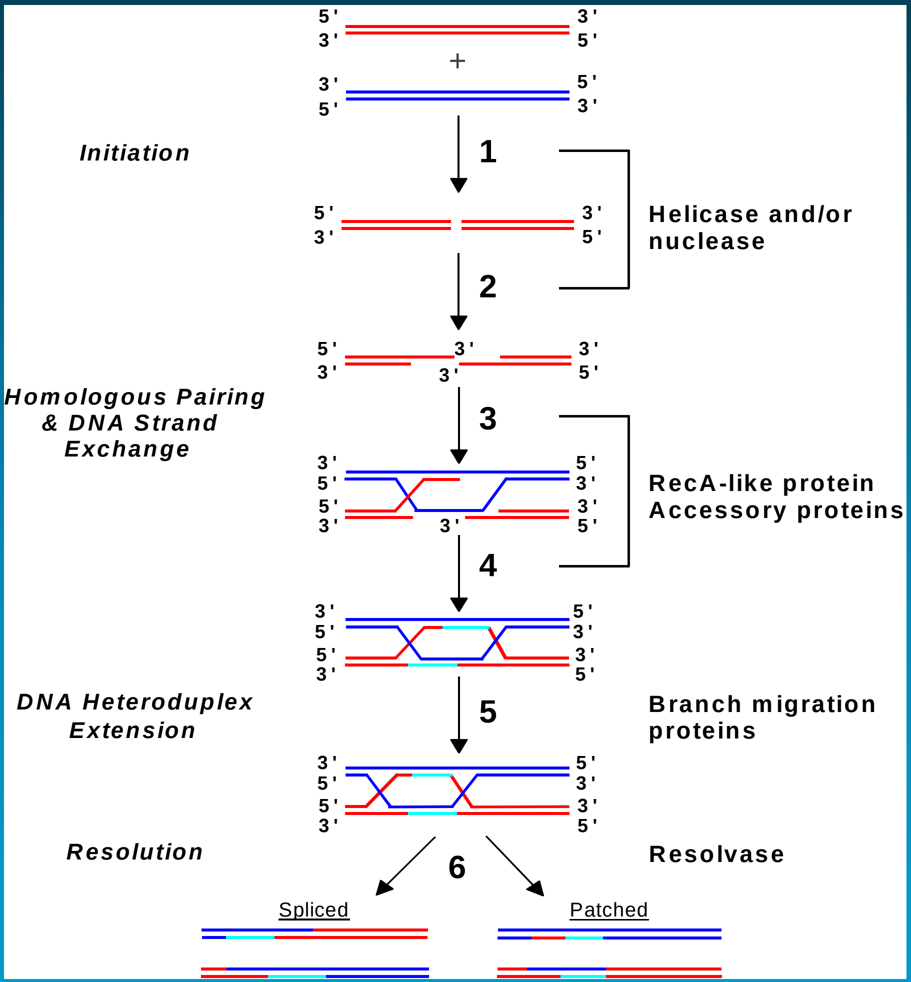
UvrD	Srs2	--
RecA	Rad51	Rad51
SSB	RPA	RPA
RecF(R)	Rad55/57	Rad51B/C/D/Xrcc2/3
RecO	Rad52	Rad52
--	Rad59	--
--	Rad54/Rdh54	Rad54/54B
--	--	Brca2
RuvAB	Rad54	Rad54
RecG	--	--
RecQ	Sgs1(?)	RecQL/4/5/BLM/WRN(?)
RuvC	--	--
--	Mus81/Mms4(?)	Mus81/Mms4(?)

Structurally Related Proteins Common to Prokaryotes and Eukaryotes



E. coli	*S. cerevisiae*	Human
SbcCD RecQ	Mre11/Rad50/Xrs2 Sgs1	Mre11/Rad50/Nbs1 RecQ1/4/5/BLM/WRN
RecA SSB	Rad51 RPA	Rad51 RPA
RecQ	Sgs1(?)	RecQ1/4/5/BLM/WRN(?)

Proteins that are Related to the RecBCD-Pathway of *E. coli*



E. coli	*S. cerevisiae*	Human
RecA SSB	Rad51 RPA	Rad51 RPA

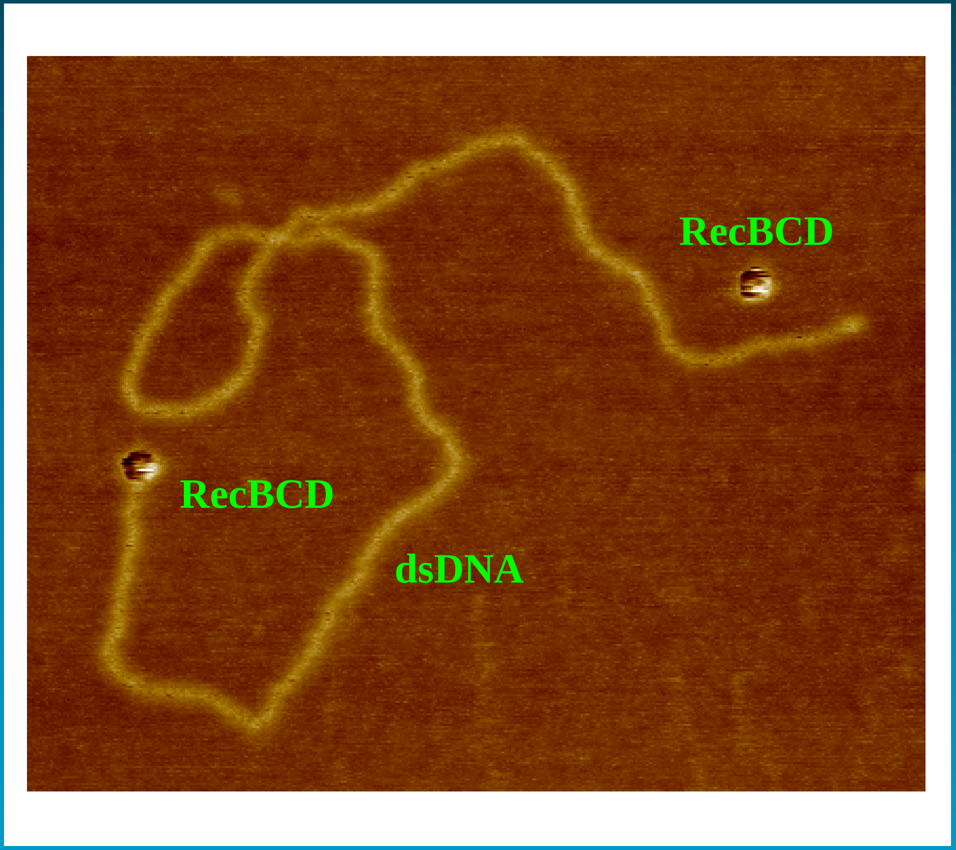
Does Recombination in Prokaryotes Proceed by the Same Mechanism as in Eukaryotes?

There are two pathways of recombination in wild-type *E. coli*:

1) RecBC(D)-pathway

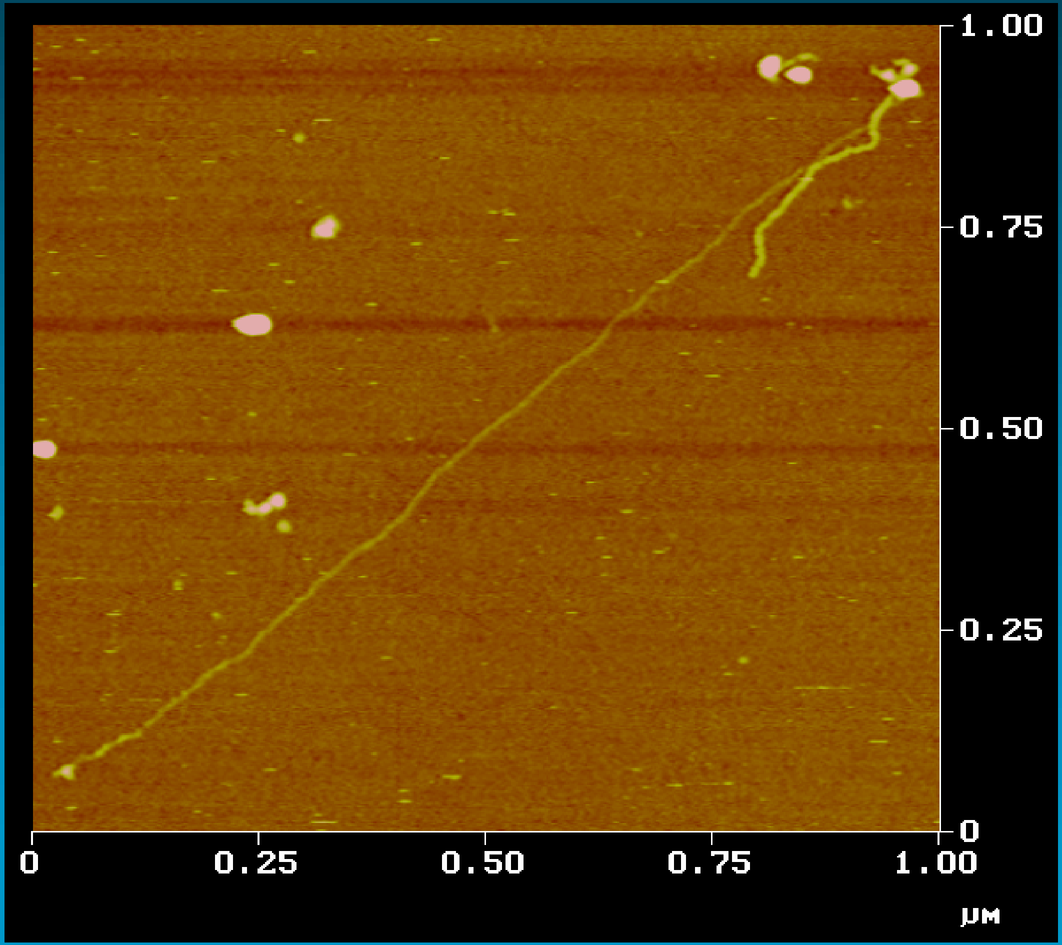
2) RecF(OR)-pathway

RecBCD Enzyme bound to a DNA End

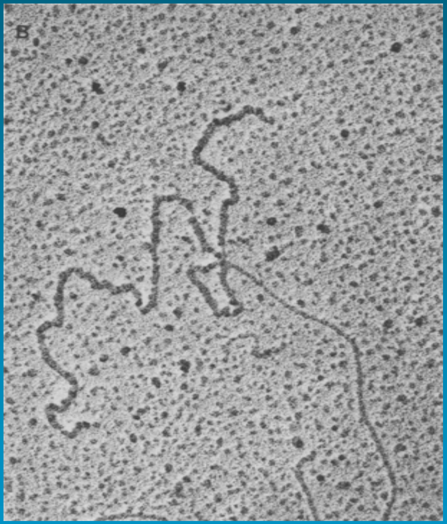


RecBCD Enzyme Unwinding dsDNA

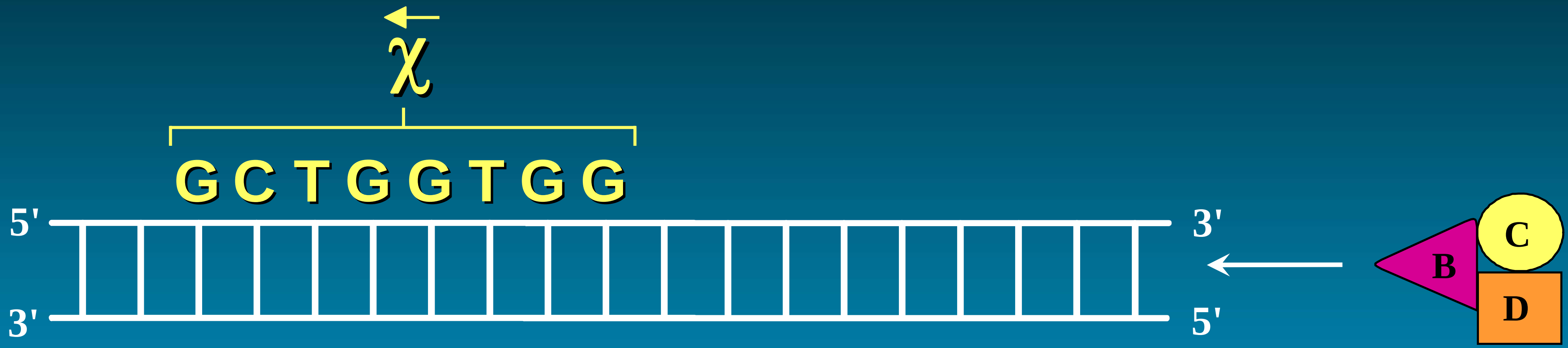
AFM



EM

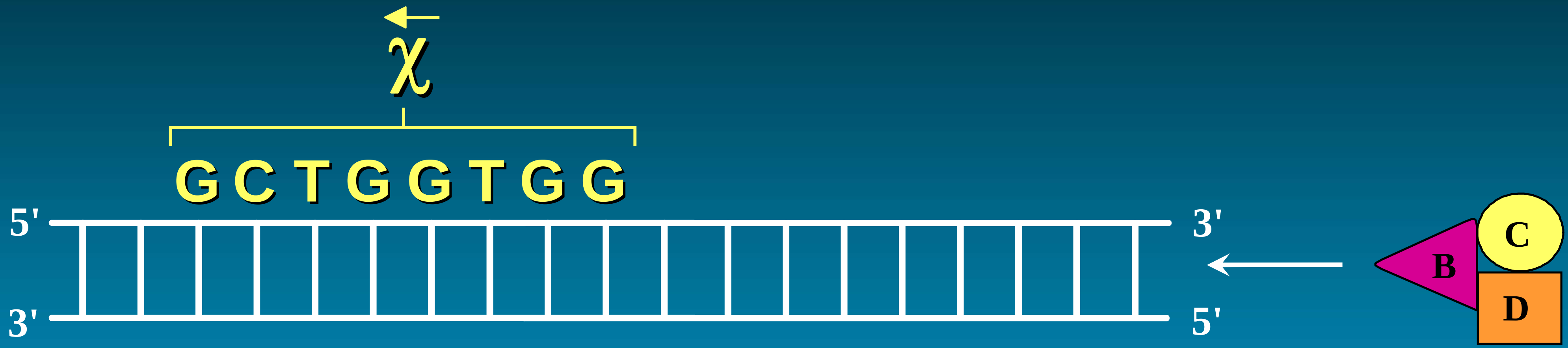


The *E. coli* Recombination Hotspot, Chi (<u>C</u>rossover <u>h</u>otspot <u>i</u>nstigator), χ



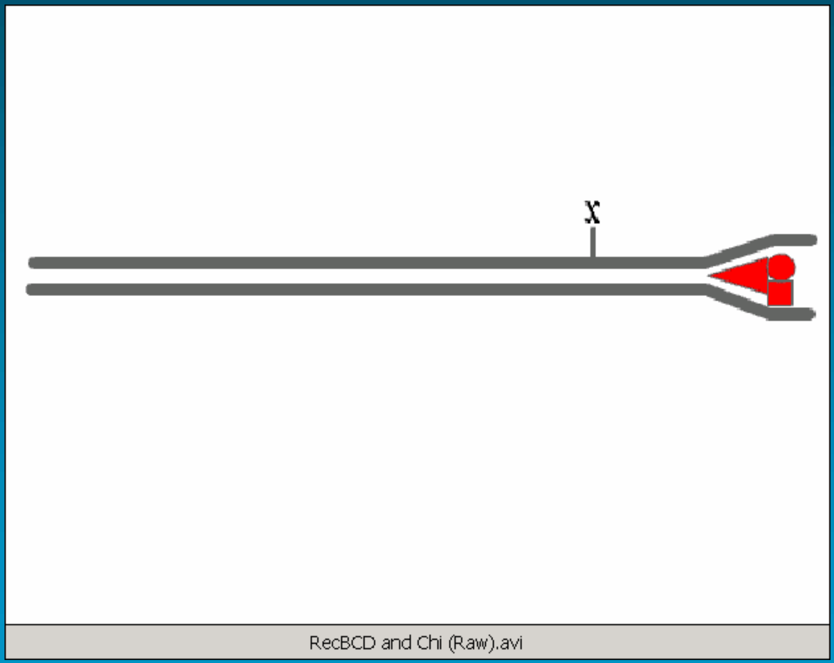
- Is an asymmetric sequence.
- Must be encountered in correct orientation (from the 3'-side).
- Requires information only on the 3'-terminated strand.
- Stimulates recombination downstream of itself.
- Is the most over-represented octamer in the *E. coli* genome.

The *E. coli* Recombination Hotspot, Chi (χ), is a Regulatory Sequence



- Chi regulates the biochemical activities of RecBCD enzyme:
  - It attenuates cleavage of the DNA strand that is 3' at the entry site.
  - It switches the polarity of DNA degradation onto the 5'-strand.
  - The result is preservation of the 3'-strand, with Chi at its terminus.
- Chi directs RecBCD enzyme to load RecA protein onto the Chi-containing ssDNA.

Chi is a Molecular Switch: It Down-regulates and Switches the Nuclease Activity of RecBCD Enzyme



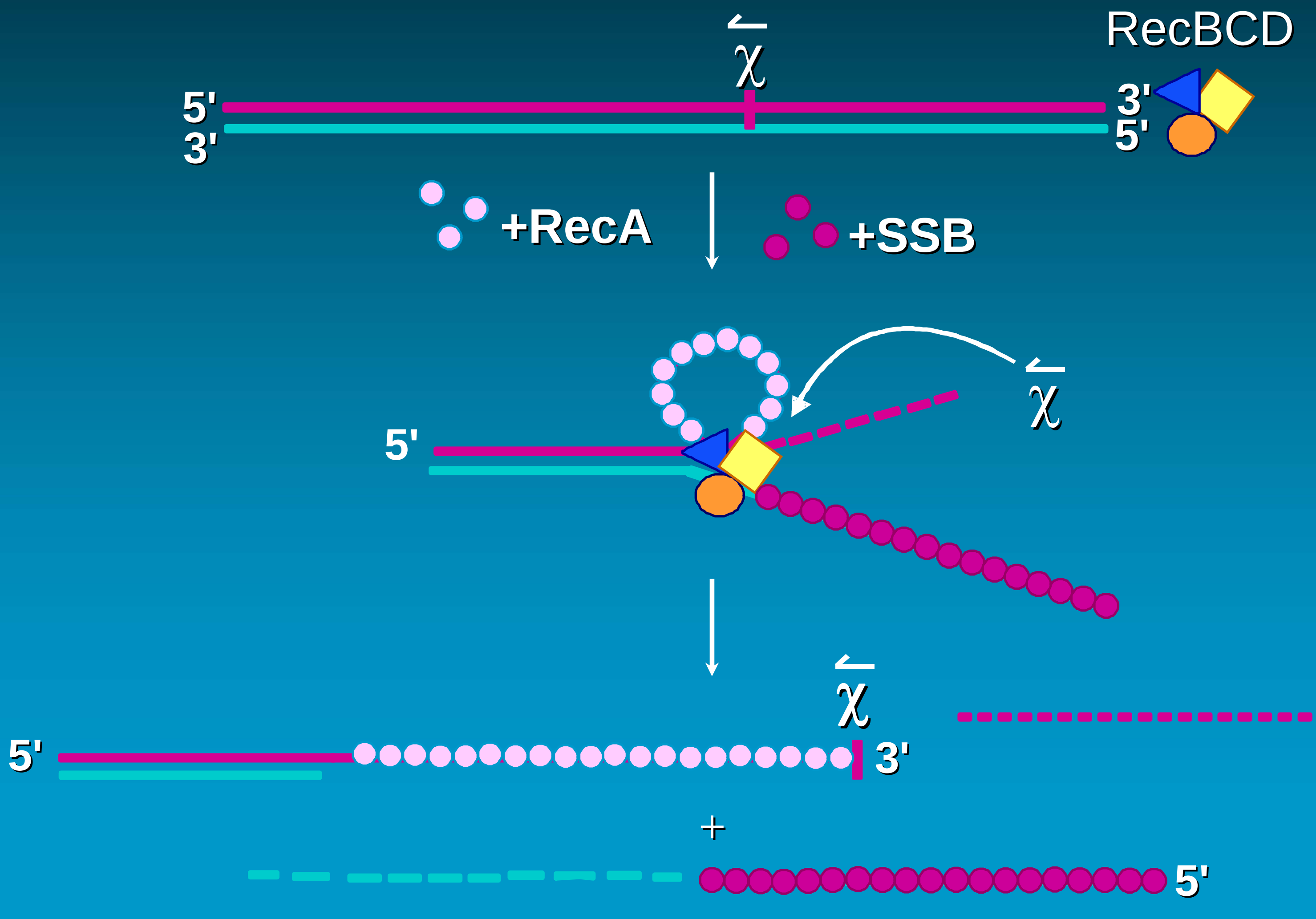
Upon recognition of χ:

1) The vigorous 3′ to 5′ nuclease activity is attenuated.

2) A weaker 5′ to 3′ nuclease activity is up-regulated.

Thus, nuclease activity is <u>reduced</u>, and the polarity of DNA strand degradation is <u>switched</u>.

χ Both Regulates the Nuclease Activity of RecBCD Enzyme and Coordinates the Loading of RecA Protein Onto the χ-Containing ssDNA



Functionally Similar Recombinational Repair Pathways

RecF(OR)-pathway ≈ Rad52-epistasis group

Common "Myths" Associated with the RecF-Pathway of Recombination

- Myth #1: Because 95-99% of conjugal recombination occurs *via* the RecBCD-pathway, this *must* mean the RecF-pathway is just a minor recombinational repair pathway in *E. coli*.
- ***Wrong!***
- Why? Because the RecBCD-pathway is responsible for all ***dsDNA-break repair***, whereas the RecF-pathway is responsible for ***all ssDNA-gap repair***.

Common "Myths" Associated with the RecF-Pathway of Recombination

- Myth #2: Because the RecF-pathway is responsible for all ssDNA-gap repair, that must mean it can't repair dsDNA breaks.
- ***Wrong!***
- Why? Because when the RecBCD-pathway is eliminated, suppressor mutations allow the RecF-pathway to repair ***all dsDNA-breaks***, with an efficiency comparable to wild-type cells.

Common “Myths” Associated with the RecF-Pathway of Recombination

- Myth #3: Because the RecF-pathway was regarded to be pathway of minor significance, which was specific only to bacteria, that meant that it couldn't be relevant to eukaryotes.
- ***Wrong!***
- Why? Because the enzymes of the RecF-pathway are biochemically similar to the Rad52-group of dsDNA break repair enzymes.

Common "Myths" Associated with the RecF-Pathway of Recombination

- Myth #4: Because the RecF-pathway was regarded to be pathway of minor significance and was thought to be specific to bacteria, not many people appreciated it.
- ***Unfortunately, this is true…***

E. coli RecO Protein

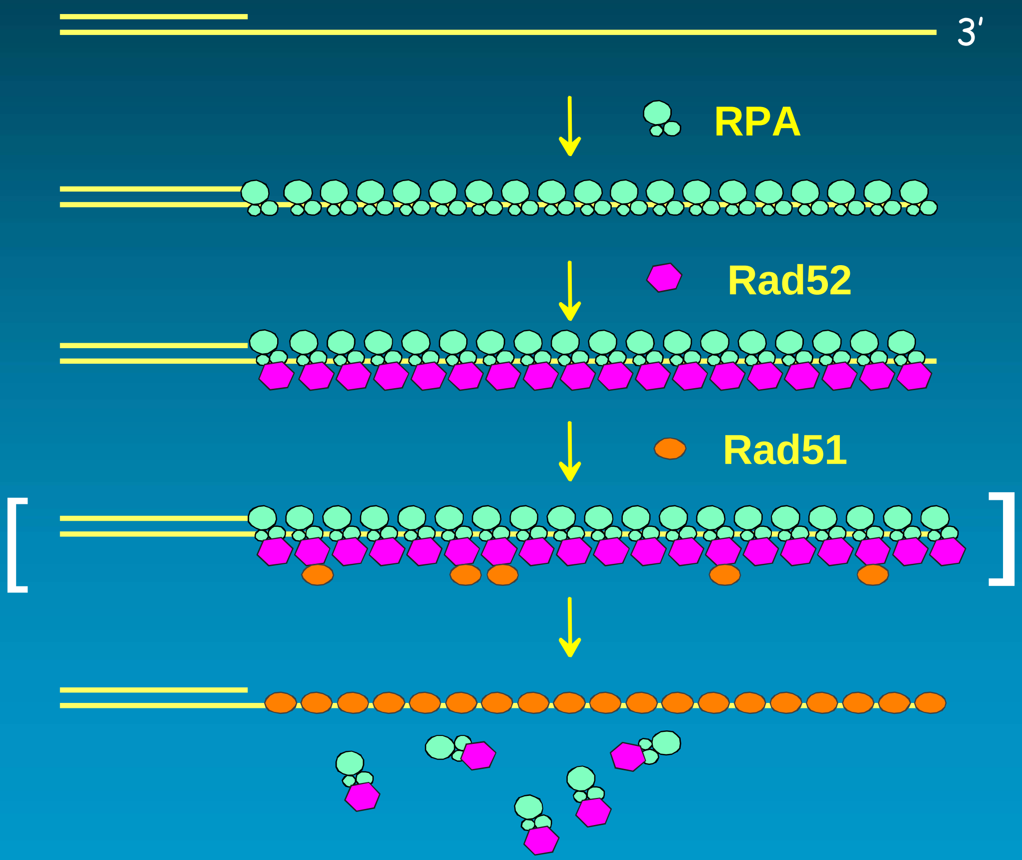
- Acts in the RecF-pathway of homologous recombination.
- Physically interacts with RecF, RecR, SSB proteins.
- Mediates replacement of SSB bound to ssDNA with RecA protein.
- Anneals ssDNA that is complexed with SSB protein.

RecO Protein Has Two Functions

- RecO is a “recombination mediator” protein: it promotes displacement of SSB by RecA to facilitate RecA nucleoprotein filament formation (Kolodner lab).
- RecO anneals ssDNA that is complexed with SSB: it promotes ssDNA annealing (SSA) and “second-end” capture in DSBR.

Kantake, N., Madiraju, M.V.V.M., Sugiyama, T., and Kowalczykowski, S.C. (2002) ***Proc. Natl. Acad. Sci. USA, 99***, 15327-15332

The Temporal Order of Presynaptic Complex Formation (*S. cerevisiae*)



Processed dsDNA with 3'-ssDNA overhang

RPA-ssDNA complex

Rad52 binds to the RPA-ssDNA complex

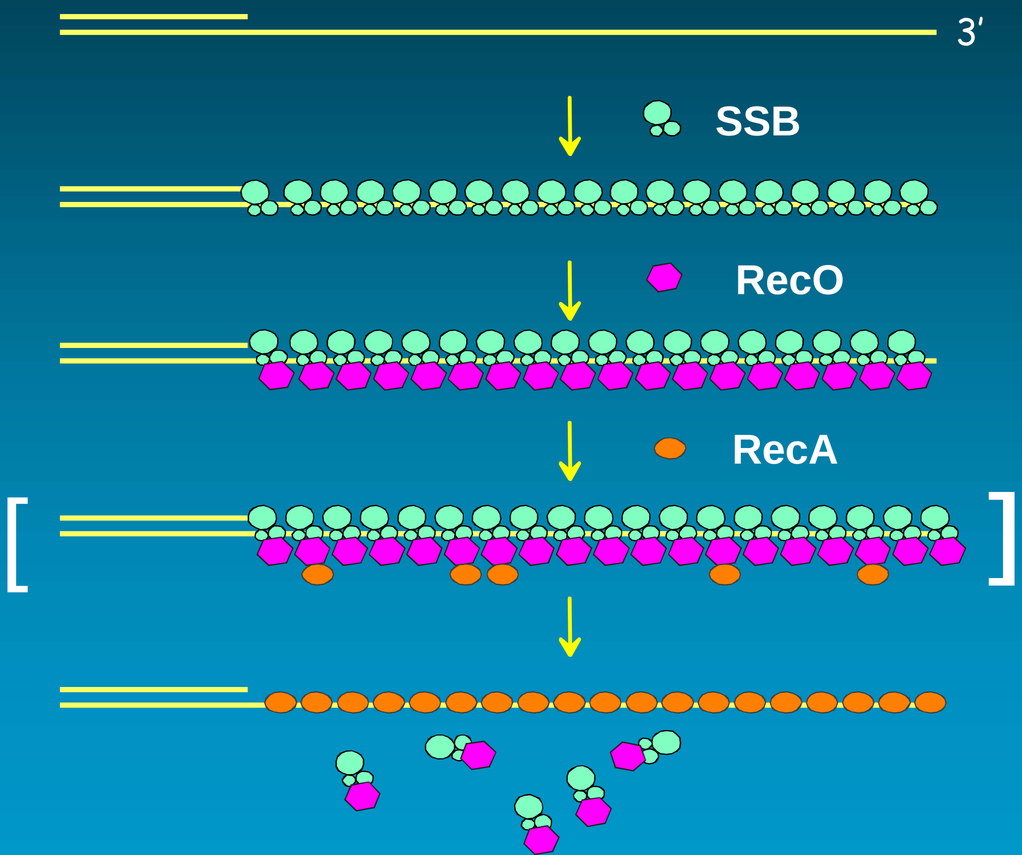
Rad51 is recruited by Rad52

Rad51 uniformly coats ssDNA to form the presynaptic complex

New, J.H., Sugiyama, T., Zaitseva, E., and Kowalczykowski, S.C. (1998) ***Nature, 391***, 407-410

Kowalczykowski, S.C. (2000). Some assembly required…. ***Nature Struct. Biol., 7,*** 1087-1089

The Temporal Order of Presynaptic Complex Formation (*E. coli*)



Processed dsDNA with 3’-ssDNA overhang

SSB-ssDNA complex

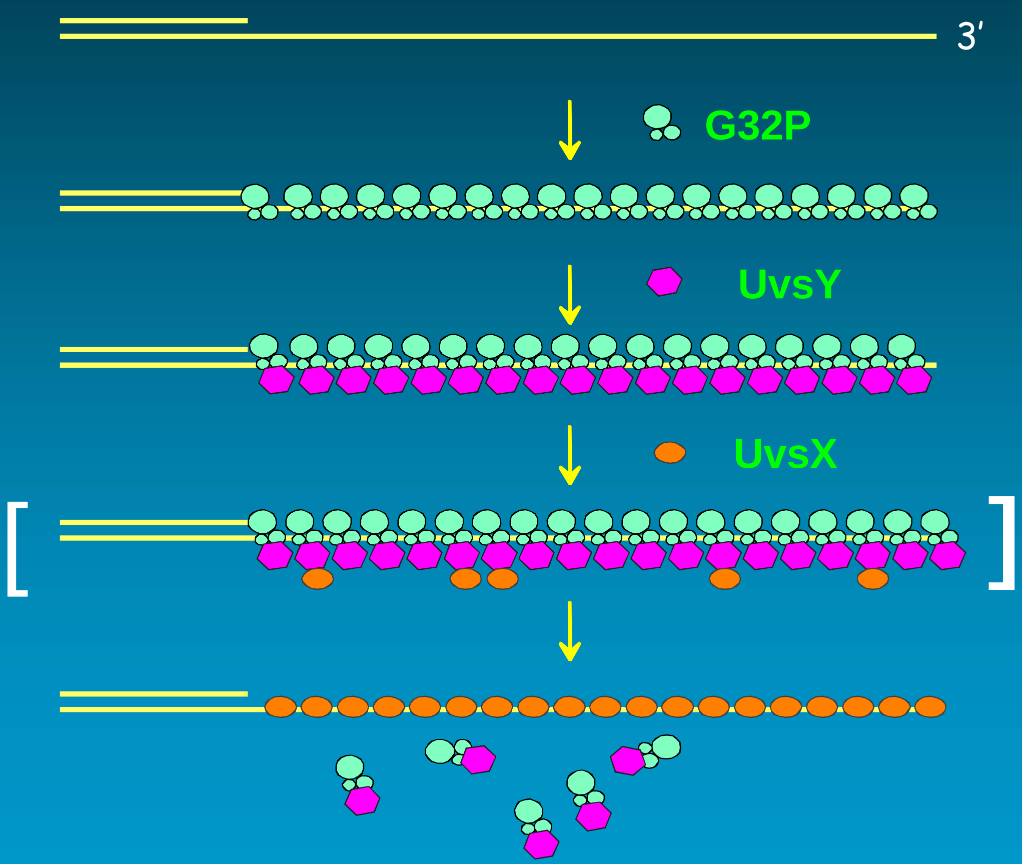
RecO binds to the SSB-ssDNA complex

RecA is recruited by RecO

RecA uniformly coats ssDNA
to form the presynaptic complex

Kolodner Lab

The Temporal Order of Presynaptic Complex Formation (T4 phage)



Processed dsDNA with 3’-ssDNA overhang

G32P-ssDNA complex

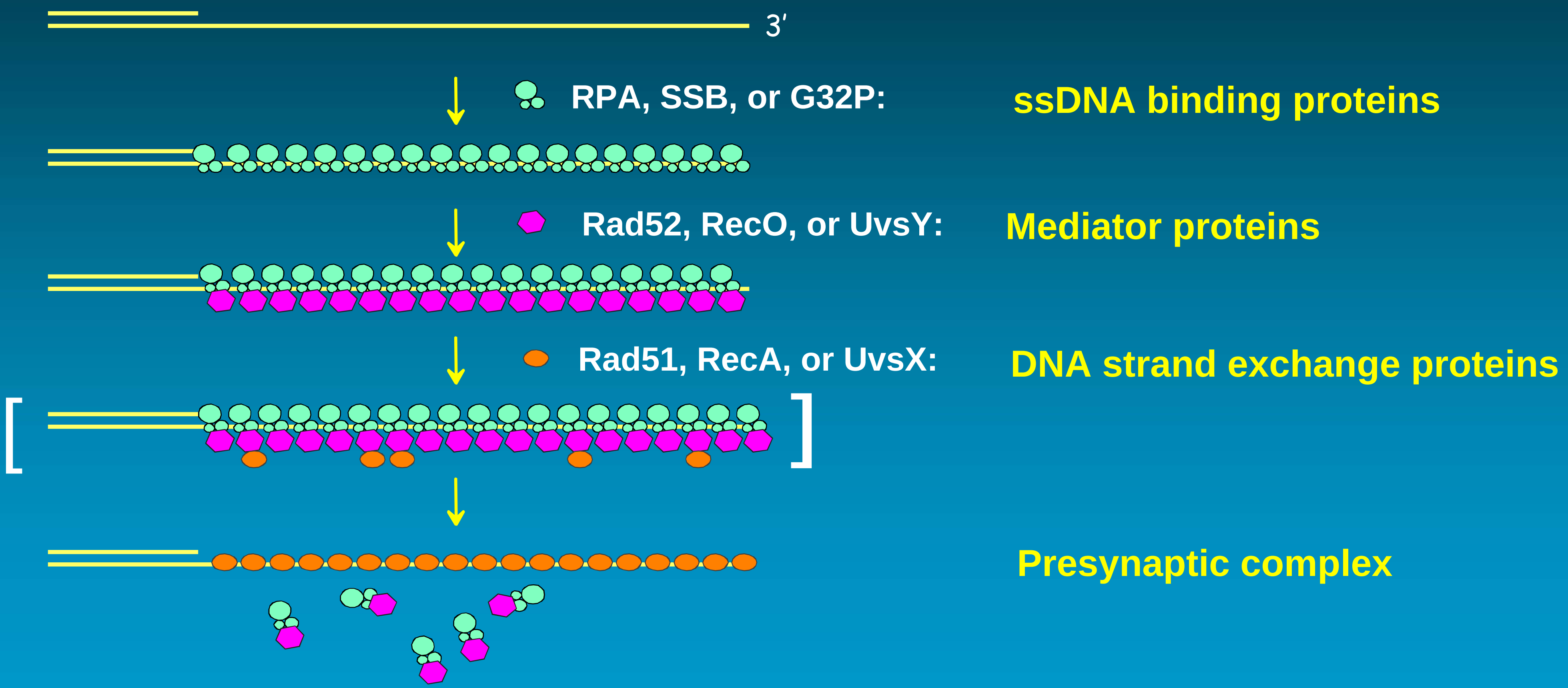
UvsY binds to the G32P-ssDNA complex

UvsX is recruited by UvsY

UvsX uniformly coats ssDNA to form the presynaptic complex

Morrical Lab

Mediator Proteins Promote the Exchange of an ssDNA-binding Protein for its Cognate DNA Strand Exchange Protein



Proteins that Mediate RecA/Rad51/UvsX Nucleoprotein Filament Formation *and* Promote Annealing of ssDNA Complexed with the Cognate SSB Protein

S. cerevisiae Rad52

E. coli RecO

T4-phage UvsY

Sugiyama, T., New, J.H., and Kowalczykowski, S.C. (1998). ***Proc. Natl. Acad. Sci. USA, 95,*** 6049-6054

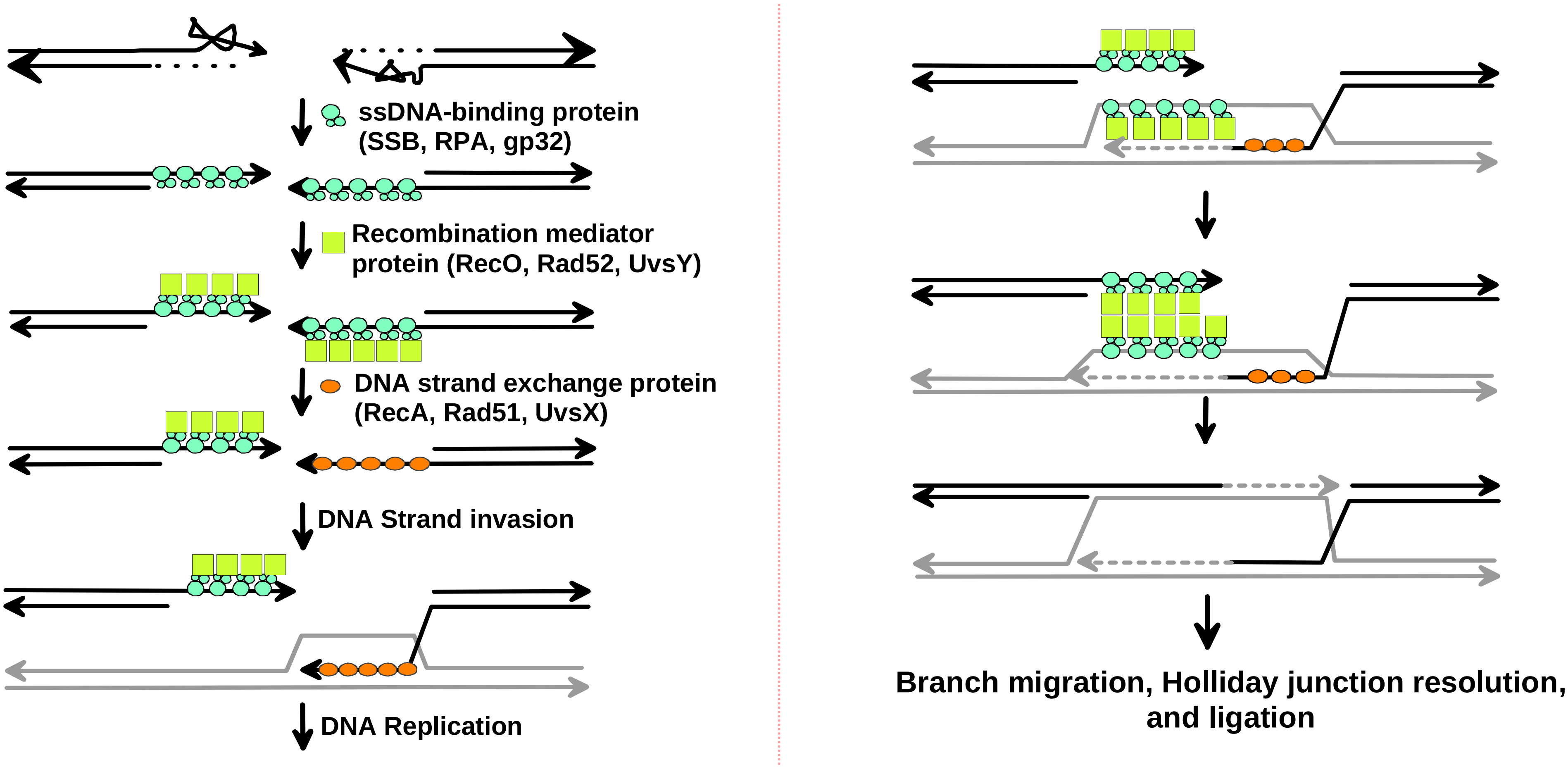
Kantake, N., Madiraju, M.V.V.M., Sugiyama, T., and Kowalczykowski, S.C. (2002). ***Proc. Natl. Acad. Sci. USA, 99***, 15327-15332

SSB-Displacing Proteins Are Also ssDNA Annealing Proteins

What is the biological significance of having SSB-displacement and DNA annealing functions in one protein?

Answer: SSB-displacement and DNA annealing are likely to be coupled events.

Annealing of an SSB-ssDNA Complex is a Universal Step in DSBR



Functional Counterparts in Bacteria, Eucarya, and Phage

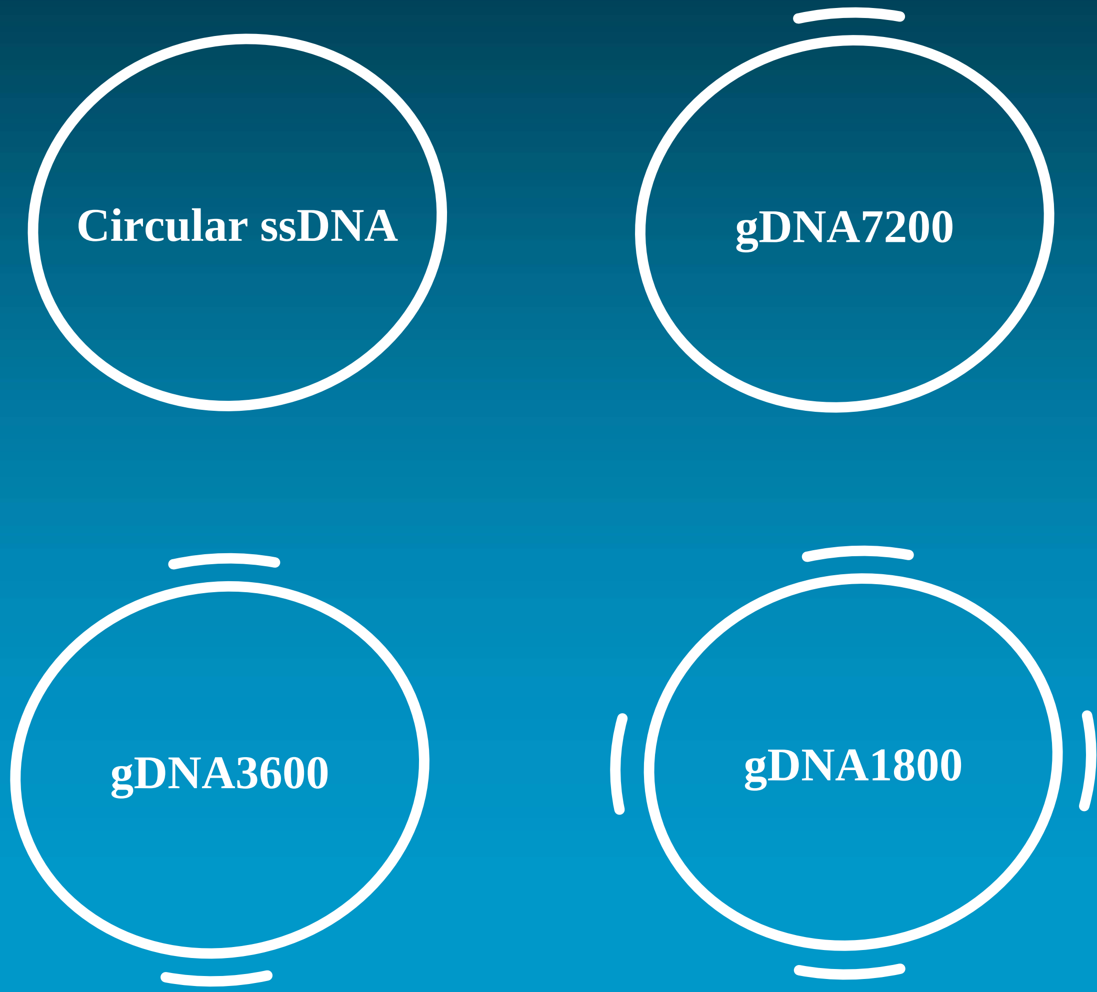
Function:	*S. cerevisiae*	*E. coli*	T4 phage
DNA strand exchange	**Rad51**	**RecA**	**UvsX**
ssDNA binding	**RPA**	**SSB**	**Gene 32p**
“Mediator” for presynaptic complex formation	**Rad52**	**RecO**	**UvsY**
DNA annealing	**Rad52**	**RecO**	**UvsY**

RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: A universal step of recombinational repair

Morimatsu, K. and Kowalczykowski, S.C. (2003).

Mol. Cell, 11, 1337-1347

"Gapped" DNA Substrates



RecA-loading Activity Can Be Followed by Measuring ATPase Activity of RecA Protein

gDNA
+SSB protein

↓

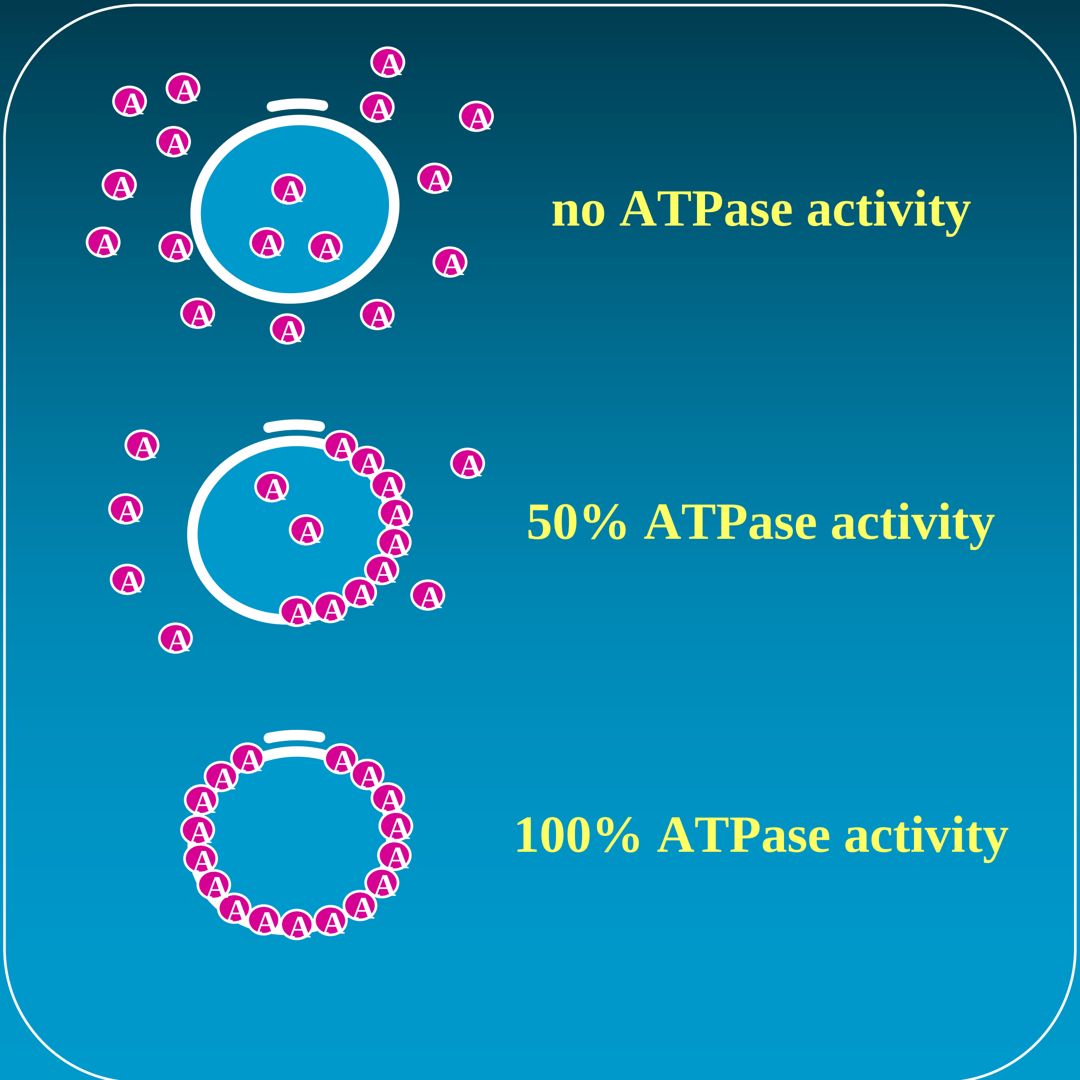
+RecFOR proteins

↓

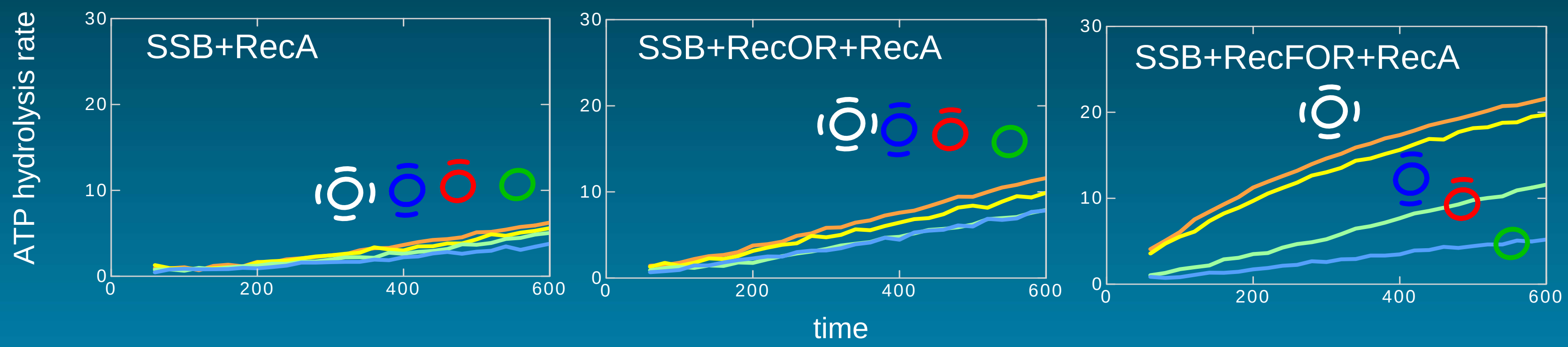
+RecA protein

↓

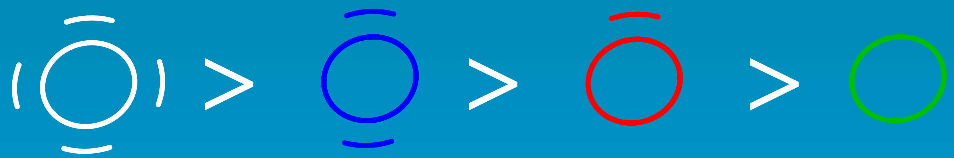
follow
RecA filament assembly
by measuring the DNA-dependent ATPase activity of RecA protein



The ssDNA-dsDNA Junction is the Site of RecFOR-Mediated Loading of RecA Protein

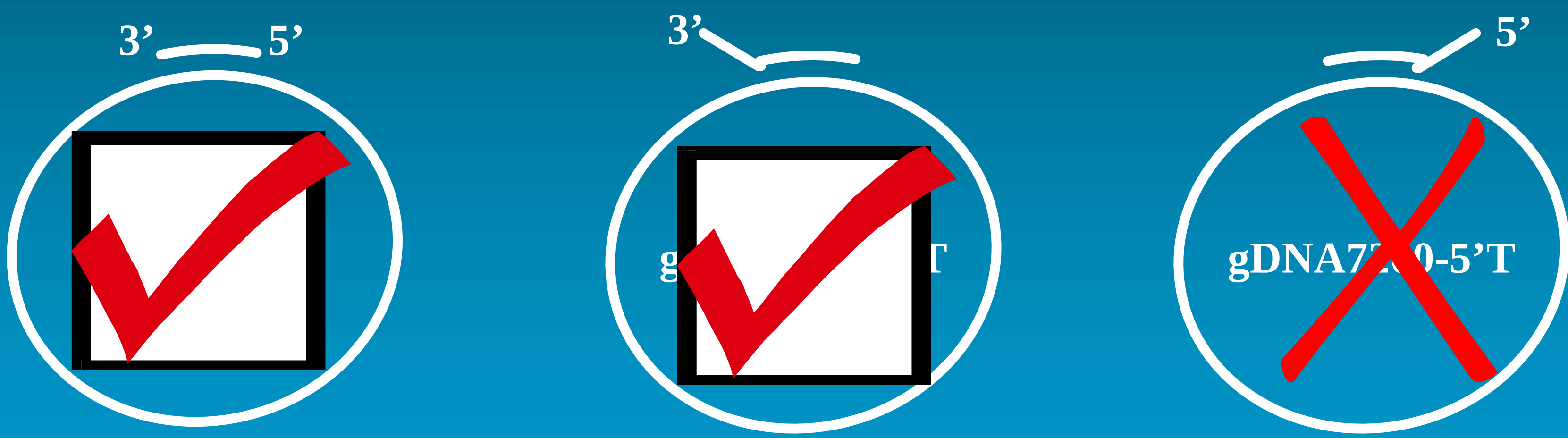


Loading efficiency:



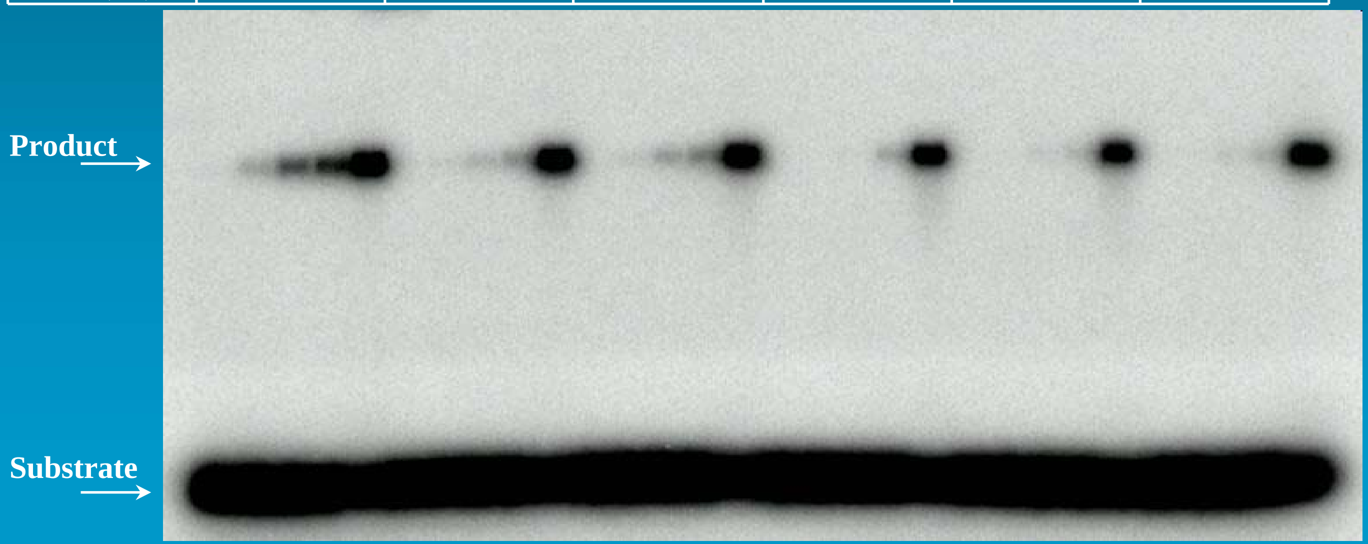
RecA-Loading by RecFOR proteins Requires a Complementary 5’-End

Possible recognition sites:



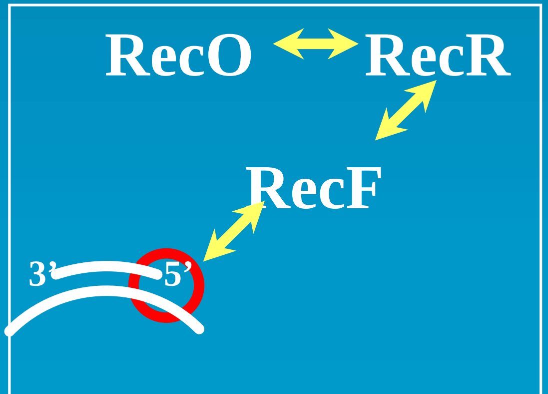
RecFOR Proteins Accelerate DNA Strand Exchange by RecA Protein Between SSB-gDNA and dsDNA

DNA	gDNA					ssDNA
SSB	+	+	+	+	+	+
RecF	+	-	-	+	+	+
RecO	+	-	+	-	+	+
RecR	+	-	+	+	-	+
RecA	+	+	+	+	+	+
Time (min)	0 3 6 9 90	0 3 6 9 90	0 3 6 9 90	0 3 6 9 90	0 3 6 9 90	0 3 6 9 90

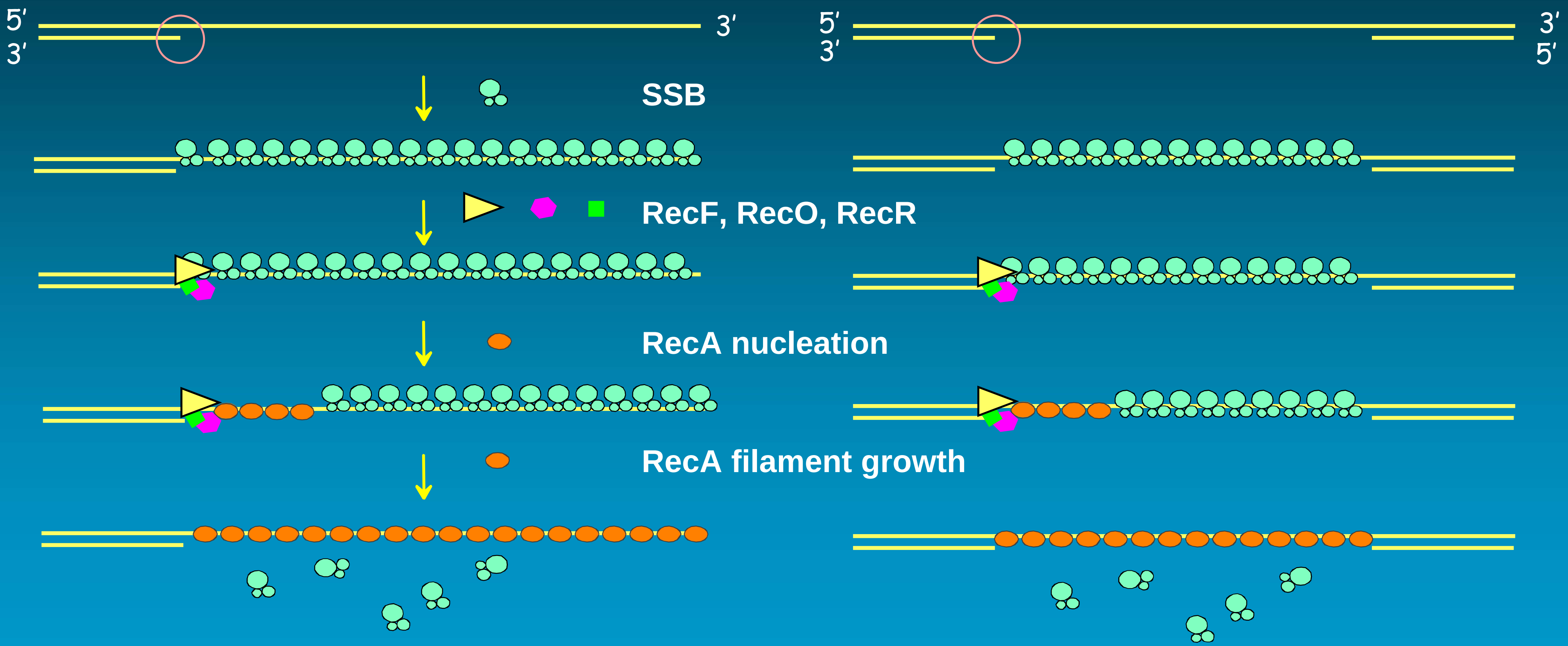


The RecFOR Proteins Load RecA Protein Specifically onto Gapped DNA

- RecF, RecO, and RecR proteins are all required for this loading reaction.
- The interactions between RecFOR and both RecA and SSB are species-specific.
- RecFOR proteins recognizes a base-paired 5’-end at ssDNA-dsDNA junction.
- RecF protein binds to the ssDNA-dsDNA junction.
- RecR protein binds to the RecF protein.
- RecO protein binds the RecR protein.



The RecFOR Complex Binds to the 5’-end of a dsDNA-ssDNA Junction to Nucleate RecA Assembly



Functionally Similar Recombinational Repair Pathways

	E. coli RecF-pathway	*S. cerevisiae* Rad52-epistasis group	Human Rad52-group
Initiation	SbcCD RecQ RecJ UvrD	Mre11/Rad50/Xrs2 Sgs1 ExoI Srs2	Mre11/Rad50/Nbs1 RecQL/4/5 BLM/WRN ExoI --
Homologous Pairing & DNA Strand Exchange	RecA SSB RecF(R) RecO	Rad51 RPA Rad55/57 Rad52	Rad51 RPA Rad51B/C/D/Xrcc2/3 Rad52
DNA Heteroduplex Extension	RuvAB RecQ	Rad54 Sgs1	Rad54 RecQL/4/5 BLM/WRN
Resolution	RuvC	Mus81/Mms4/?	Mus81/Mms4/?

Functionally Similar Recombinational Repair Pathways

RecF(OR)-pathway ≈ Rad52-epistasis group

Bacterial/Archaeal/Yeast/Mammalian Functional Homologs and Orthologs

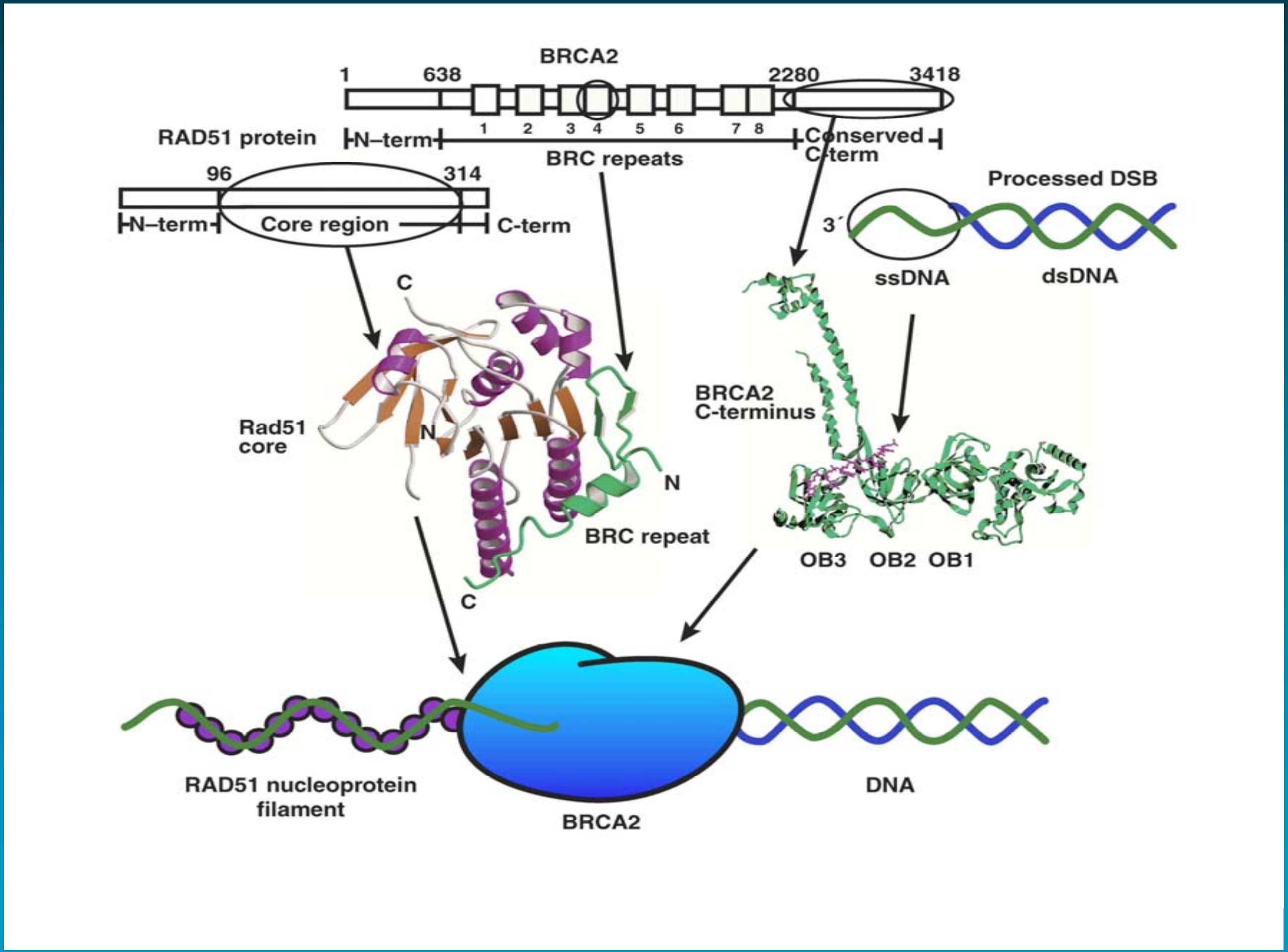
RecF
RecR

RadB/
RadB2

Rad55
Rad57

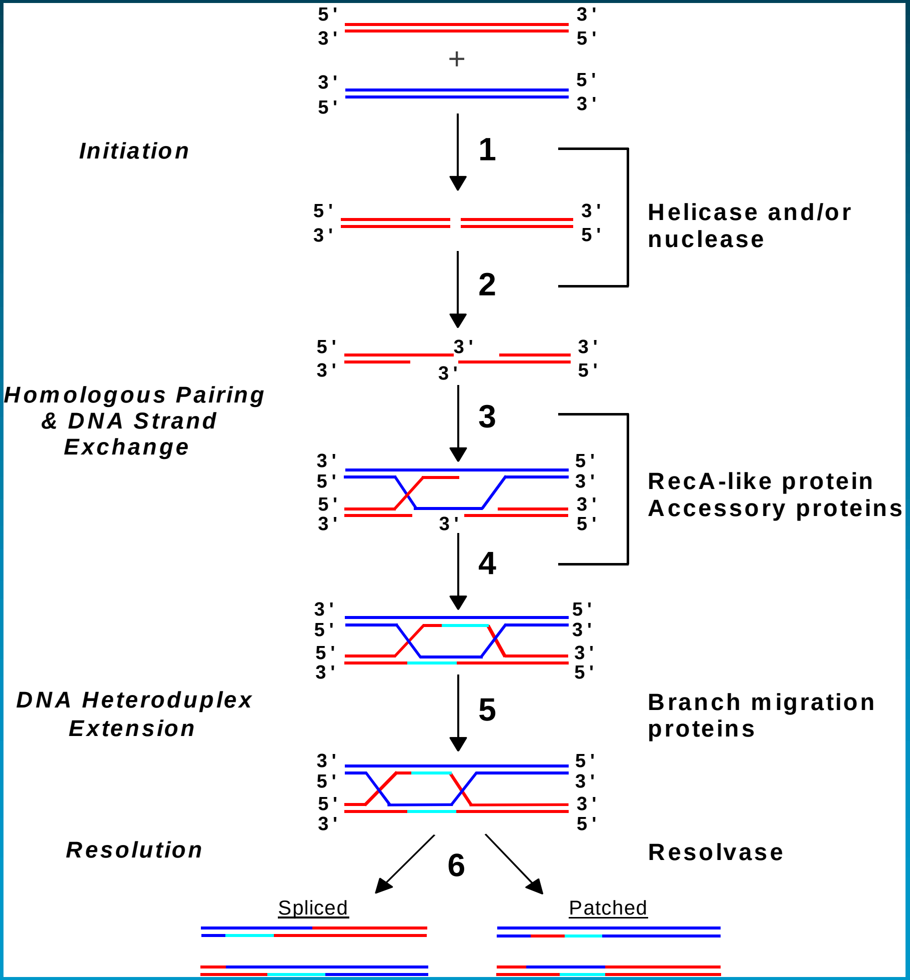
Rad51B
Rad51C
Rad51D
Xrcc2
Xrcc3

Is Brca2 a Mediator Protein, that Functions Similarly to the Rad51 Paralogs?



Kowalczykowski, S.C. (2002) ***Nature Struct. Biol., 9,*** 897-899

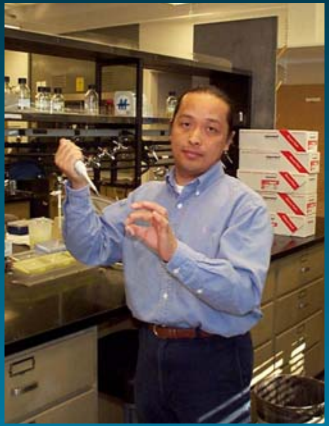
Proteins Involved in Recombinational DNA Repair

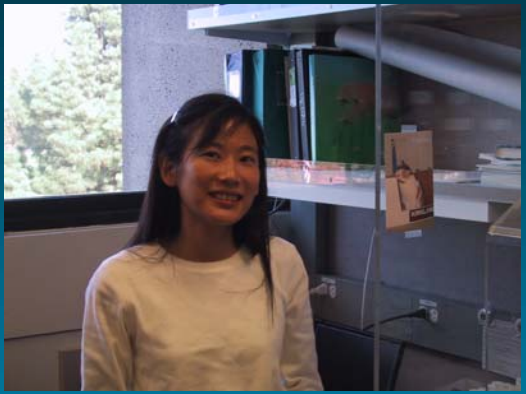


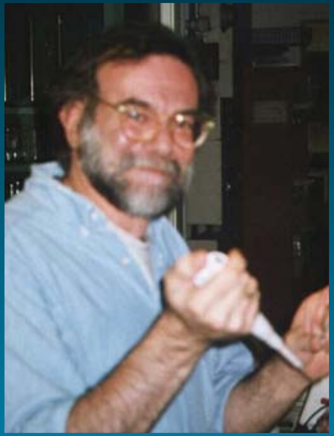
E. coli	*S. cerevisiae*	Human
RecBCD	--	--
SbcCD	Mre11/Rad50/Xrs2	Mre11/Rad50/Nbs1
RecQ	Sgs1(?)	RecQ1/4/5/BLM/WRN(?)
RecJ	ExoI	ExoI
UvrD	Srs2	--
RecA	Rad51	Rad51
SSB	RPA	RPA
RecF(R)	Rad55/57	Rad51B/C/D/Xrcc2/3
RecO	Rad52	Rad52
--	Rad59	--
--	Rad54/Rdh54	Rad54/54B
--	--	Brca2
RuvAB	Rad54	Rad54
RecG	--	--
RecQ	Sgs1(?)	RecQL/4/5/BLM/WRN(?)
RuvC	--	--
--	Mus81/Mms4(?)	Mus81/Mms4(?)

The People Who Did the Work:


Alex Mazin


Katsumi Morimatsu


Yun Wu


Jim New


Cynthia Haseltine


Tomohiko Sugiyama


Noriko Kantake


Andrei Alexeev