HUNPROTEXC

BE THERE ON TIME AND DO THE RIGHT THING: NOVEL MECHANISMS OF GENOME STABILITY

MIHÁLY KOVÁCS

NEMZETI KUTATÁSI, FEJLESZTÉSI ÉS INNOVÁCIÓS HIVATAL

AZ INNOVÁCIÓ LENDÜLETE

AZ NKFI ALAPBÓL MEGVALÓSULÓ PROGRAM

Be there on time and do the right thing: novel mechanisms of genome stability

Mihály Kovács

ELTE-MTA „Momentum” Motor Enzymology Research Group

Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

www.mk-lab.org

HunProtExc Conference

23.11.2019

1. Mihez kezdjünk az invázióval?

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2. Hogyan lehet hasznos az „egy lépést előre, kettőt hátra” stratégia?

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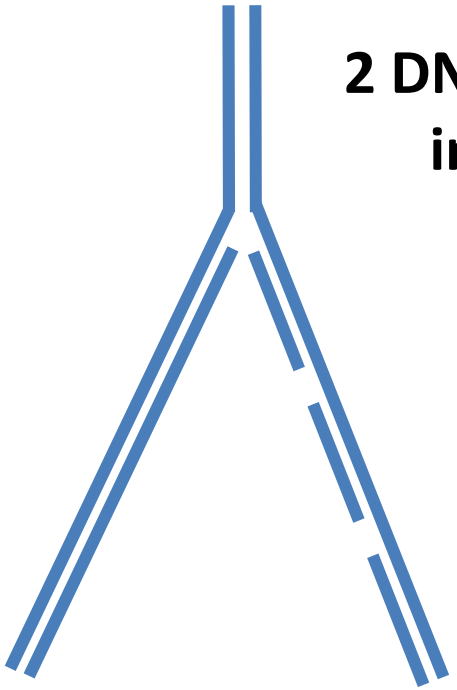
6. Hogyan lőhetünk célzottan egyszerre 18 mélyedésbe, és hogyan szolgálhatja ez az emberiséget?

There is no life without this



2 DNA strands interact

REPLICATION

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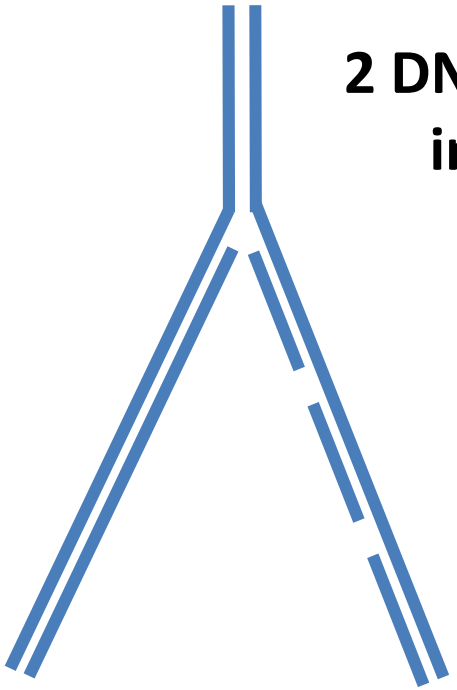

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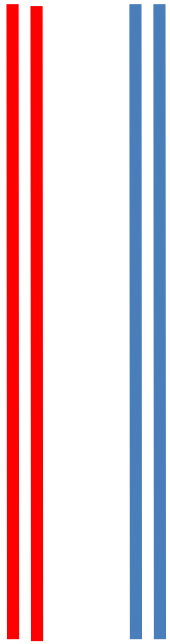

2 DNA duplexes interact

REPLICATION

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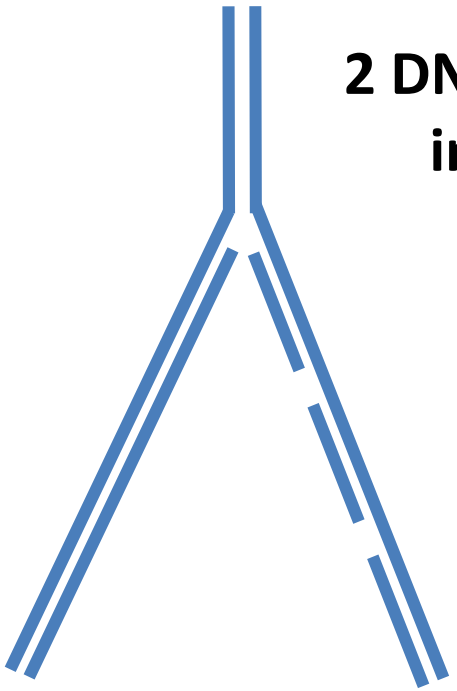
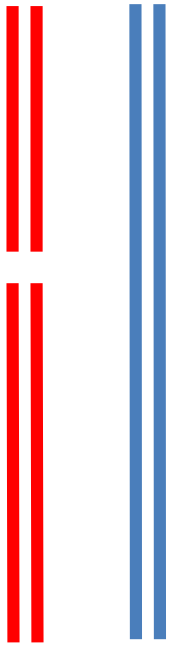


2 DNA strands interact

DNA strands break



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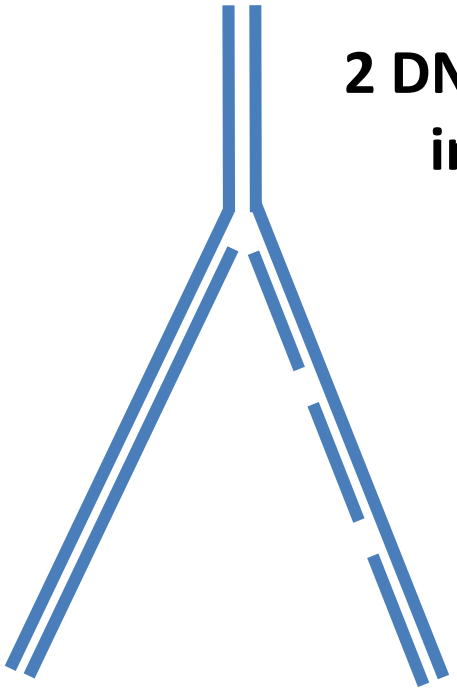
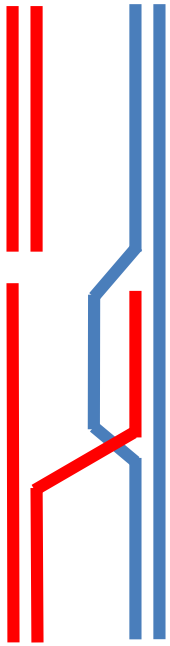


2 DNA strands interact

DNA strands break invade



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REPLICATION

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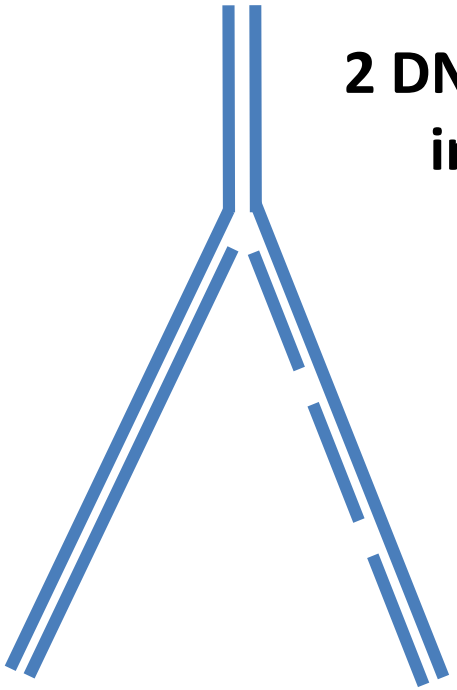
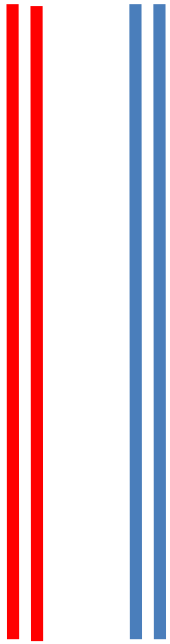


2 DNA strands
interact

DNA strands break
invade
and then get repaired



2 DNA duplexes
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REPLICATION

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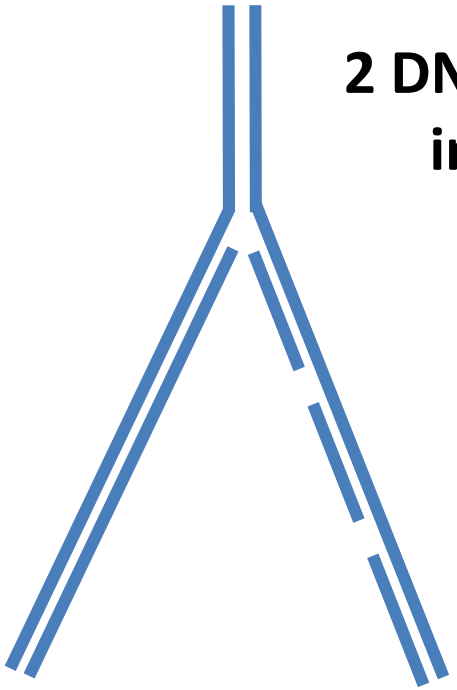

2 DNA strands
interact

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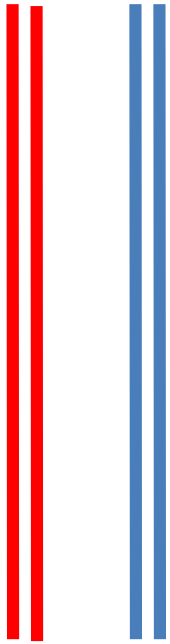
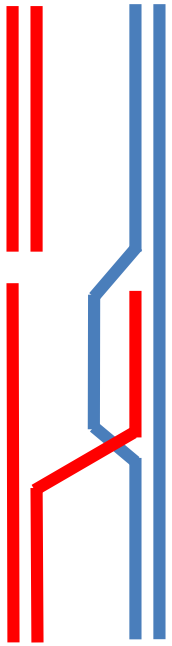


2 DNA duplexes
interact

REPLICATION

HOMOLOGOUS
RECOMBINATION
(HR)

There is no life without this



2 DNA duplexes interact

HR maintains genome integrity
Restart of stalled replication
Repair of DNA breaks
Chromosome segregation Generation of new combinations

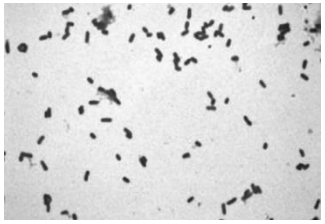
HOMOLOGOUS RECOMBINATION (HR)

Why is HR crucial?

HR

Bacteria

- Spread of antibiotic resistance (horizontal gene transfer)
- Target for antibiotics

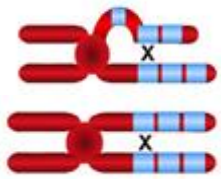


Genome engineering

- Induced DNA breaks (e.g. CRISPR/Cas9): trigger HR mechanisms for repair

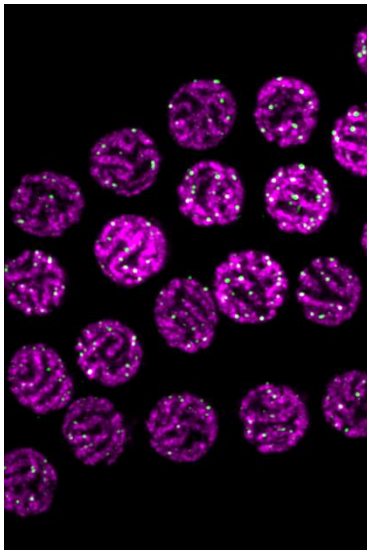
Eukaryotes (somatic)

- Carcinogenesis (HR quality control)
- Alternative lengthening of telomeres (resistance to radiotherapy)



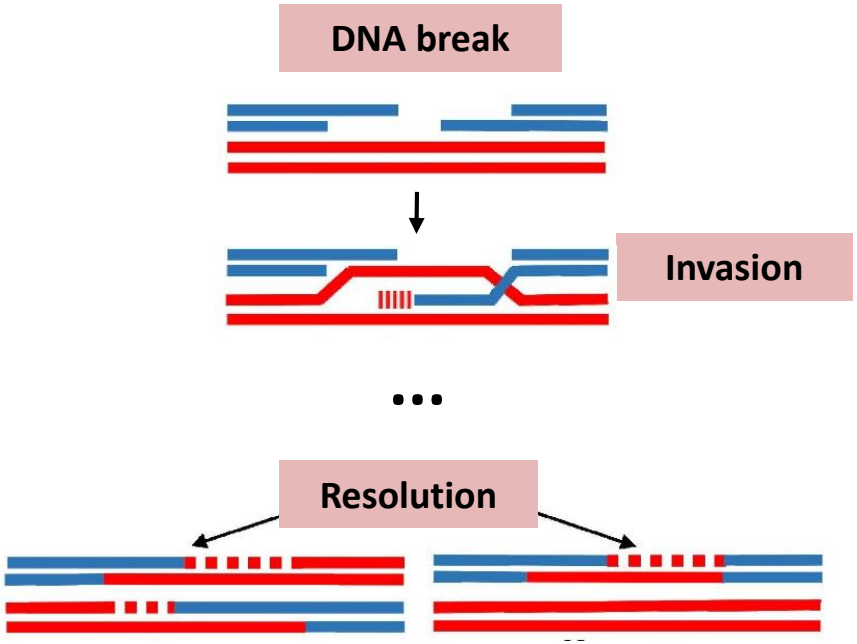
Eukaryotes (germ line)

- Meiotic chromosome segregation
- Infertility
- Aneuploidy



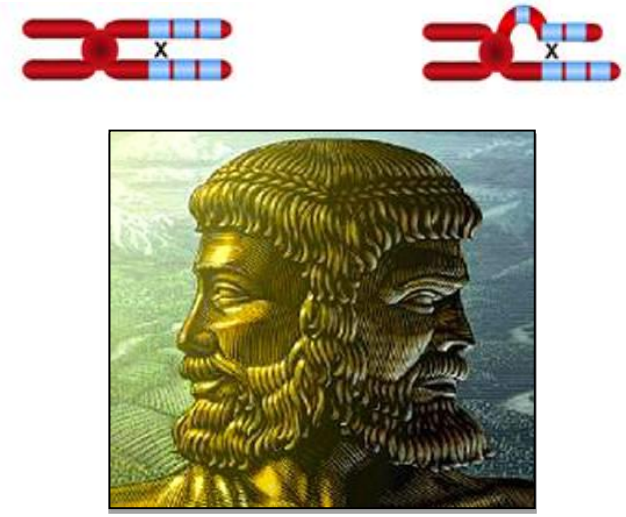
Martina et al., unpublished

But… HR comes with risks



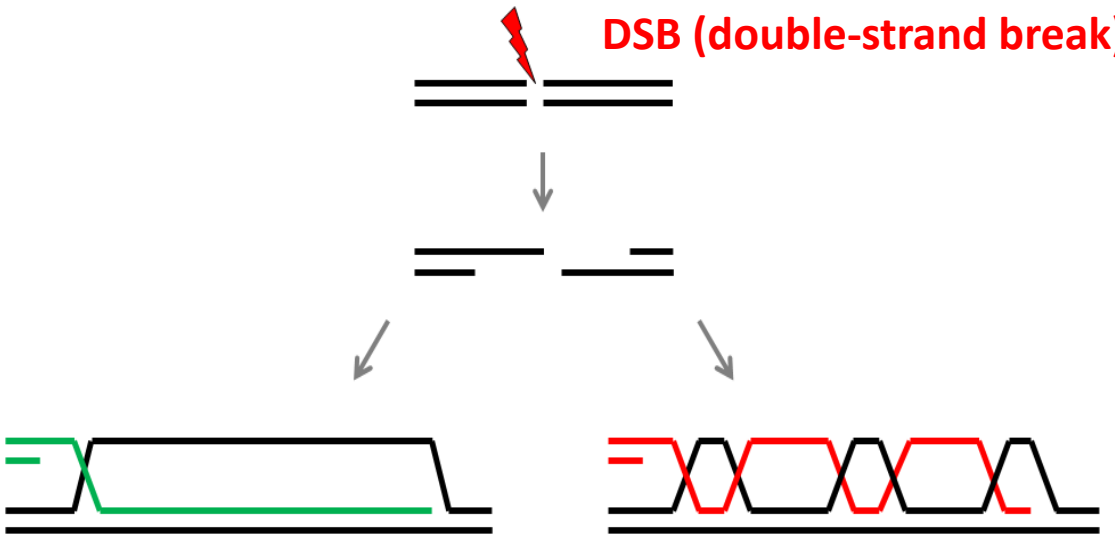
crossover *noncrossover*

Legitimate (allelic) **Illegitimate**



Harmful genomic rearrangements
Cell death
Cancer

Central role of HR quality control



Error-free repair

Genome rearrangements
Cell death / CANCER

How are these distinguished and differentially processed?

LEGITIMATE recombination

ILLEGITIMATE recombination

How are these distinguished and differentially processed?

LEGITIMATE
recombination

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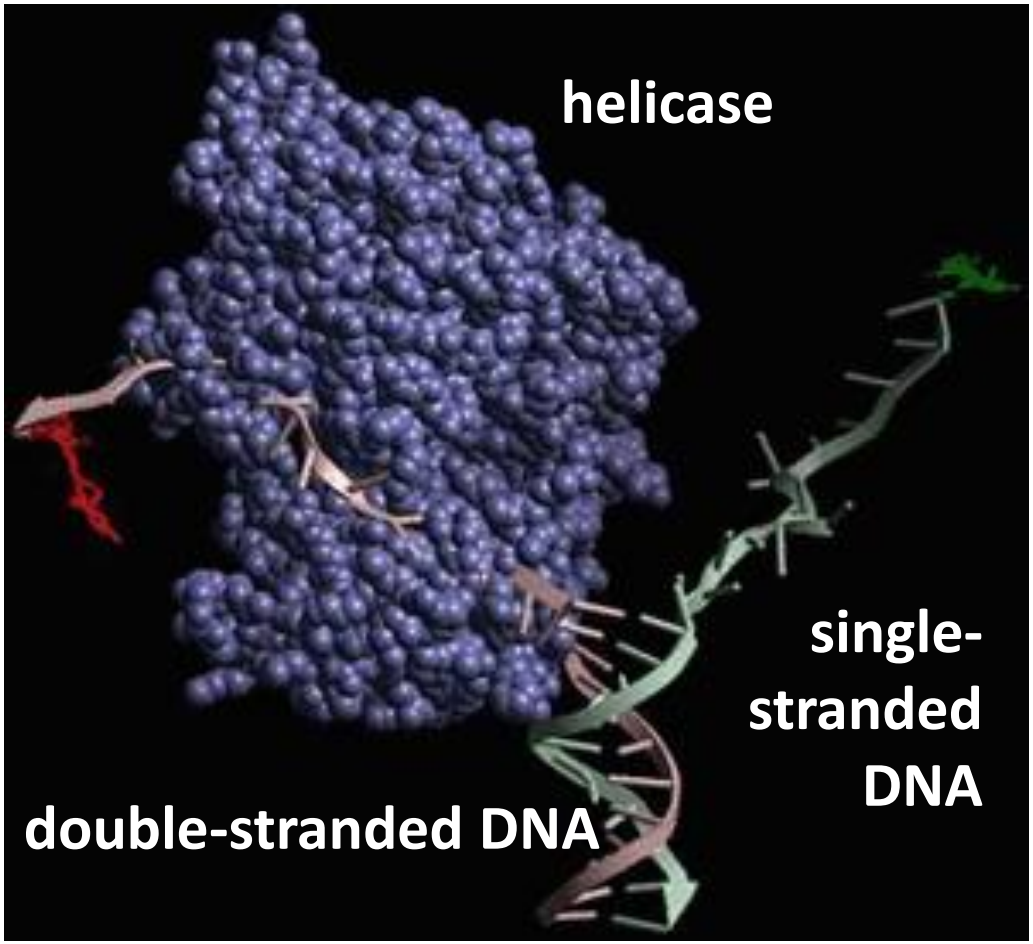
recombination

There is no obvious structural determinant for usual mechanisms of molecular recognition

Helicases unwind DNA duplexes



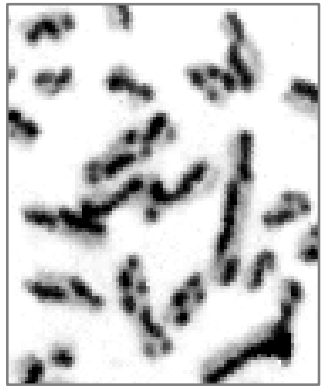
RecQ-family helicases are the „guardian angels” of the genome

Conserved from bacteria to humans

E. coli	RecQ
Human	RECQ1 BLM (Bloom’s syndrome) WRN (Werner’s syndrome) RECQ4 (Rothmund-Thompson syndrome) RECQ5

RecQ deficiencies -> hyperrecombination
CANCER PREDISPOSITION
ACCELERATED AGEING

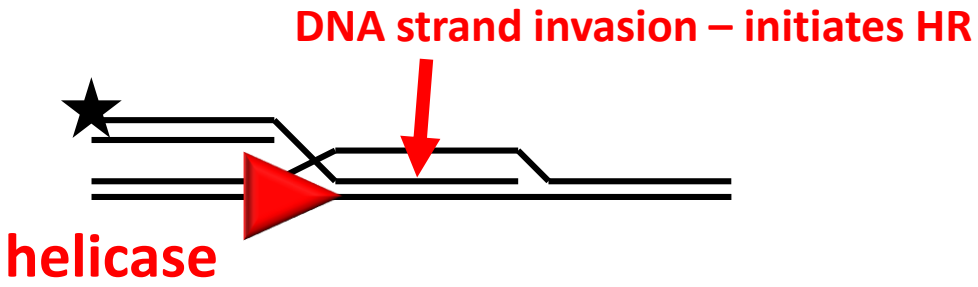
normal



deficient

Image: atlasgeneticsoncology.org

So… how do RecQ helicases control HR initiation?



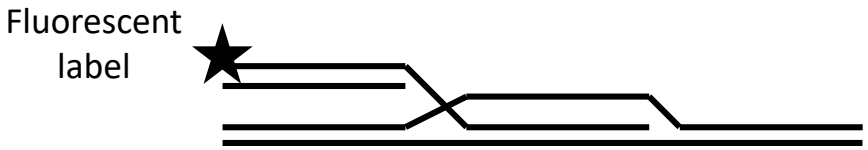
PNAS

Shuttling along DNA and directed processing of D-loops by RecQ helicase support quality control of homologous recombination

Gábor M. Harami[a,1], Yeonee Seol[b,1], Junghoon In[b], Veronika Ferencziová[a], Máté Martina[a], Máté Gyimesi[a], Kata Sarlós[a], Zoltán J. Kovács[a], Nikolett T. Nagy[a], Yuze Sun[b,2], Tibor Vellai[c], Keir C. Neuman[b,3], and Mihály Kovács[a,3]

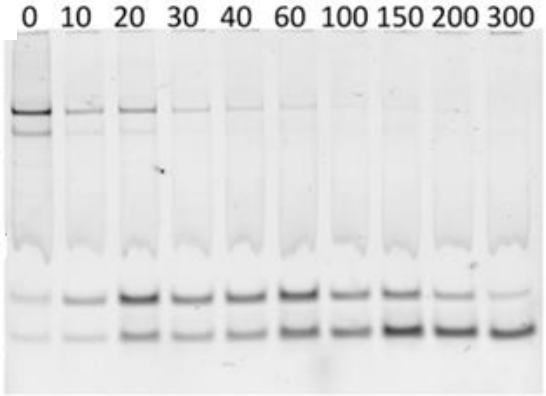
[a]Department of Biochemistry, Eötvös Loránd University–Hungarian Academy of Sciences "Momentum" Motor Enzymology Research Group, Eötvös Loránd University, H-1117 Budapest, Hungary; [b]Laboratory of Single Molecule Biophysics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892; and [c]Department of Genetics, Eötvös Loránd University, H-1117 Budapest, Hungary

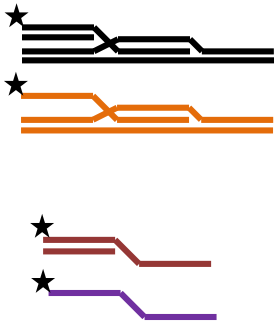
Monitoring the disruption of DNA strand invasions



Oligonucleotide-based D-loop

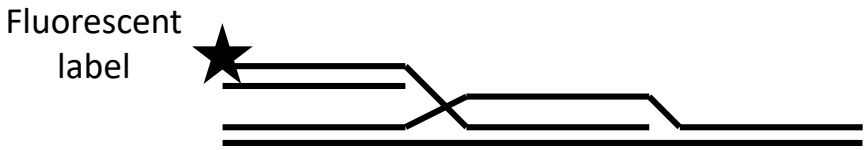
Reaction time (s)





Native PAGE of DNA species

Monitoring the disruption of DNA strand invasions



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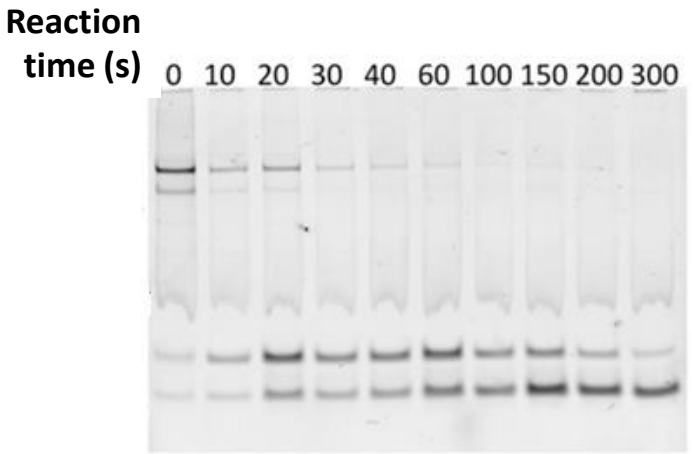

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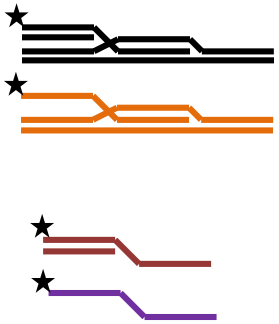
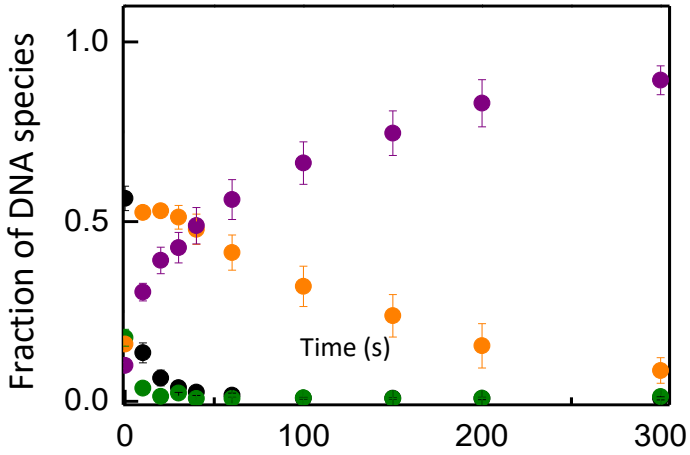
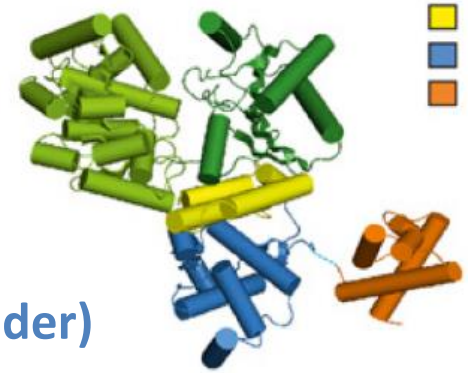




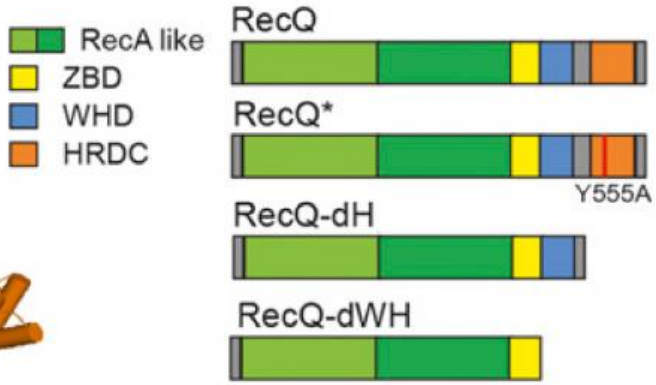
Transient kinetic analysis

RecQ helicase engineering

Motor core



WHD (dsDNA binder)



HRDC (ssDNA binder)

Review

Cell

From keys to bulldozers: expanding roles for winged helix domains in nucleic-acid-binding proteins

Gábor M. Harami, Máté Gyimesi, and Mihály Kovács

ELTE-MTA "Momentum" Motor Enzymology Research Group, Department of Biochemistry, Eötvös University, H-1117 Budapest, Pázmány P. stny. 1/C, Hungary

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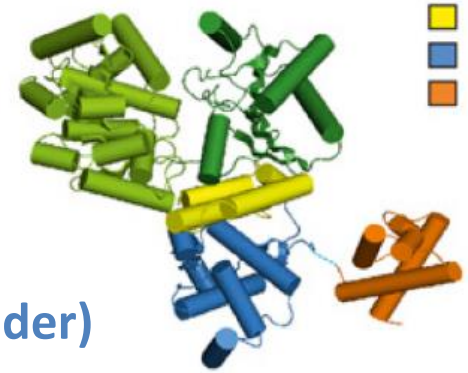
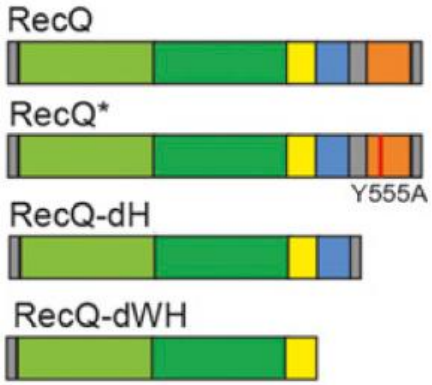
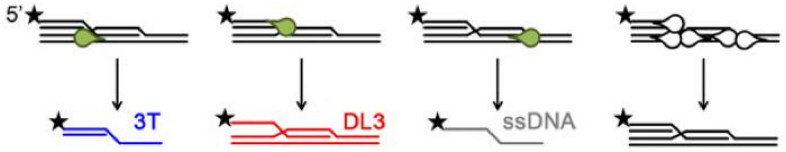
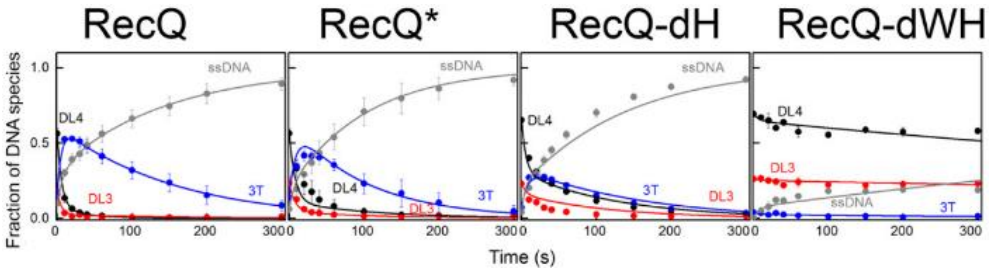


RecA like
ZBD
WHD
HRDC



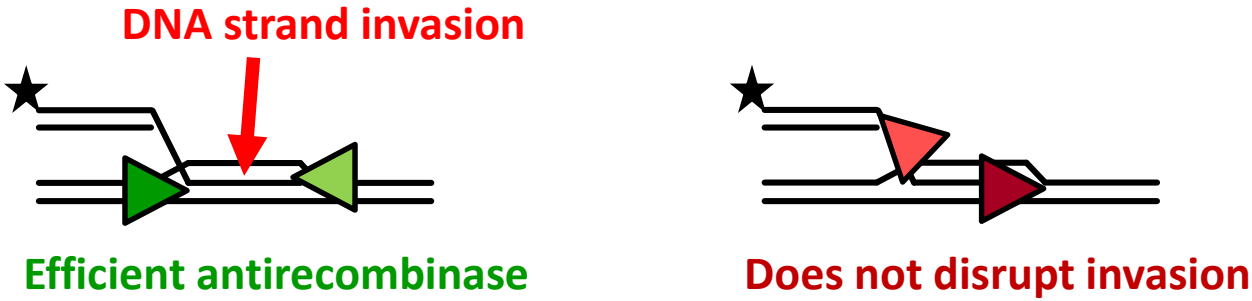
HRDC (ssDNA binder)

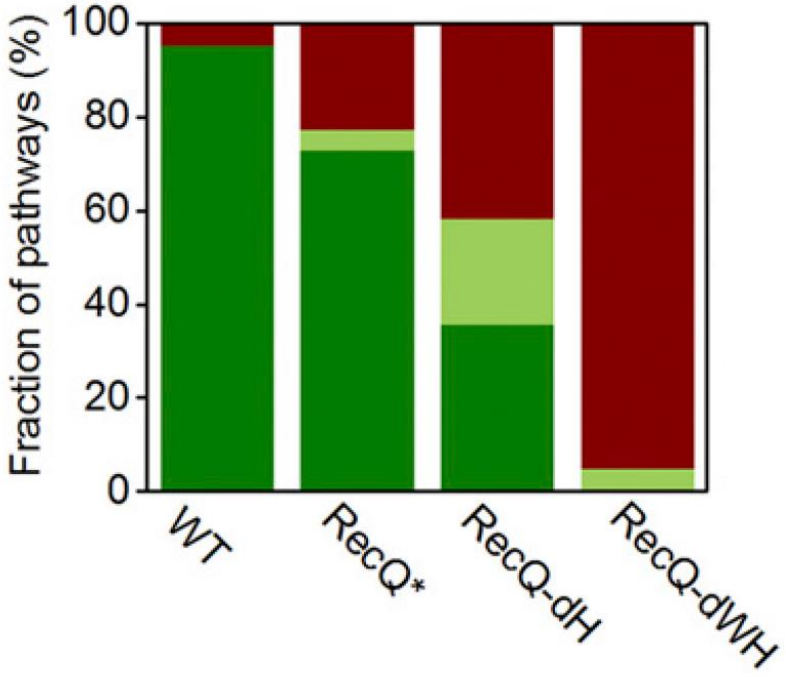




D-loop processing is specifically altered in helicase variants

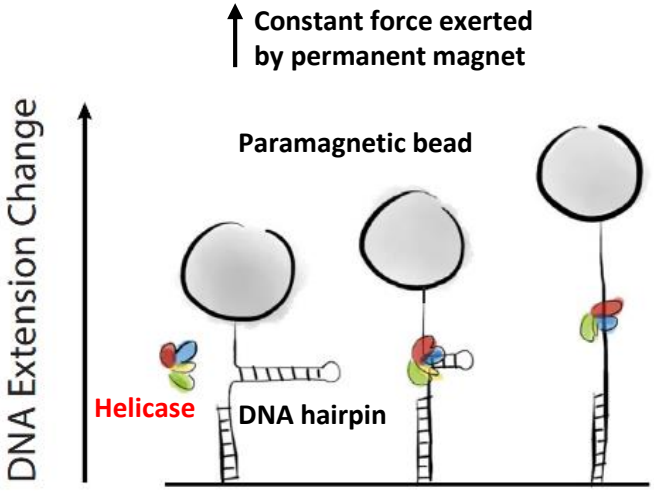
Invasion disruption can be engineered





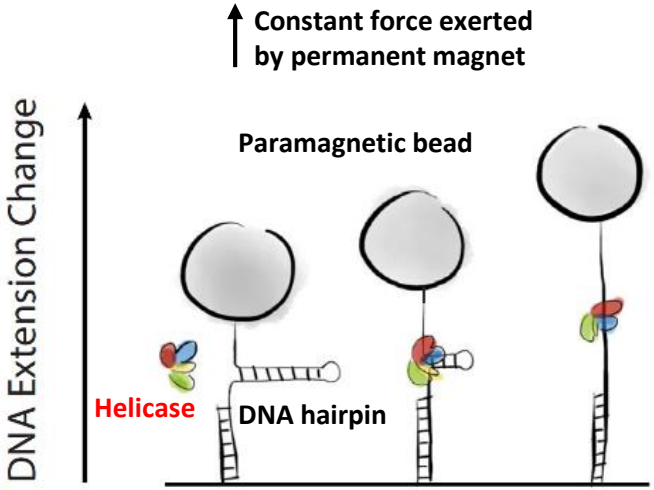
Taking a look at how DNA is unwound

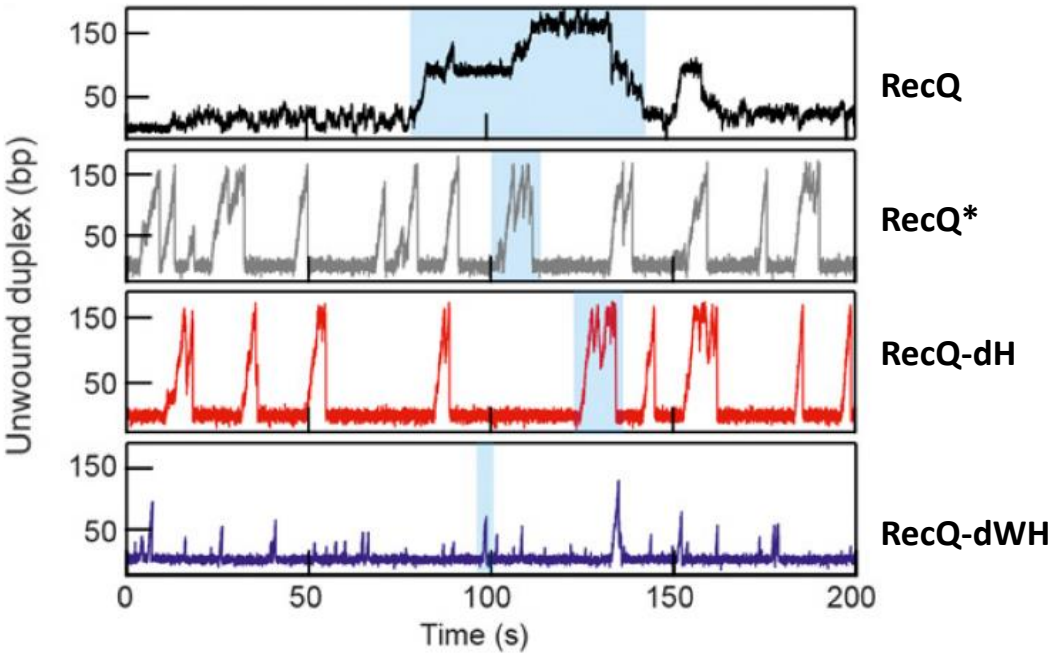
Magnetic tweezers experiment



RecQ helicase pauses and shuttles (performs repetitive local DNA unwinding)

Magnetic tweezers experiment





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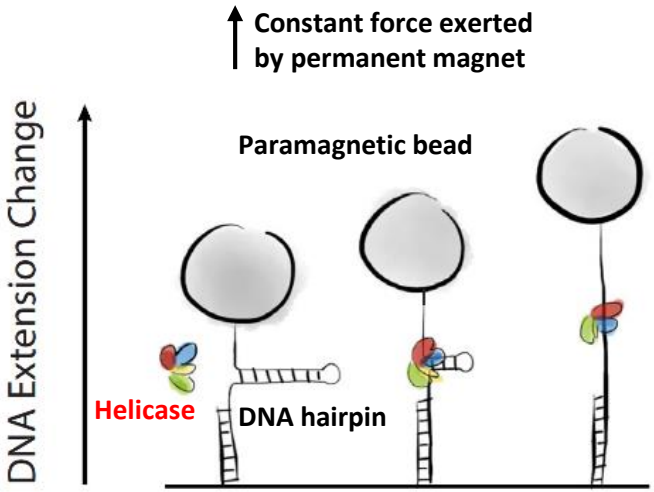
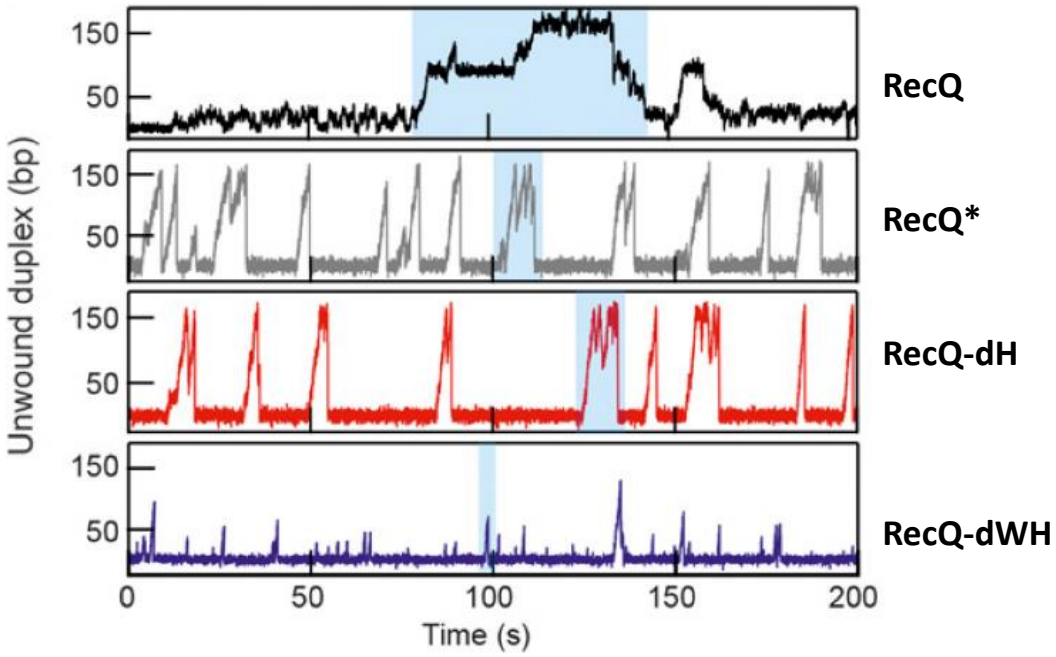
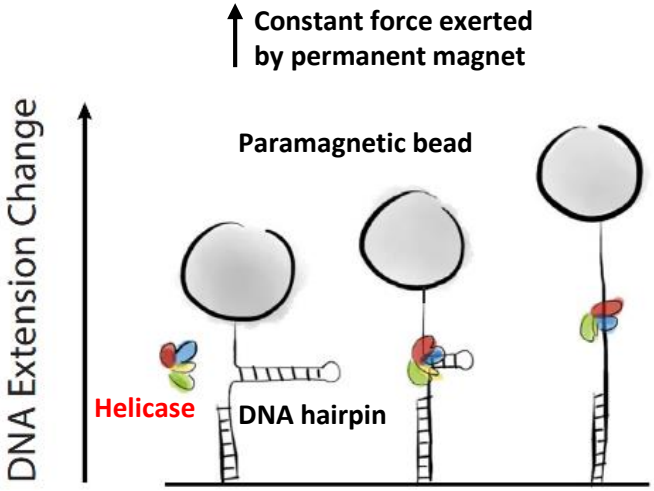


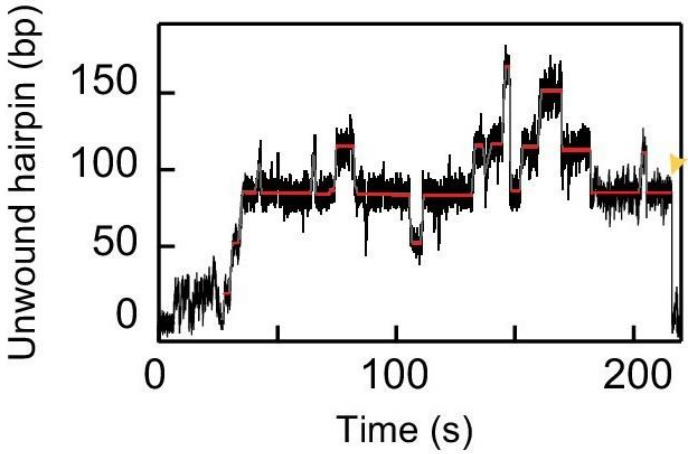


Shuttling can be engineered, too (independent of DNA unwinding)

Pauses don’t just occur anywhere

Magnetic tweezers experiment





eLIFE elifesciences.org

RESEARCH ARTICLE

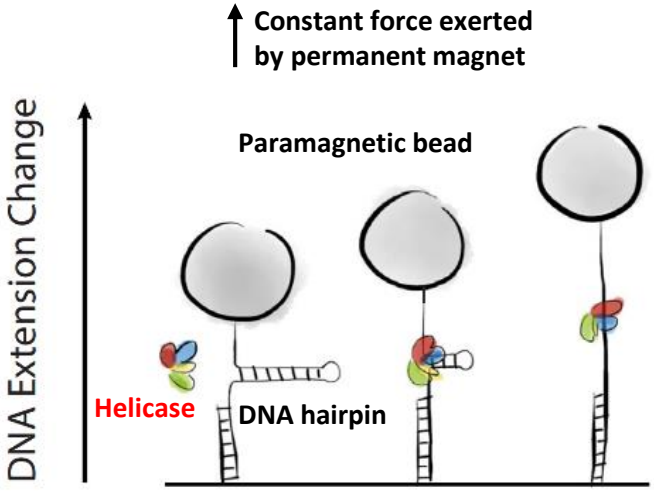
Homology sensing via non-linear amplification of sequence-dependent pausing by RecQ helicase

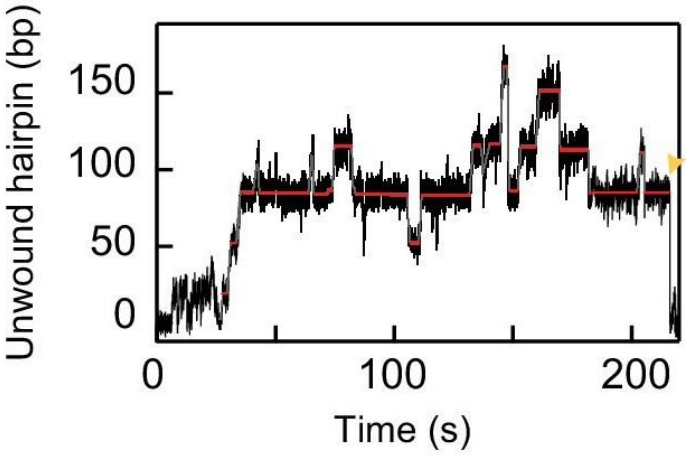
Yeonee Seol[1†], Gábor M Harami[2†], Mihály Kovács[2,3]*, Keir C Neuman[1]*

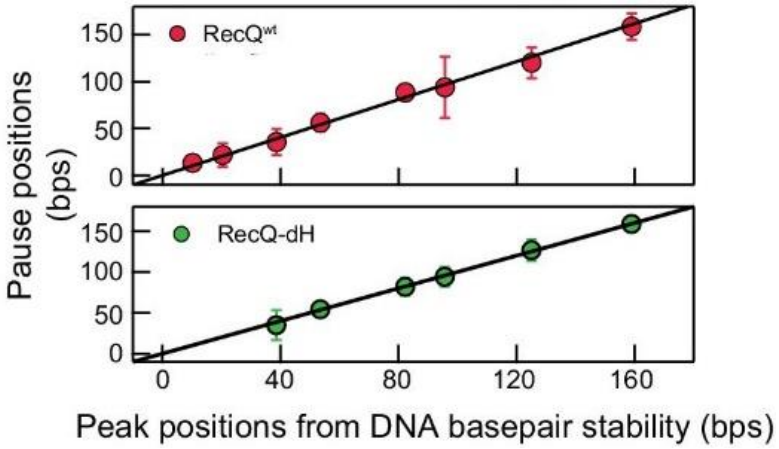
[1]Laboratory of Single Molecule Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, United States; [2]Department of Biochemistry, ELTE-MTA “Momentum” Motor Enzymology Research Group, Eötvös Loránd University, Budapest, Hungary; [3]Department of Biochemistry, MTA-ELTE Motor Pharmacology Research Group, Eötvös Loránd University, Budapest, Hungary

Pauses occur at DNA regions of high base pair stability

Magnetic tweezers experiment







(depending on GC content ahead of helicase)

How is the DNA region ahead of the enzyme „screened” for base pair energy?

Simultaneous melt: ***n*** **bp opened in a single step**

Delayed release: **1 bp opened at a time, then *n* „bp” released from protein in a single step**

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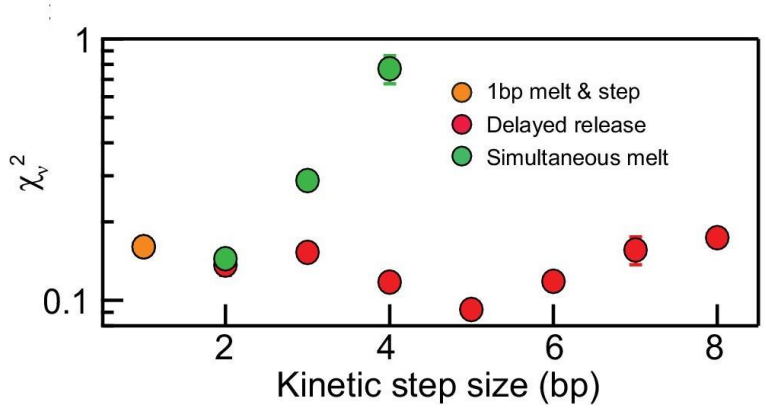
…and how long is the screened DNA region?

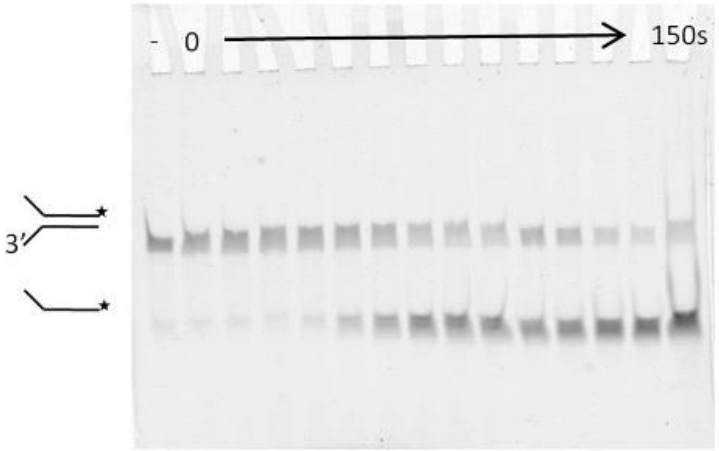
Modeling of magnetic tweezers data

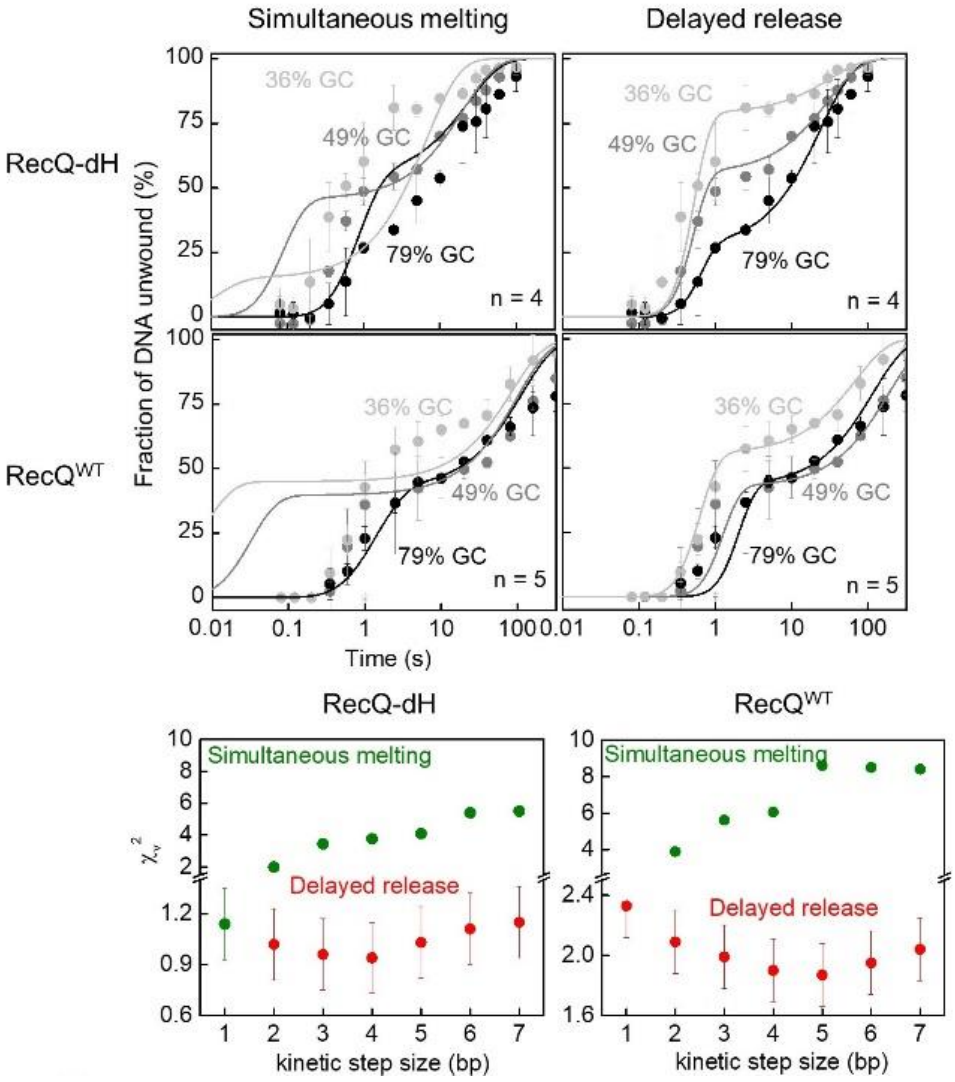


…and how long is the screened DNA region?

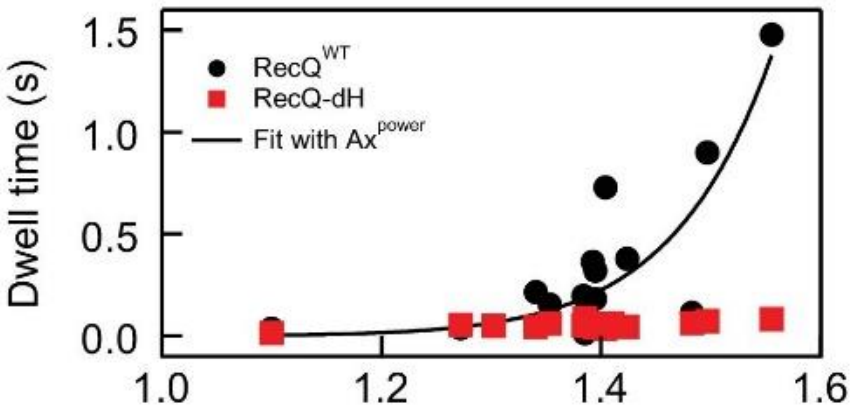
Independent evidence for the 5-bp „delayed release" steps

from rapid kinetics of oligonucleotide DNA unwinding

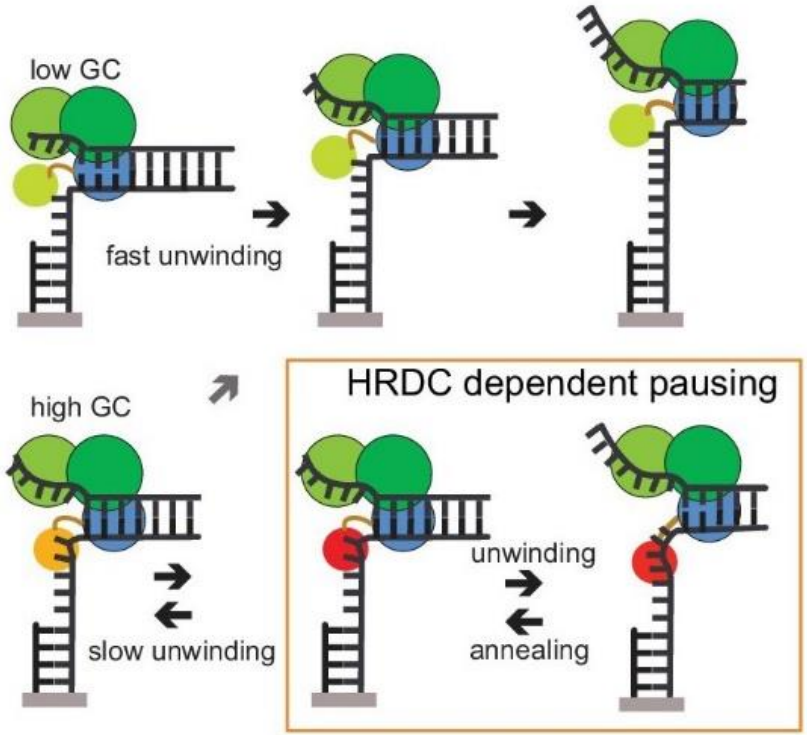




The HRDC domain amplifies pauses in a non-linear fashion



Average base pair energy over a 10-bp window (ln $\Delta G_{bp}/k_BT$)



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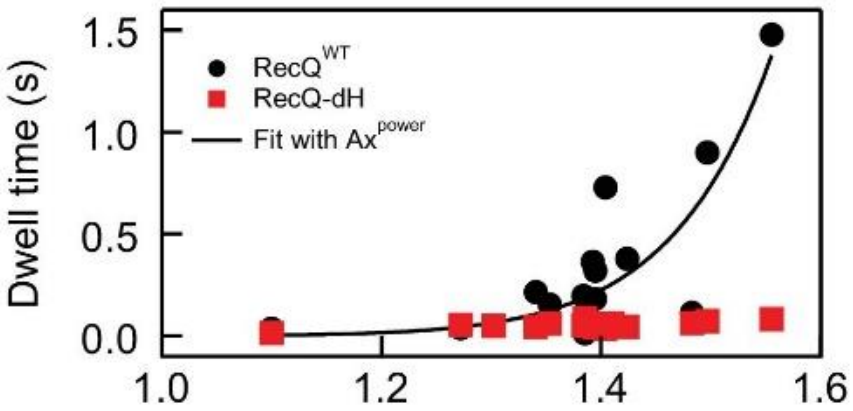

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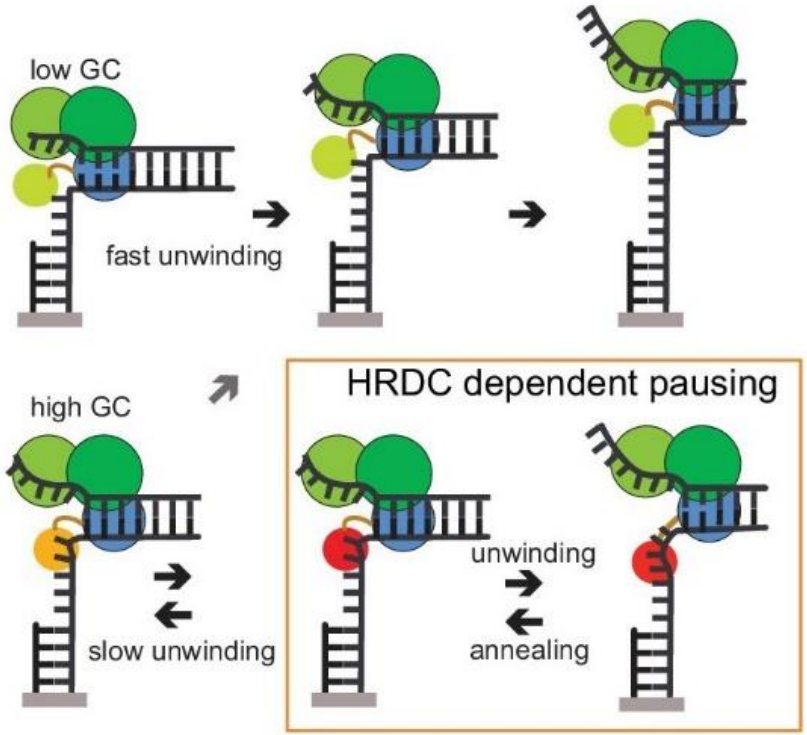
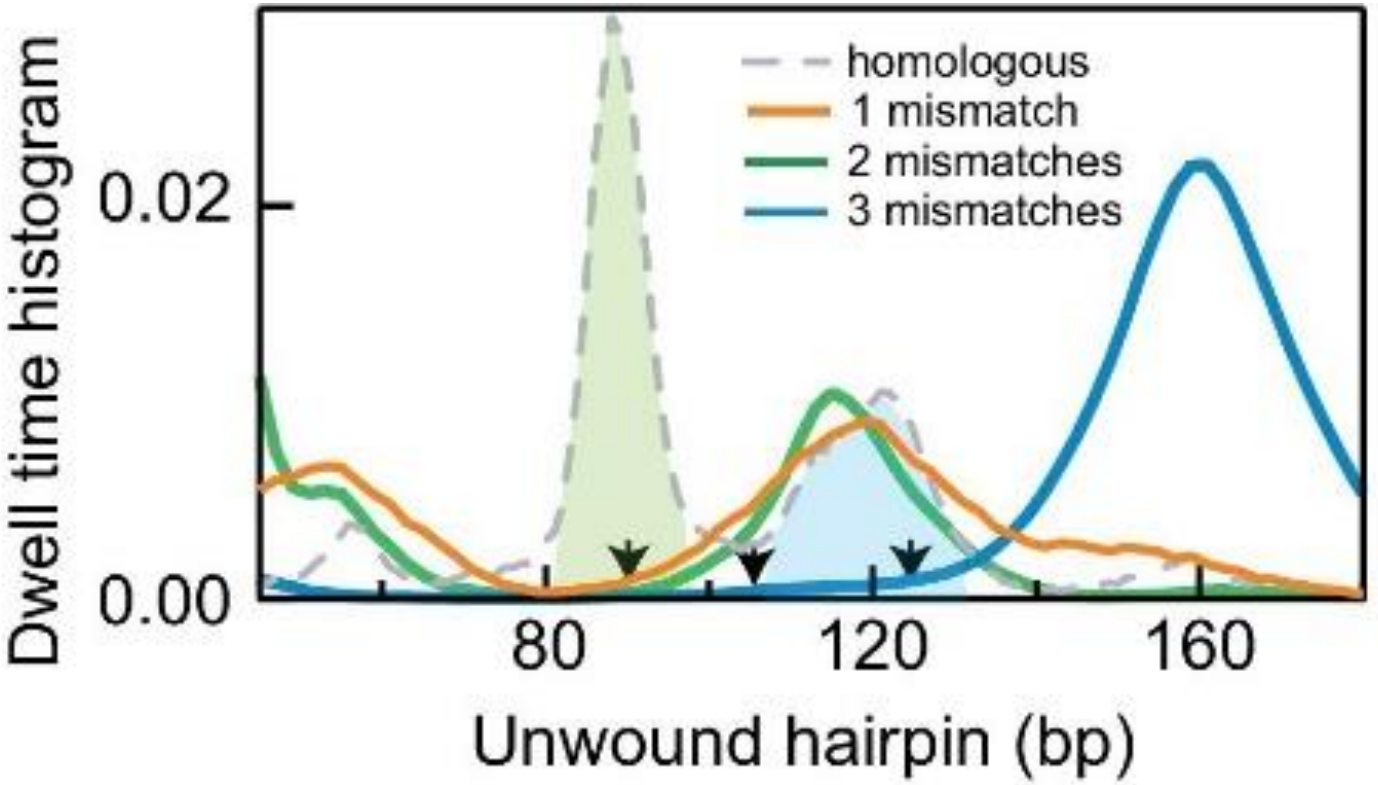
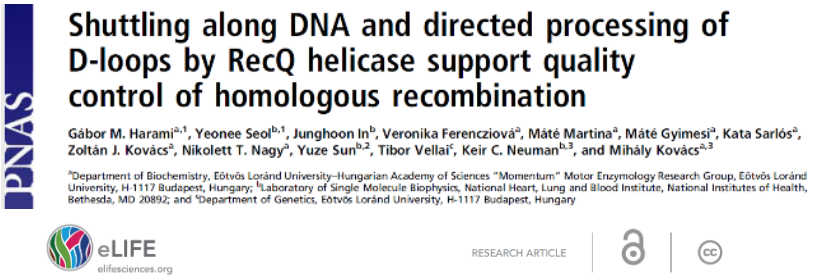


Depending on DNA base pair energy

…and this also makes the helicase slip over base pairing mismatches!



Demonstration of quality control function: RecQ activities suppress illegitimate recombination



PNAS

Shuttling along DNA and directed processing of D-loops by RecQ helicase support quality control of homologous recombination

Gábor M. Harami[a,1], Yeonee Seol[b,1], Junghoon In[b], Veronika Ferencziová[a], Máté Martina[a], Máté Gyimesi[a], Kata Sarlós[a], Zoltán J. Kovács[a], Nikolett T. Nagy[a], Yuze Sun[b,2], Tibor Vellai[c], Keir C. Neuman[b,3], and Mihály Kovács[a,3]

[a]Department of Biochemistry, Eötvös Loránd University–Hungarian Academy of Sciences "Momentum" Motor Enzymology Research Group, Eötvös Loránd University, H-1117 Budapest, Hungary; [b]Laboratory of Single Molecule Biophysics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892; and [c]Department of Genetics, Eötvös Loránd University, H-1117 Budapest, Hungary

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RESEARCH ARTICLE

Homology sensing via non-linear amplification of sequence-dependent pausing by RecQ helicase

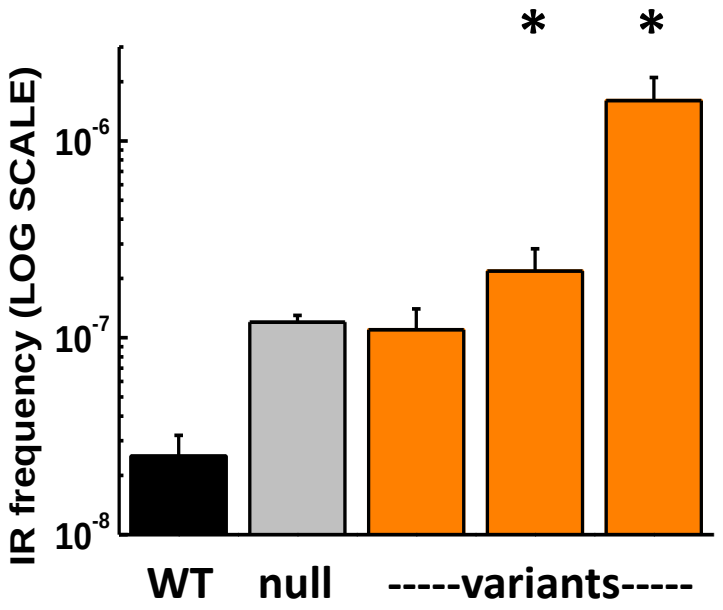
Yeonee Seol[†1], Gábor M Harami[2†], Mihály Kovács[2,3]*, Keir C Neuman[1]*

[1]Laboratory of Single Molecule Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, United States; [2]Department of Biochemistry, ELTE-MTA "Momentum" Motor Enzymology Research Group, Eötvös Loránd University, Budapest, Hungary; [3]Department of Biochemistry, MTA-ELTE Motor Pharmacology Research Group, Eötvös Loránd University, Budapest, Hungary

	WT	null	-----variants-----		
Helicase	+	-	-	+	+
Oriented disruption	+	-	-	+	-
Shuttling	+	-	-	-	-
Base pair energy-dependent pausing	+	-	-	-	-

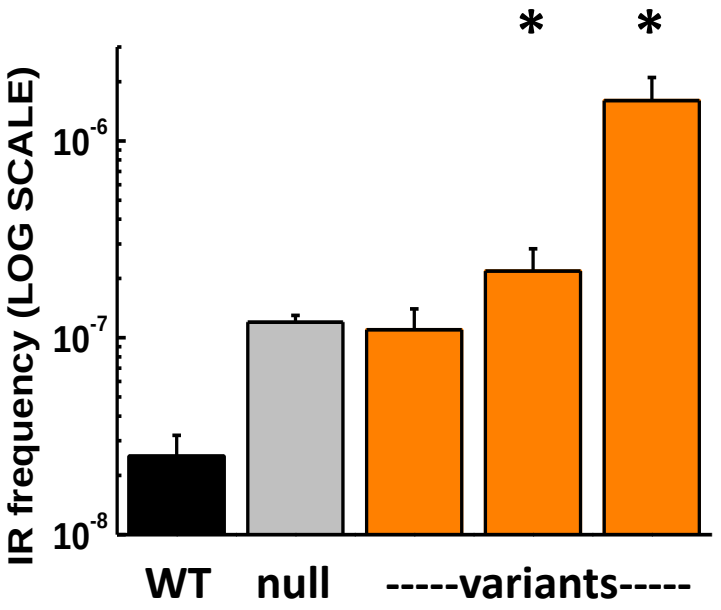
Demonstration of quality control function: RecQ activities suppress illegitimate recombination

Illegitimate recombination (IR) assay

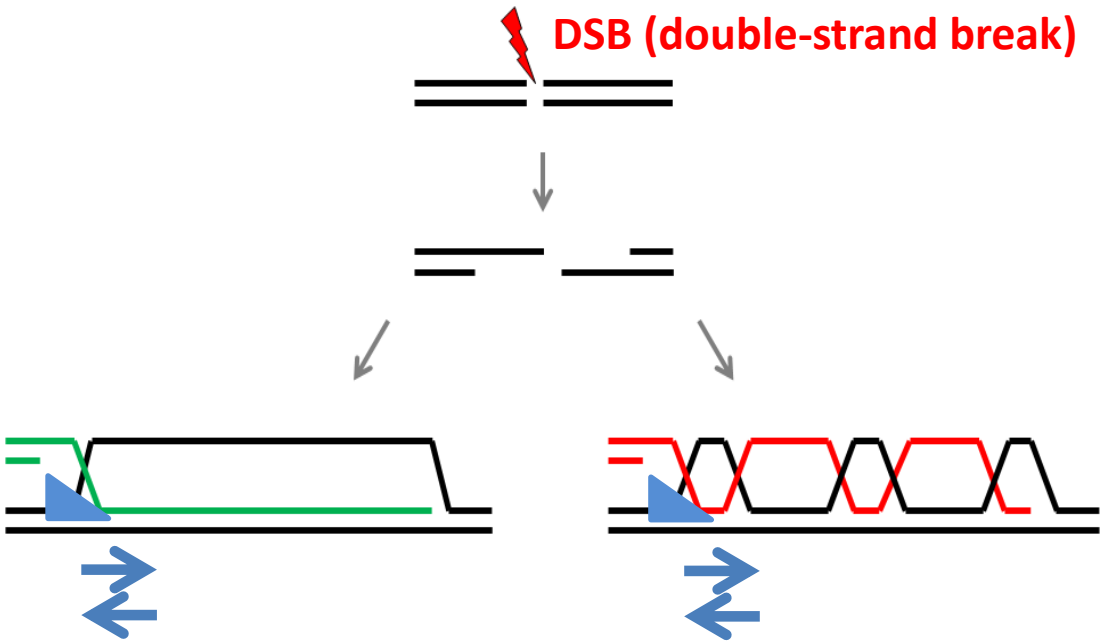


Demonstration of quality control function: RecQ activities suppress illegitimate recombination

Helicase activity is harmful without quality control



RecQ helicases thus serve HR quality control



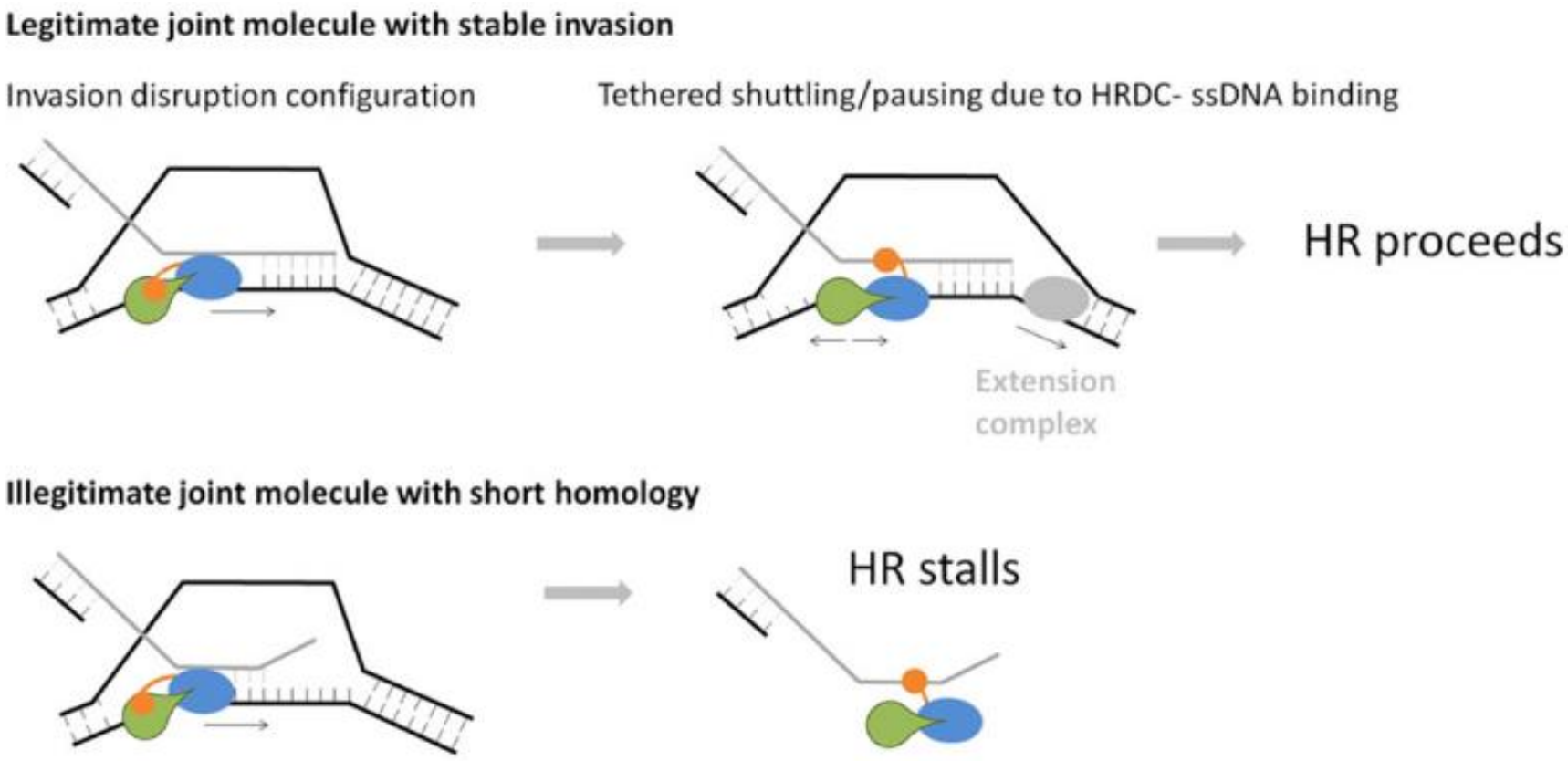
LEGITIMATE recombination

ILLEGITIMATE recombination

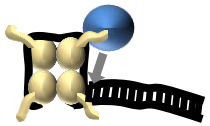
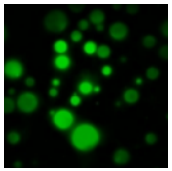
Saved by helicase

Selectively disrupted by helicase

RecQ helicases thus serve HR quality control

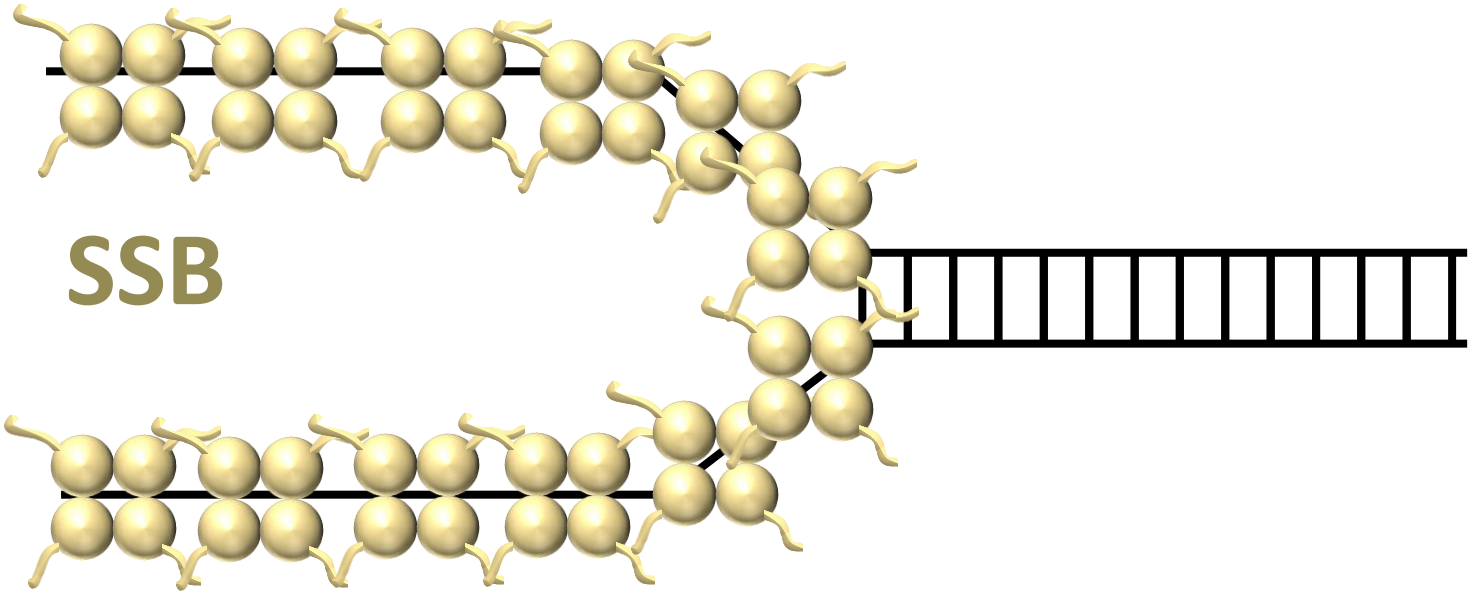


Action summary

	Discovered feature	Mechanistic determinant	Physiological function
	**Helicase shuttling** (local repetitive DNA unwinding)	**Mechanical tension** on displaced DNA strand	**HR quality control**
	**Oriented D-loop disruption**	**Geometry** of DNA strand junctions	
	**Non-linear pausing/stalling**	Local **base-pair energy** of DNA/mismatches	
	Access to DNA *via* **PPI-induced switch** in SSB-DNA binding mode	**Specific interaction** between SSB-CTP and SIP	**Organization/targeting of replication/repair complexes**
	**Liquid-liquid phase separation**	**Non-specific interaction** between SSB IDP regions **Specific interaction** between SSB-OB/CTP regions	**Rapid subcellular relocalization to genome replication/repair sites**

Whenever single-stranded DNA appears in the cell, SSB is there

Single-stranded (ss) DNA



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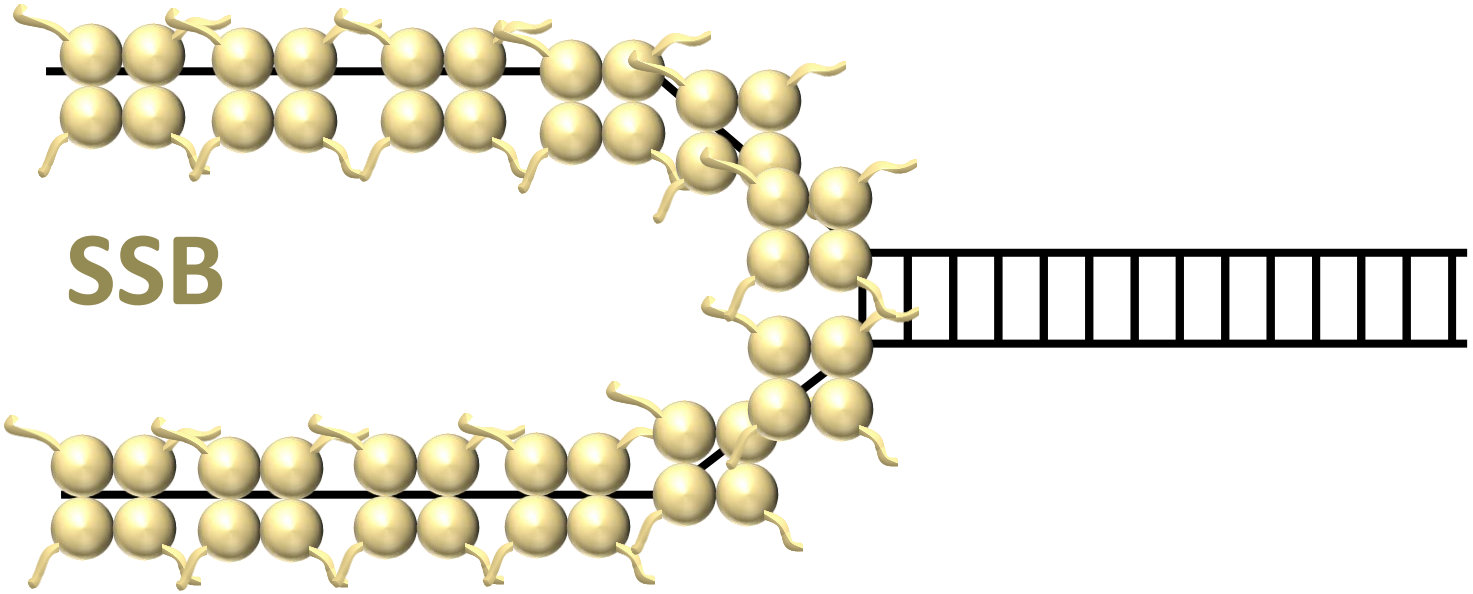
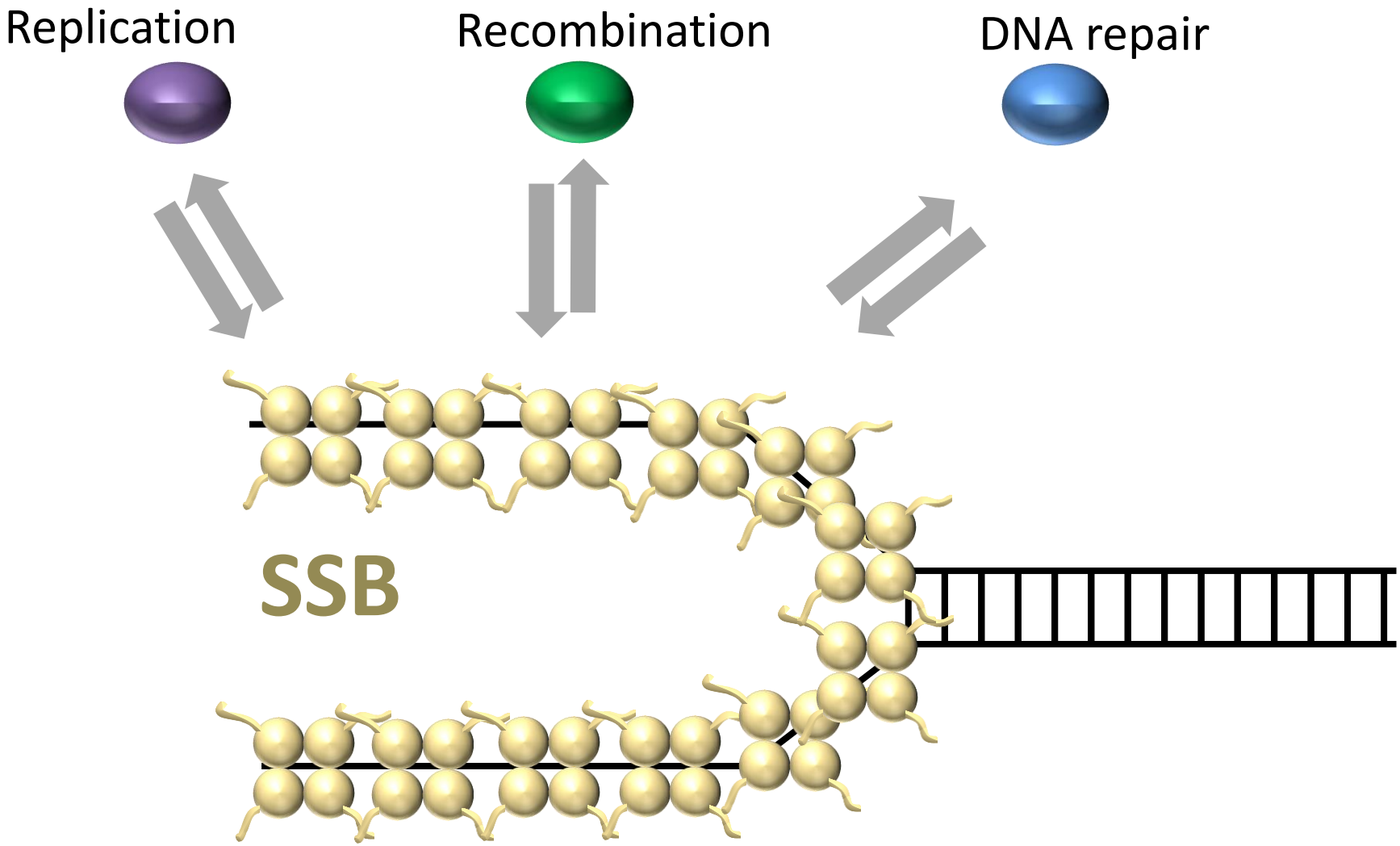
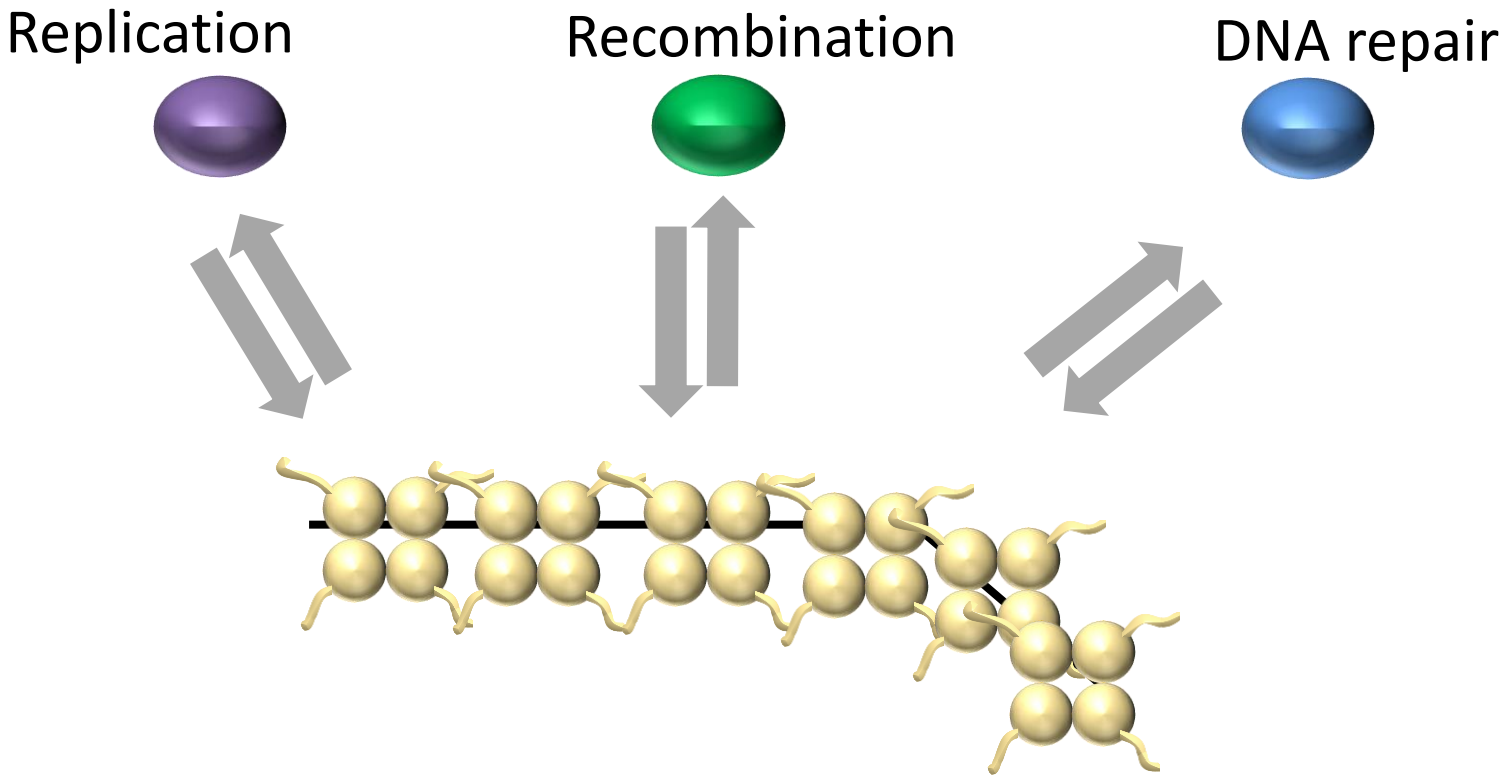


Prevents DNA secondary structure formation
Protects ssDNA from degradation

More than a cover for DNA: a central hub for DNA metabolism



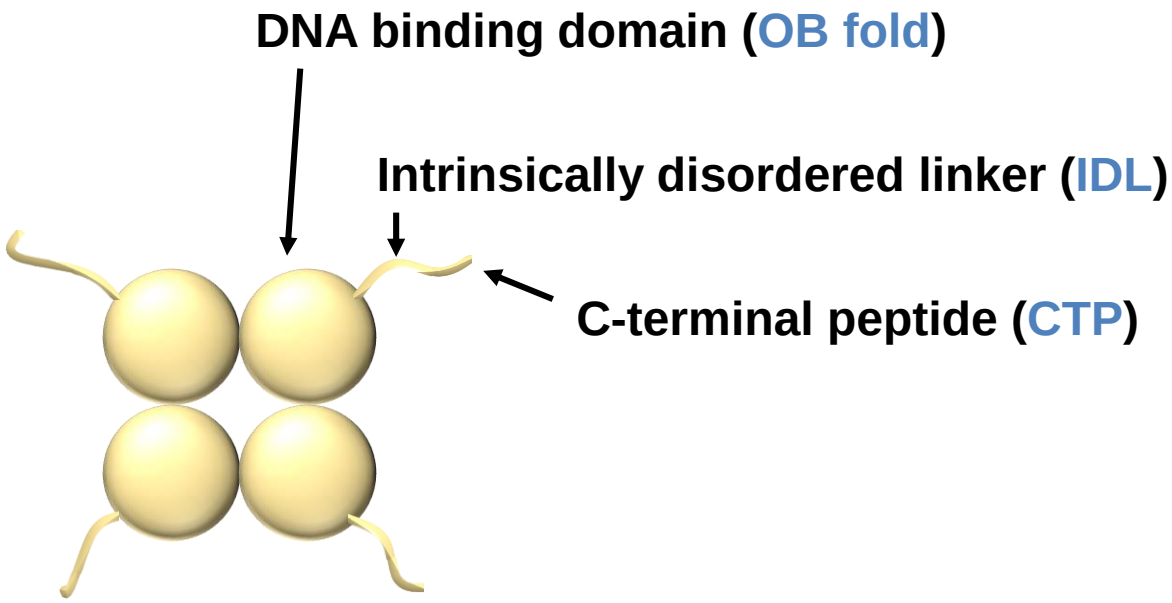
More than a cover for DNA: a central hub for DNA metabolism



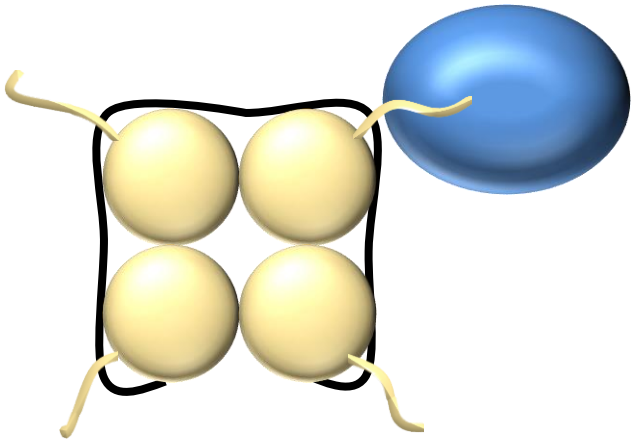
…but how can these proteins access ssDNA that is strongly bound by SSB?

How do they initiate DNA modification at the appropriate site?

Functional anatomy of the SSB homotetramer



SSB forms protein-protein interactions to „anyone who matters”…



Exonuclease I
RecQ helicase
DNA polymerase III
Uracil-DNA glycosylase
Topoisomerase III
Primase
RecO recombination mediator
etc.

18 known partners
SSB-interacting proteins (SIPs)

Sequence logo: Shereda 2009 Crit Rev Mol Biol

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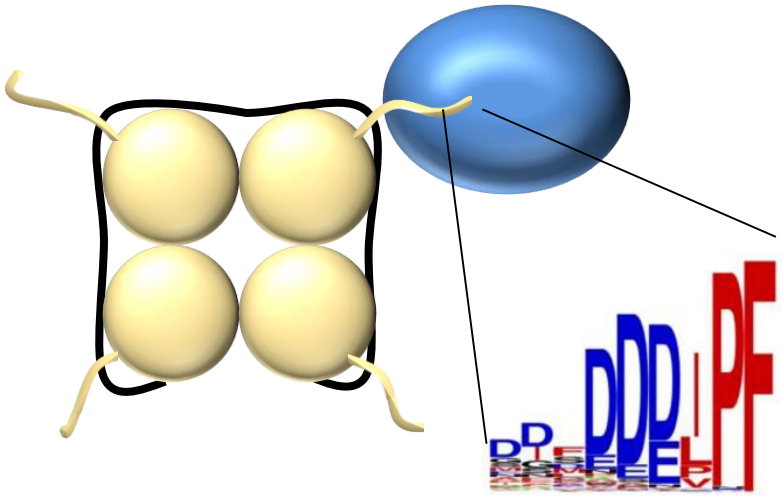

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RecQ helicase
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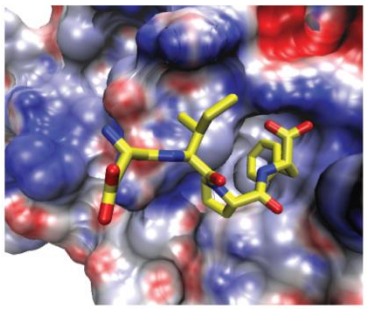
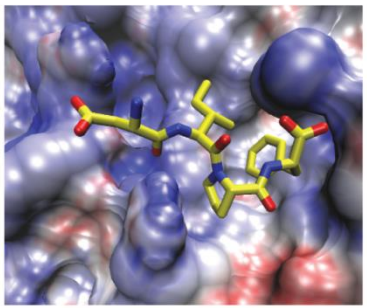
etc.

18 known partners
SSB-interacting proteins (SIPs)

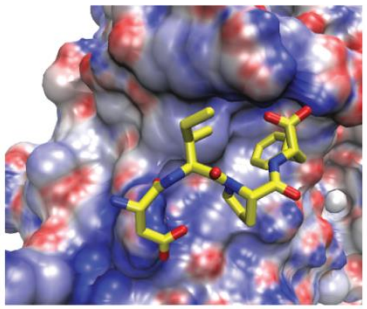
…all through its highly conserved C-terminal peptide (CTP)!



ExoI-SSB C peptide complex



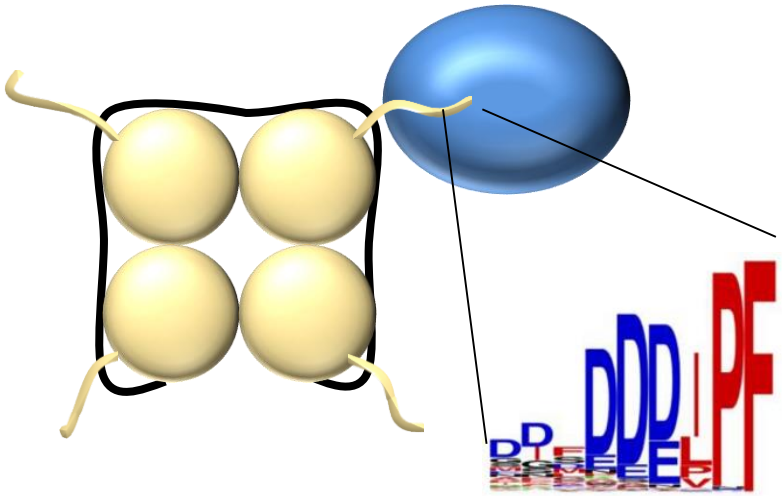
UDG-SSB C peptide complex



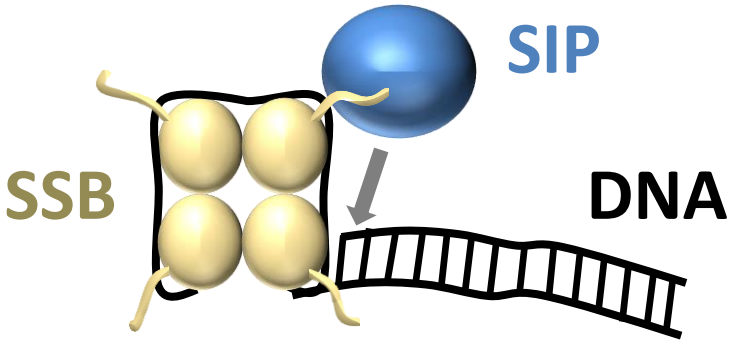
RecQ-SSB C peptide docked complex

Sequence logo: Shereda 2009 Crit Rev Mol Biol

SSB forms protein-protein interactions to „anyone who matters”

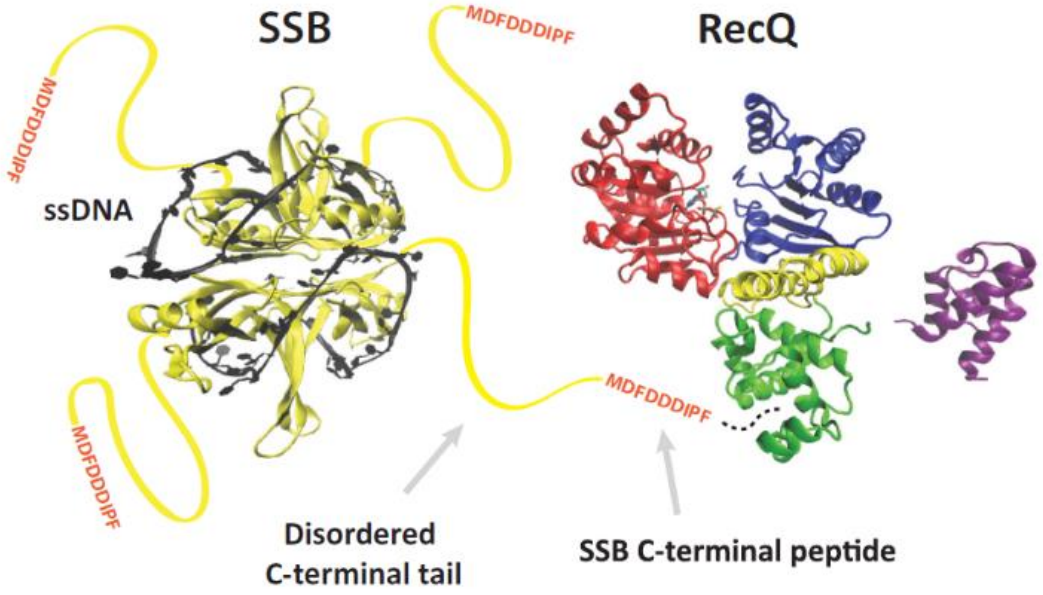


General scheme of SSB-SIP-DNA interactions



Sequence logo: Shereda 2009 Crit Rev Mol Biol

So, how does RecQ helicase gain access to ssDNA that's covered by SSB?



11878–11890 Nucleic Acids Research, 2017, Vol. 45, No. 20 Published online 20 October 2017
doi: 10.1093/nar/gkx939

RecQ helicase triggers a binding mode change in the SSB–DNA complex to efficiently initiate DNA unwinding

Maria Mills[1,†], Gábor M. Harami[2,†], Yeonee Seol[1], Máté Gyimesi[2], Máté Martina[2], Zoltán J. Kovács[2], Mihály Kovács[2,*] and Keir C. Neuman[1,*]

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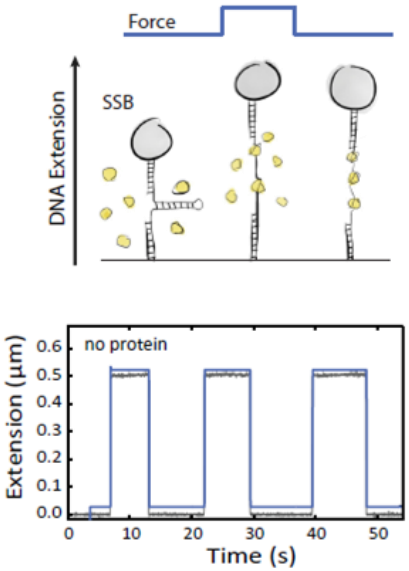
A pull on naked and SSB-coated DNA by magnetic tweezers



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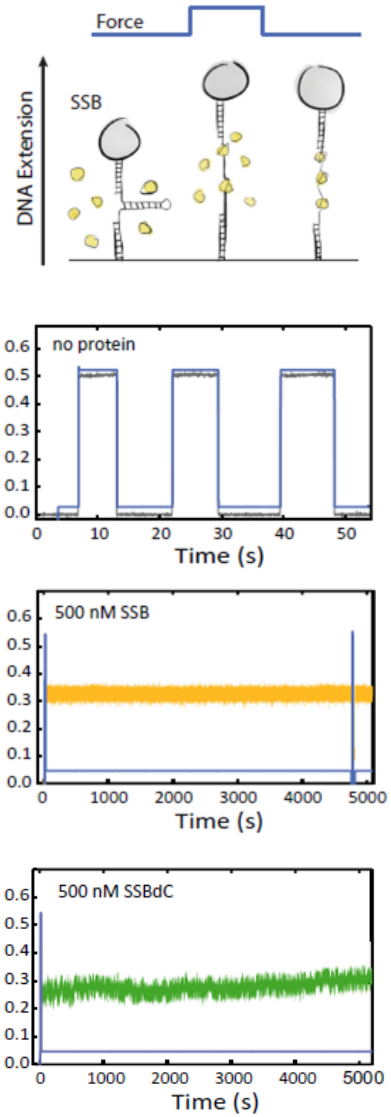
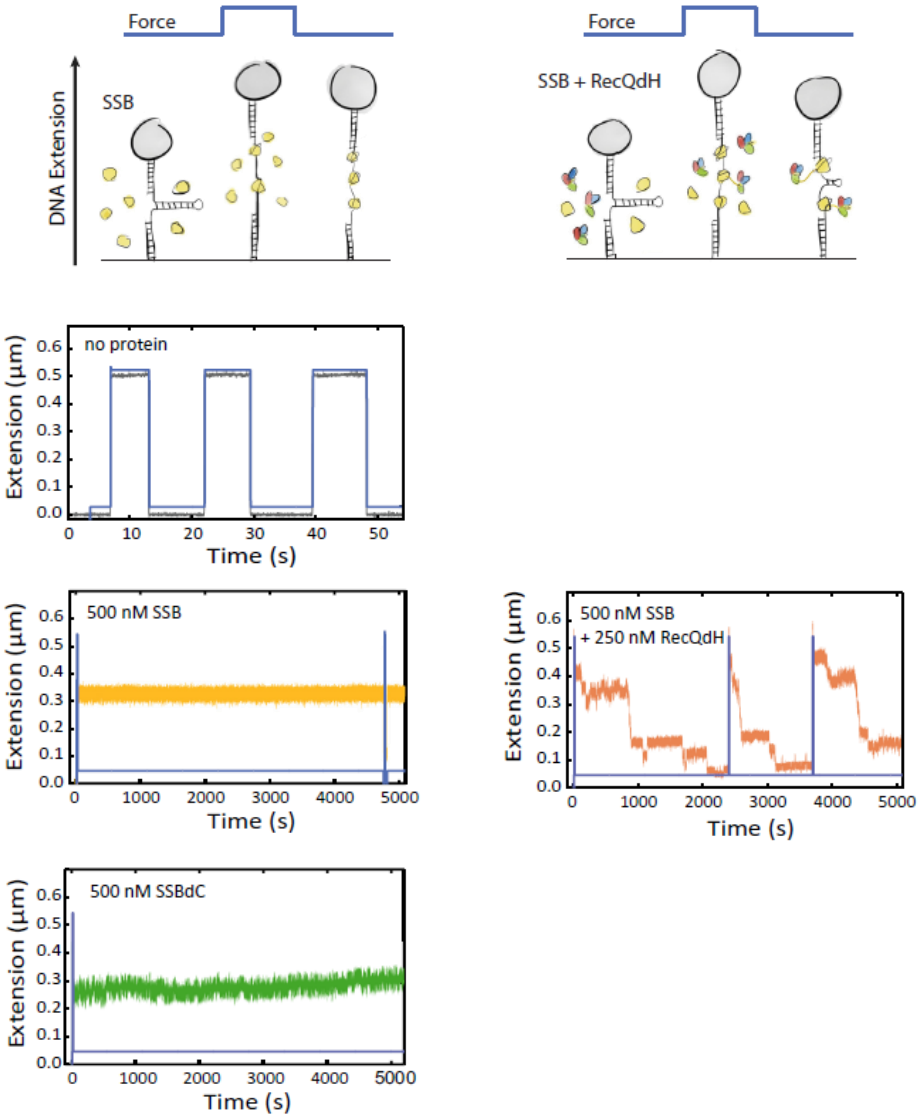
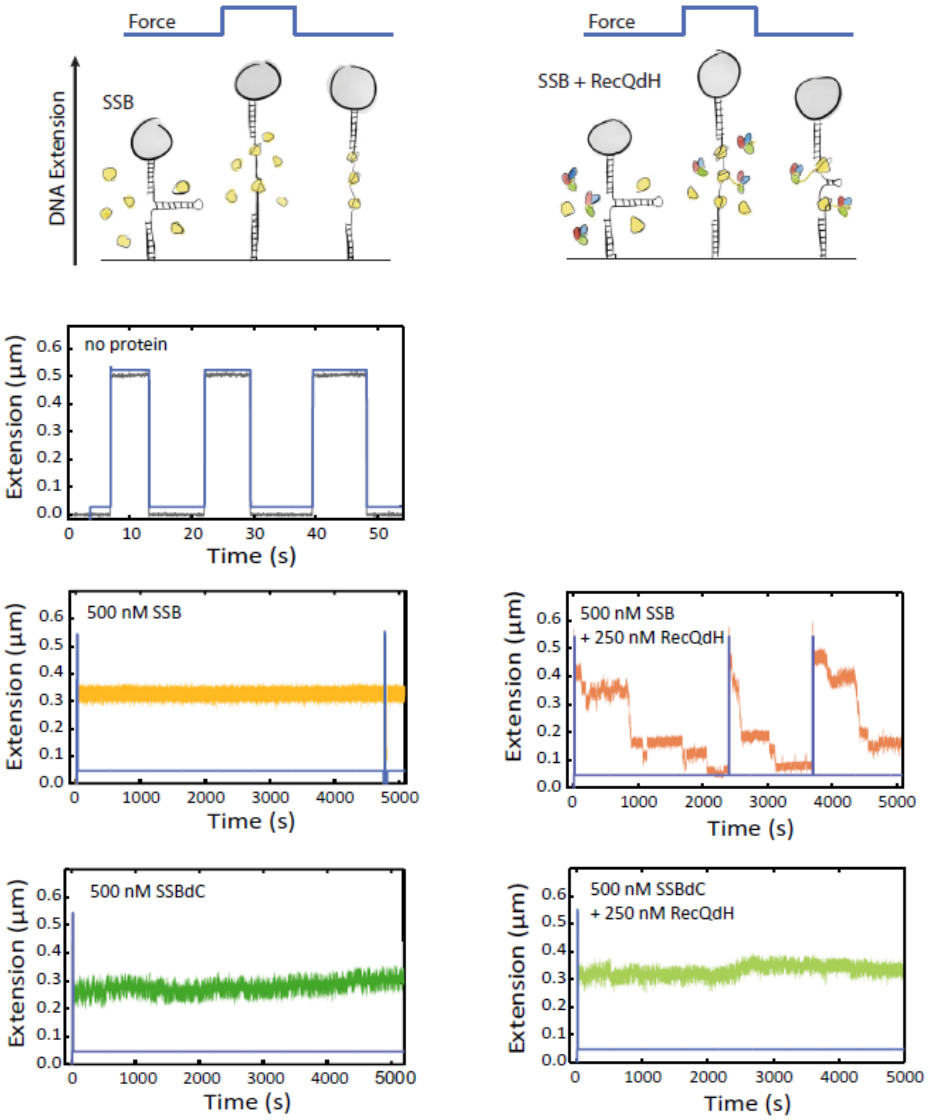


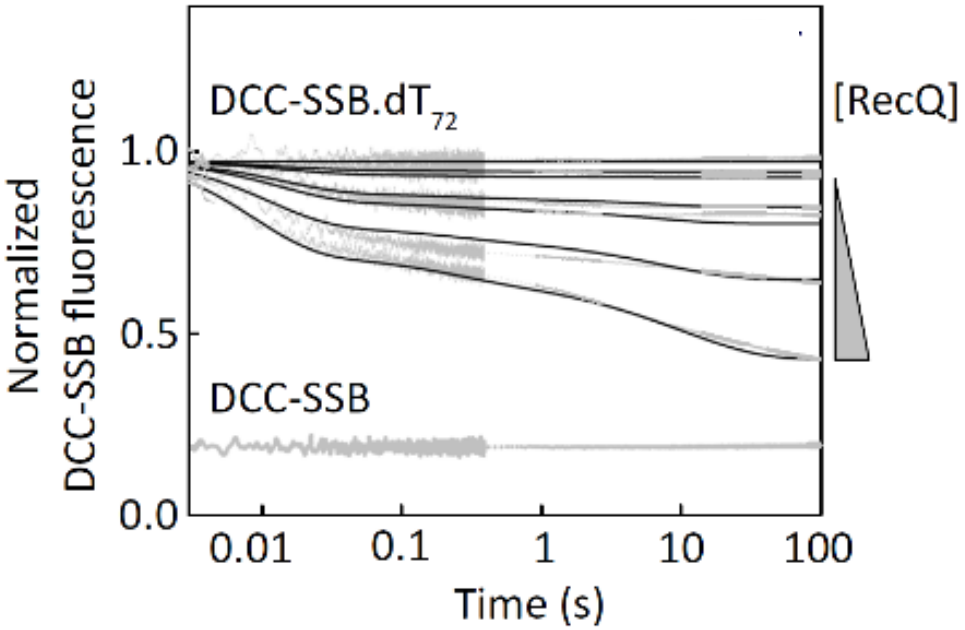
Helicase-dependent SSB removal…



Helicase-dependent SSB removal requires the presence of the CTP!



Rapid kinetics of RecQ-induced SSB removal

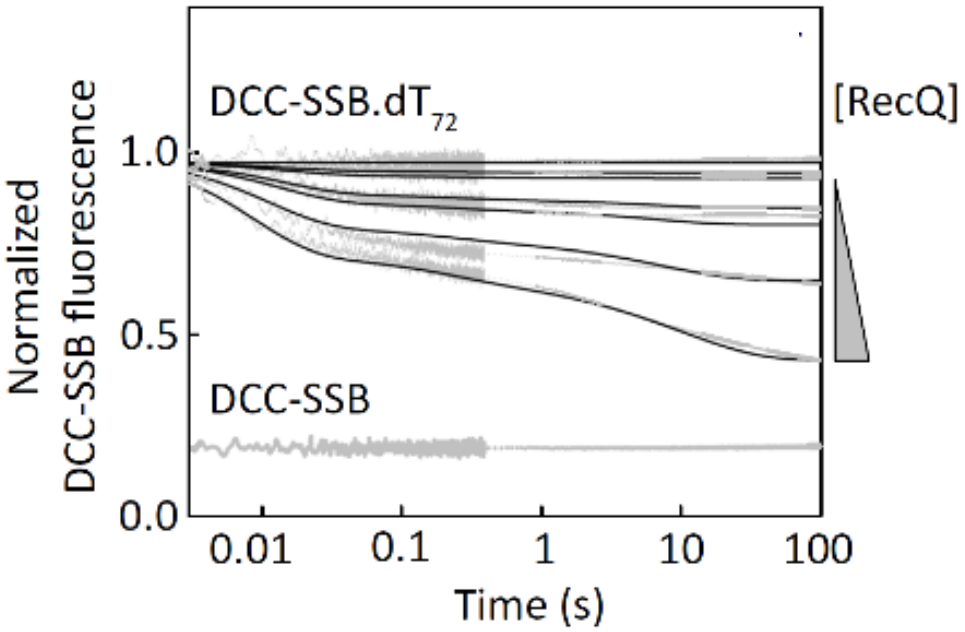


Stopped-flow experiment

DCC-SSB.dT_{72} x [RecQ]

(Fluorescent label)

Rapid kinetics of RecQ-induced SSB removal …suggesting an SSB-DNA binding mode change



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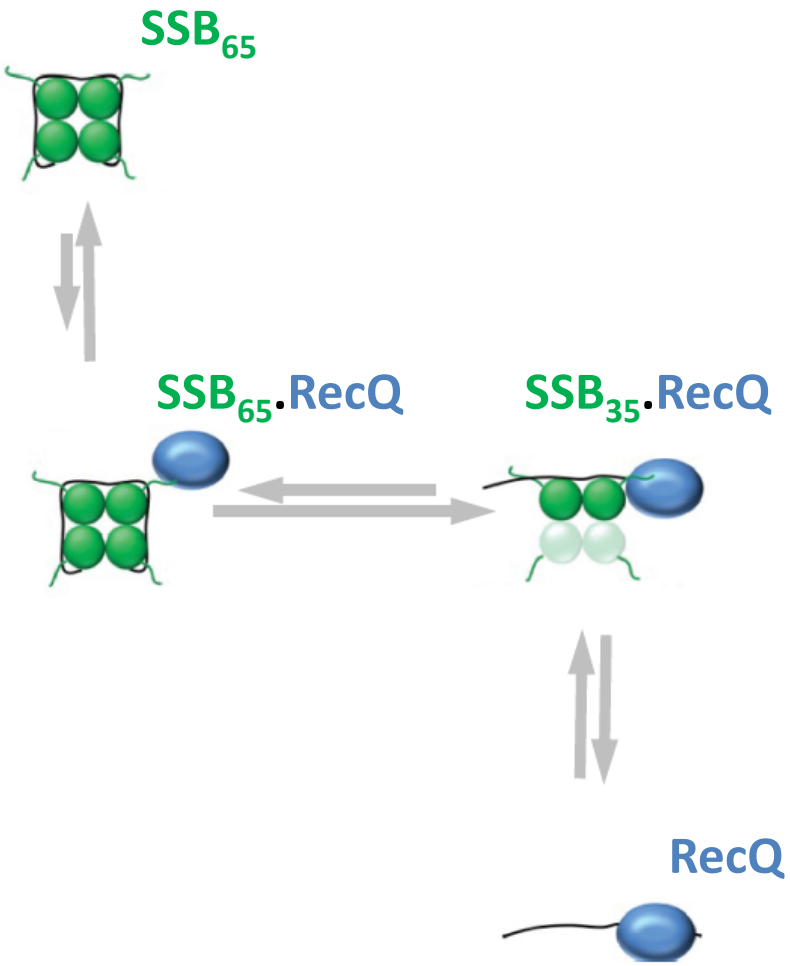
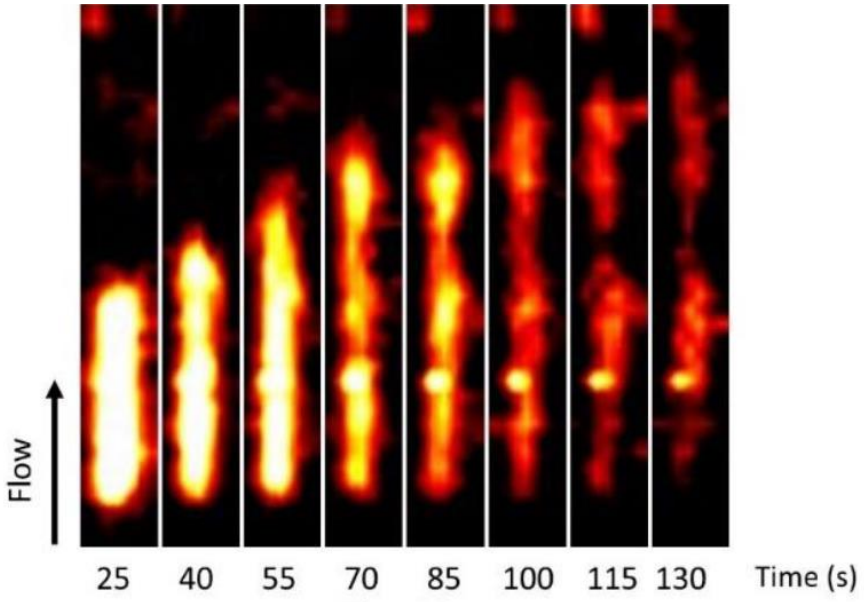


Visualization of the SSB_{65}-SSB_{35} transition *via* single-filament fluorescence microscopy



Harami GM, Kovács ZJ et al., unpublished data

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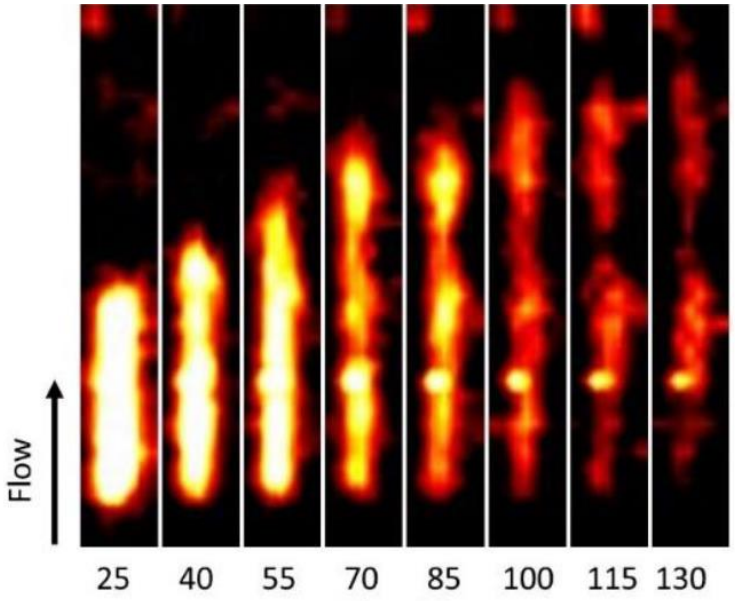
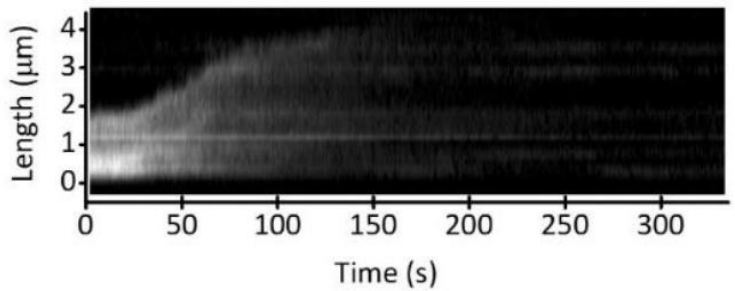
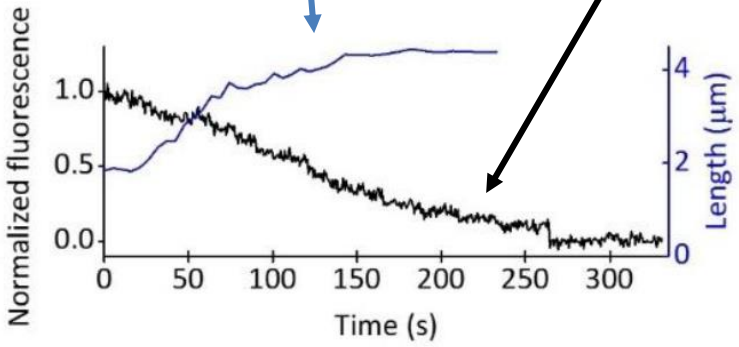


Extension of SSB nucleoprotein filament related to SSB_{65}-SSB_{35} transition

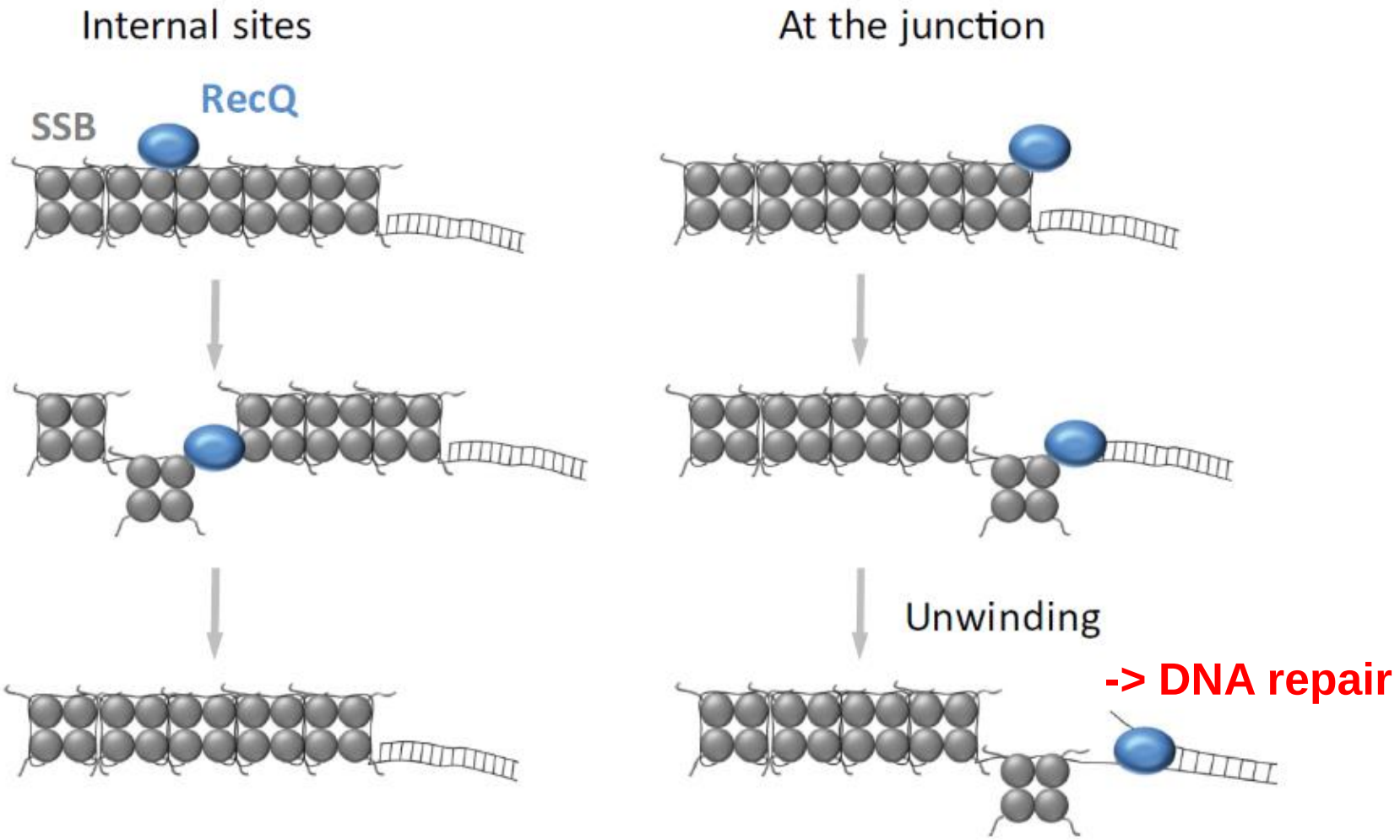
SSB removal





Harami GM, Kovács ZJ et al., unpublished data

How this becomes biology: the RecQ-CTP interaction triggers helicase activity at the effective target site



Is an SSB solution *a solution*?

~2000 SSB tetramers per *E. coli* cell (5 µM)
Only ~10 % of this is bound to ssDNA under stress-free conditions (65 nt/tetramer; ~10 kb ssDNA))
How about the rest?

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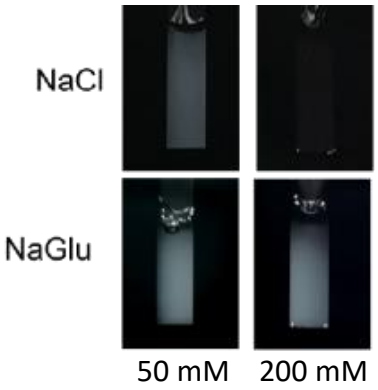

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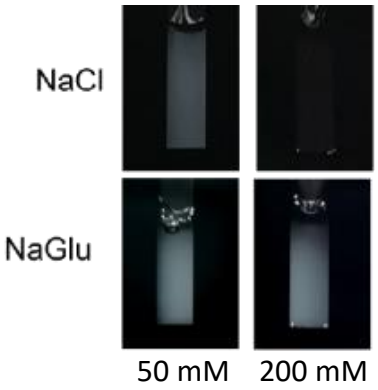
Is it insoluble?



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SSB forms phase-separated protein condensates under physiological conditions

Cold Spring Harbor Laboratory

bioRχiv

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New Results

Phase separation by ssDNA binding protein controlled *via* protein-protein and protein-DNA interactions

Gábor M. Harami, Zoltán J. Kovács, Rita Pancsa, János Pálinkás, Veronika Baráth, Krisztián Tárnok, András Málnási-Csizmadia, Mihály Kovács

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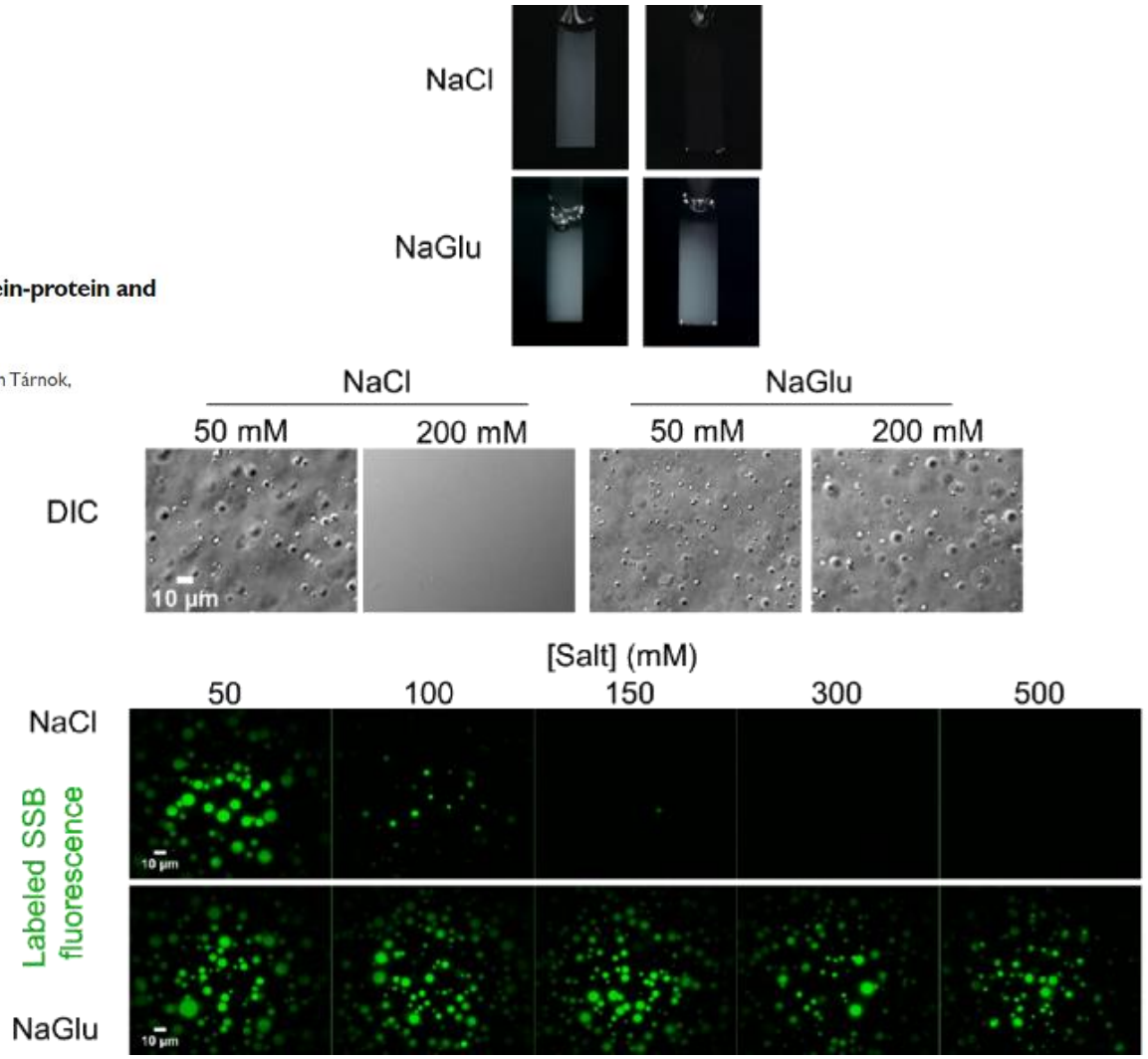
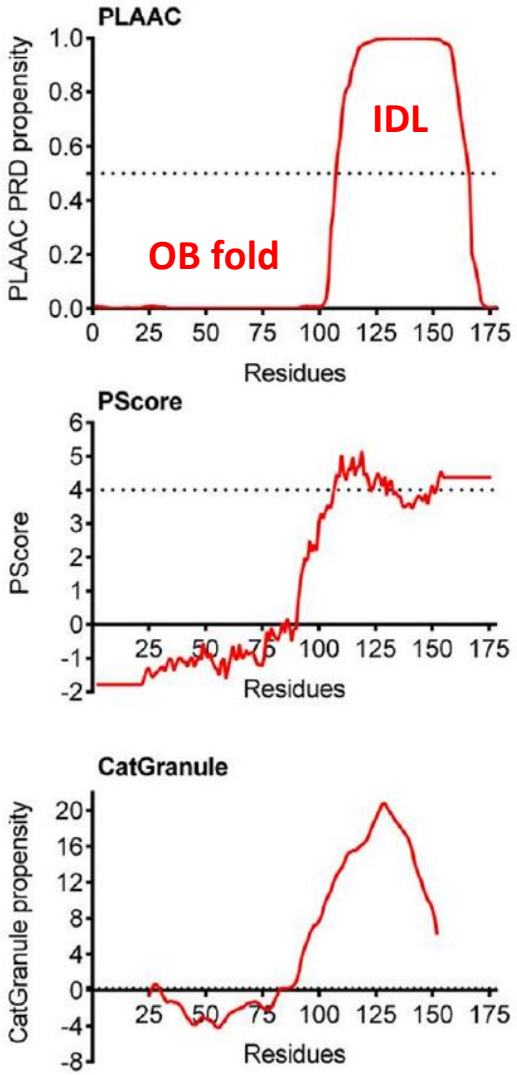


It is the IDL that makes SSB phase-separate



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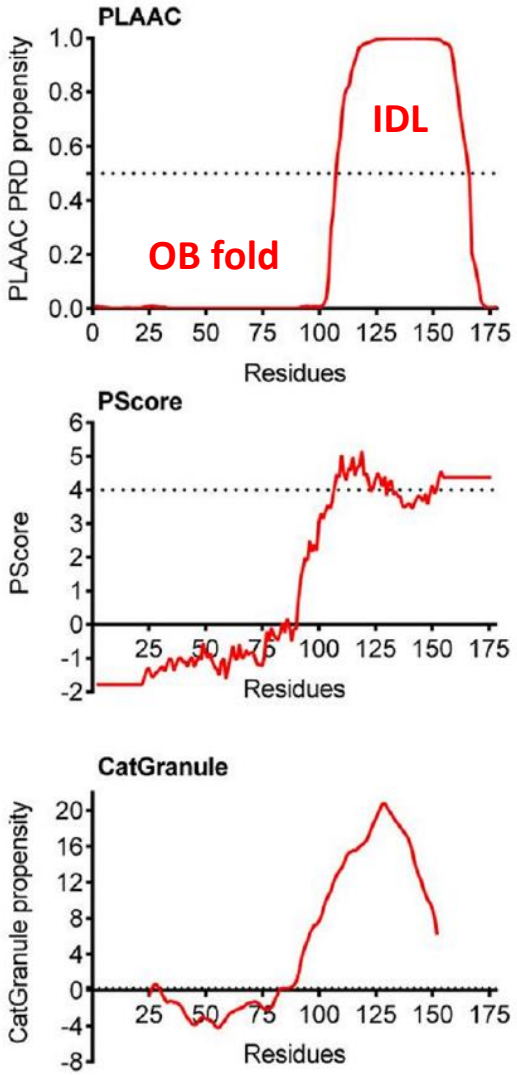
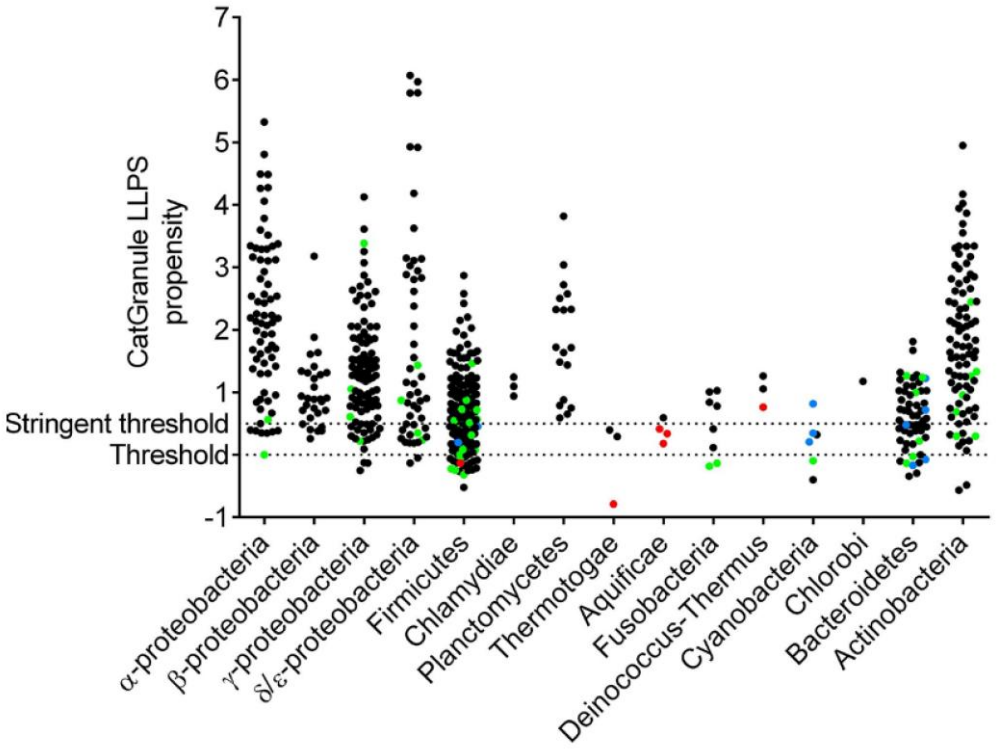
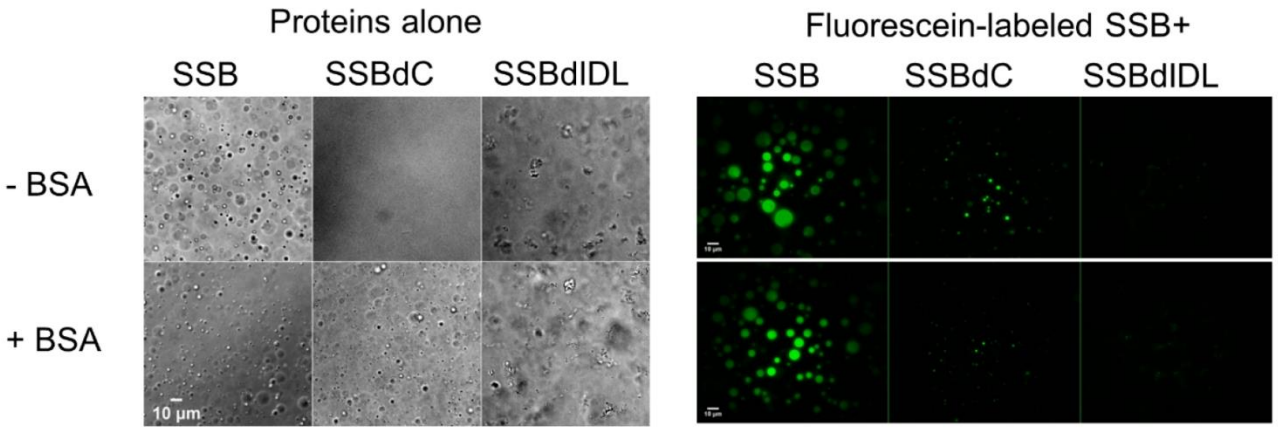
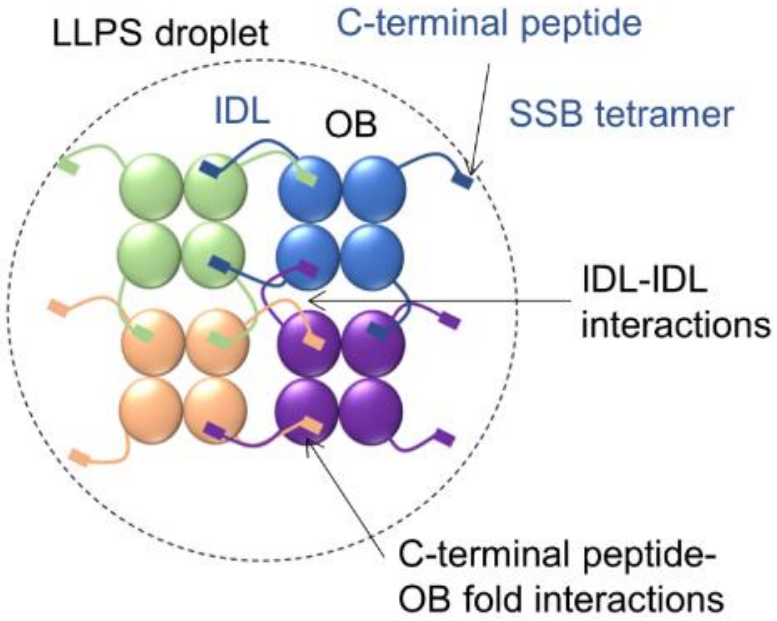




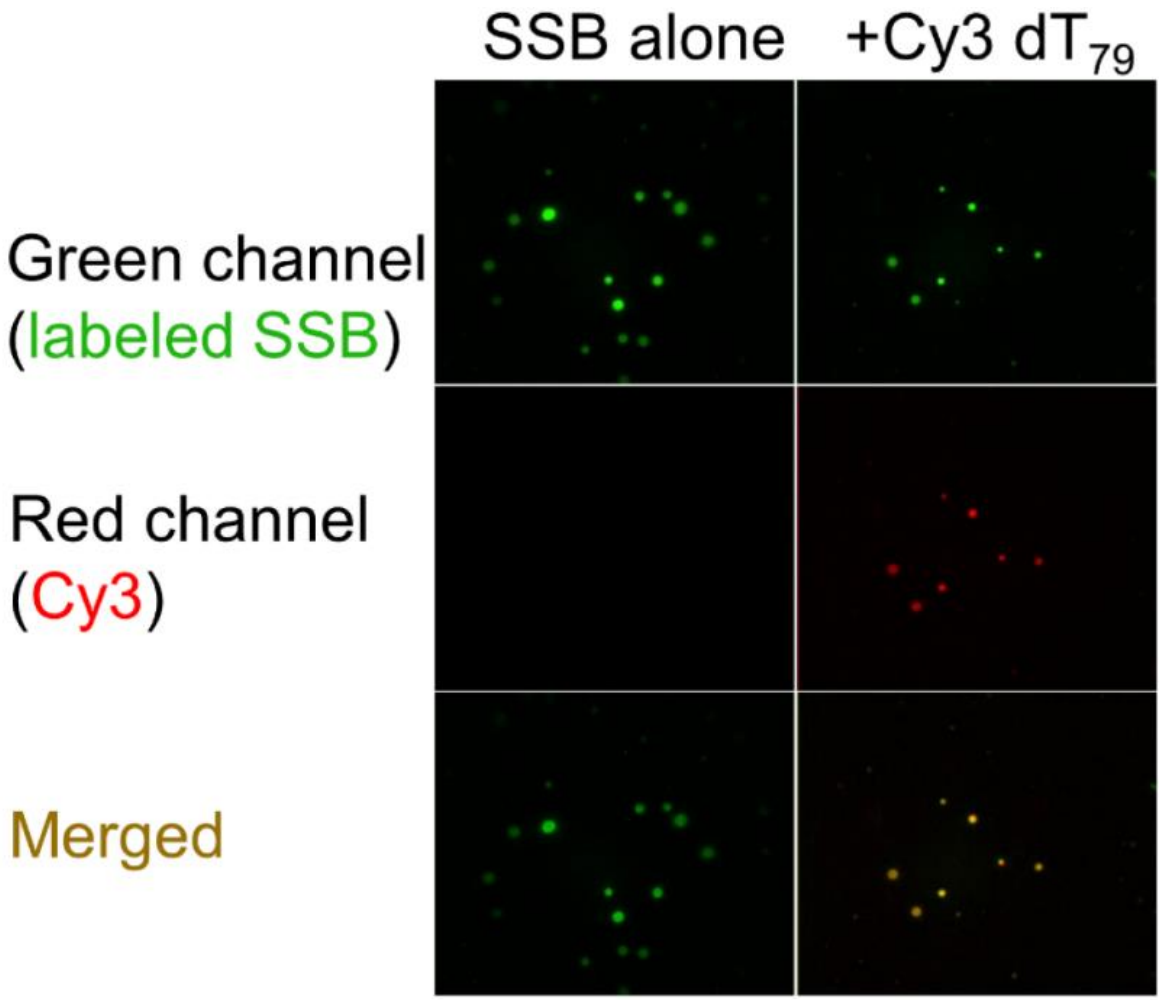
Feature conserved among most bacterial SSBs

IDL-IDL and OB-CTP interactions are both required for phase separation

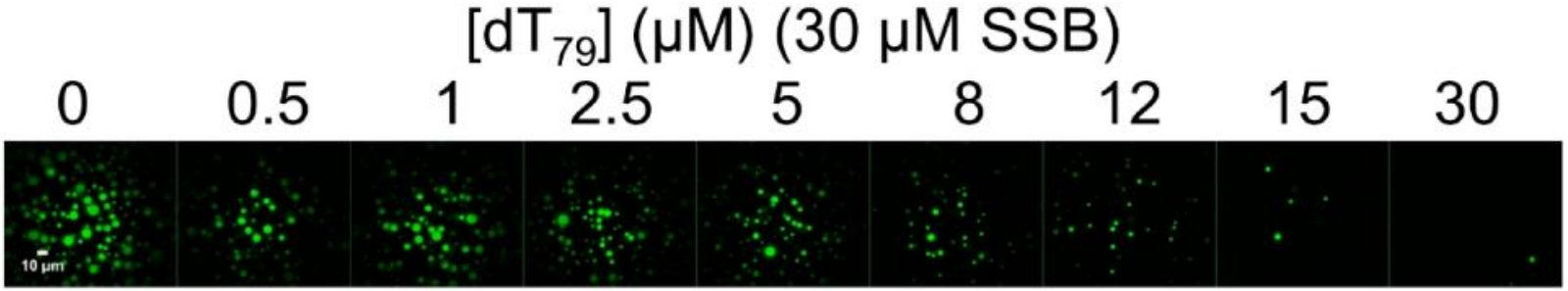


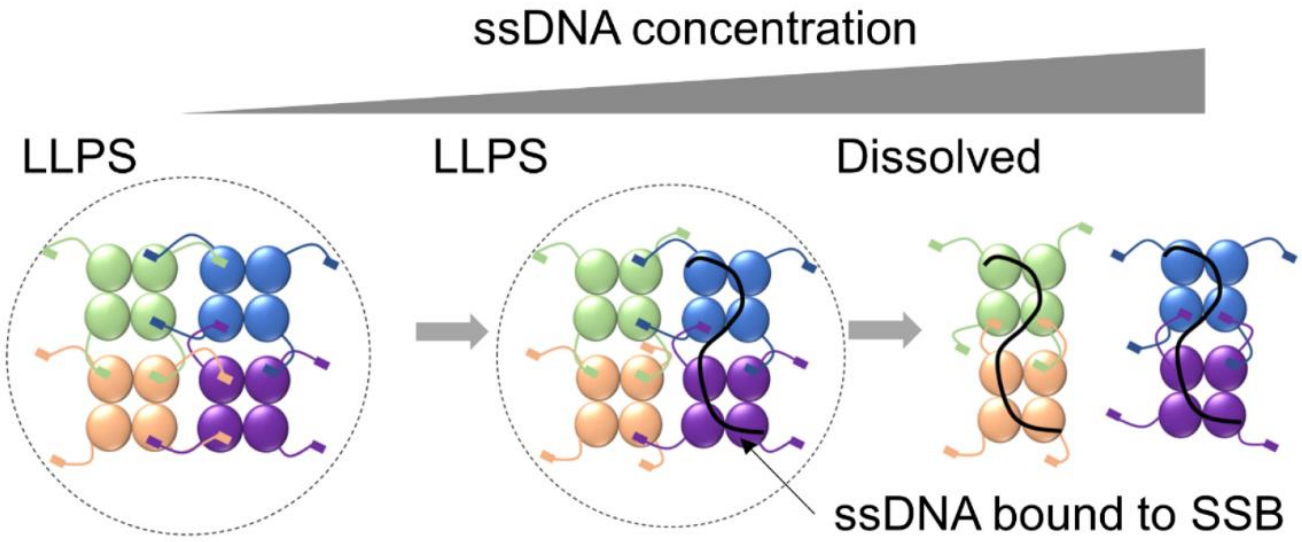


ssDNA is incorporated in the droplets…

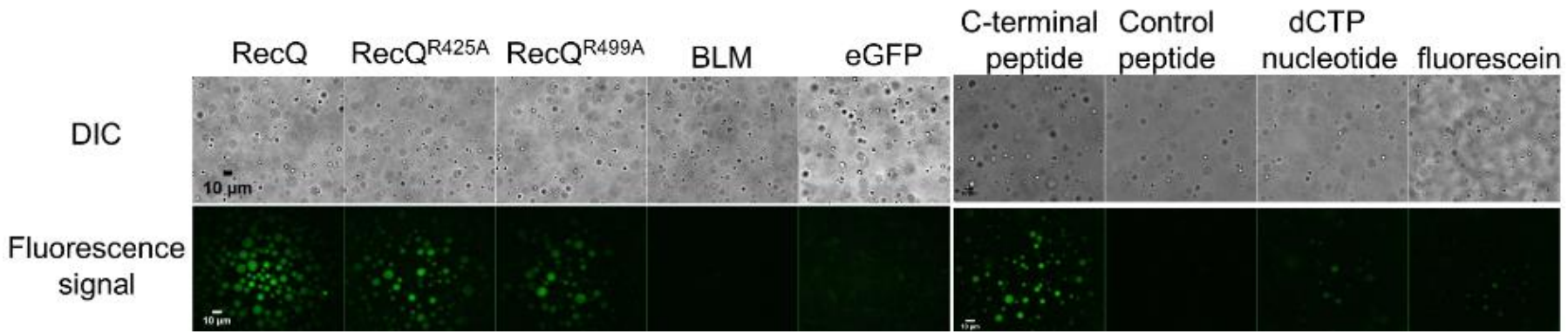


ssDNA is incorporated in the droplets…
but it disrupts them at high concentration!

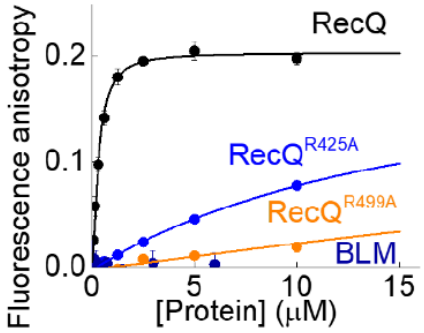




SSB binding partners (*even weak ones!*) are specifically enriched in the SSB droplets

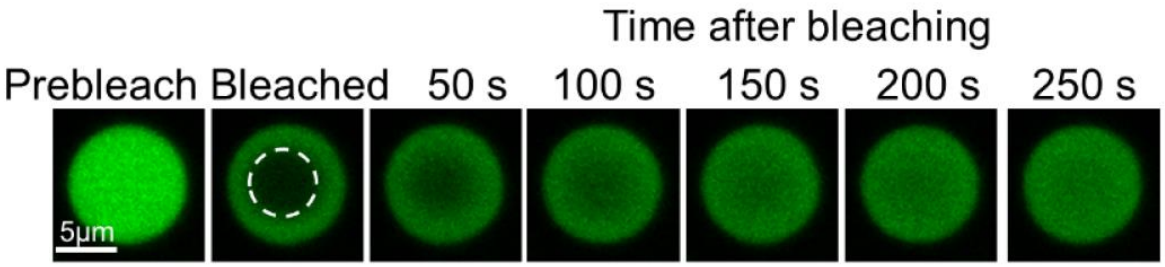


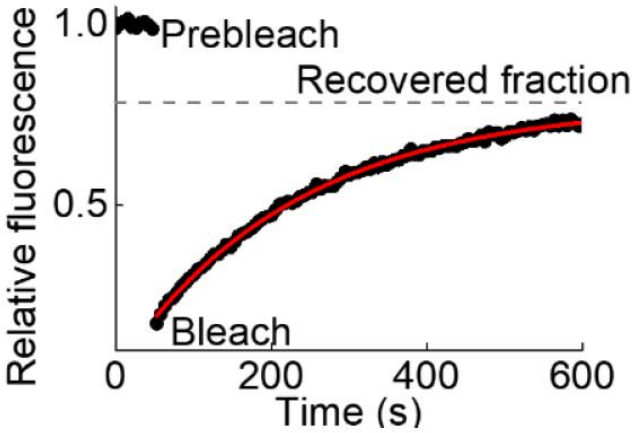
Large inert probes are excluded



Helicase binding to SSB-CTP peptide

SSB molecules are rapidly exchanged within the droplet





Recovery time calculated for total intracellular SSB content (about 2000 molecules, 100-nm diameter): **70 ms**

How this becomes biology

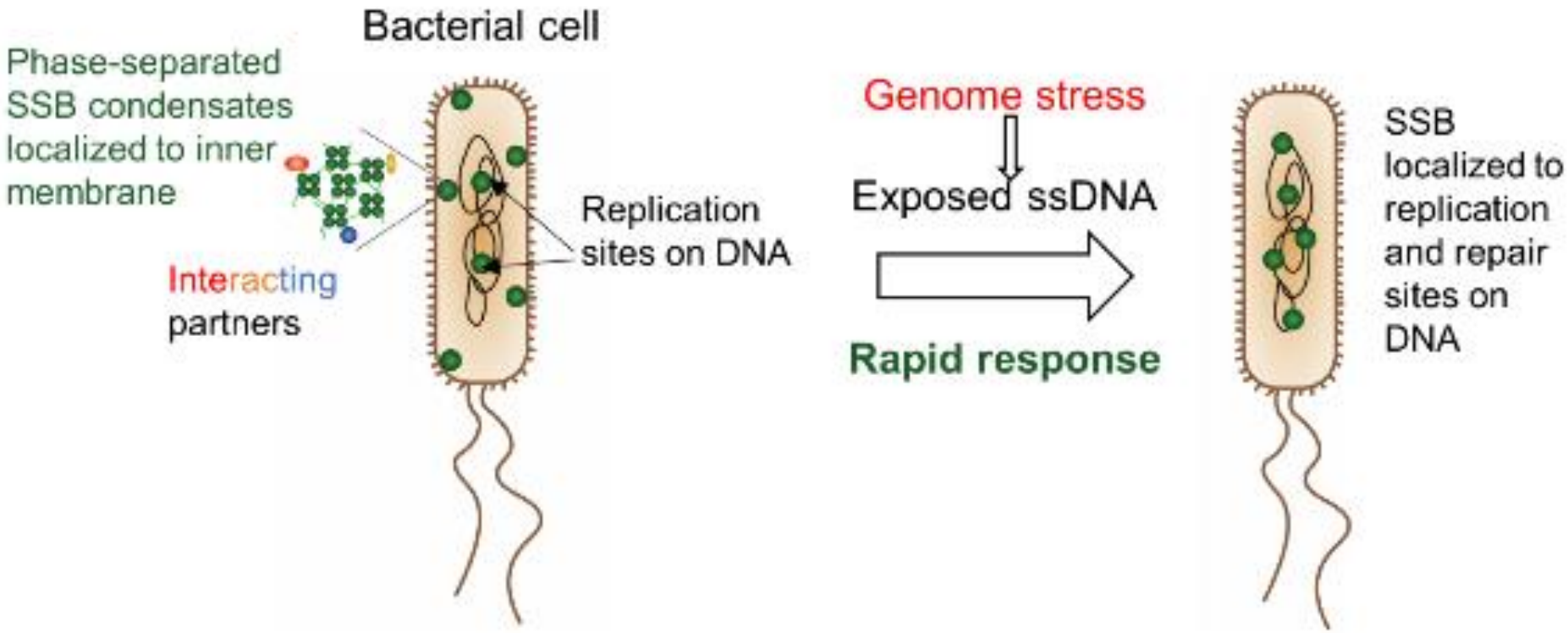
LLPS functions in eukaryotes

- Bioreactors (concentration enhancement)
- Molecular filters
- Stress sensors

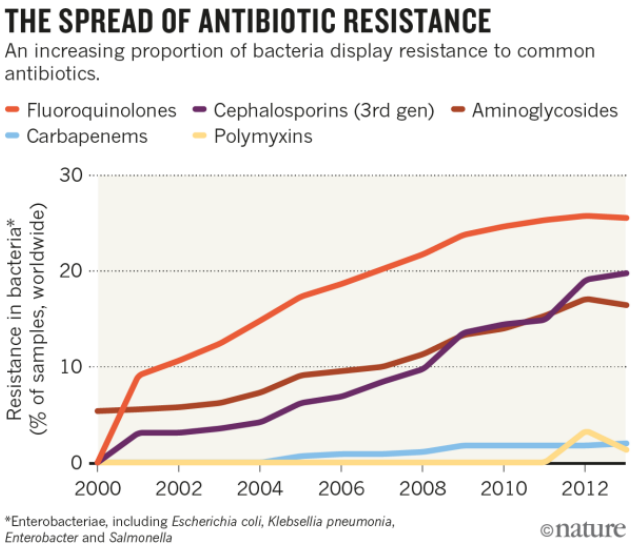
(nucleolus, stress granules, post-synaptic density, heterochromatin, super-enhancers)

Occurrence and function in bacteria unexplored

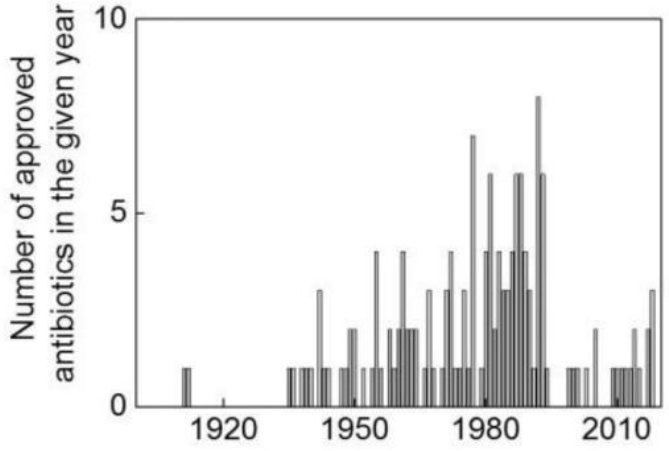
How this becomes biology: dynamic subcellular relocalization *via* phase separation

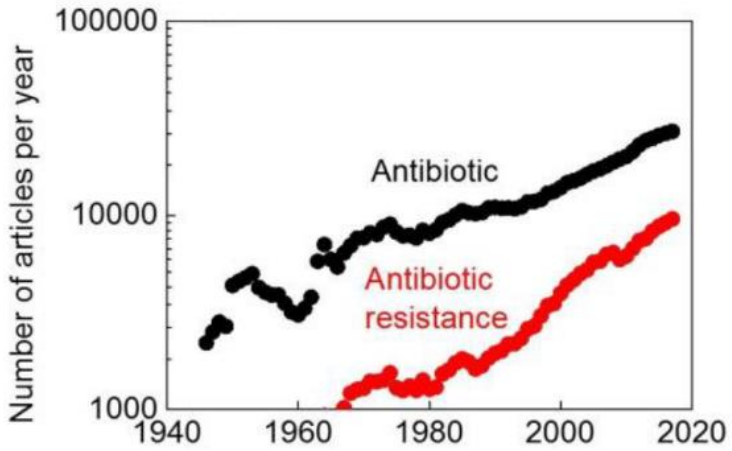


Antibiotic resistance on the rise: a global health problem



Reardon 2015 Nature





Antibiotic resistance on the rise: a global health problem

Resistance quickly develops via **mutation of target protein** (p ~10^{-8}/generation)

Suppression of resistance even by 2-3 orders of magnitude is already of clinical relevance (e.g. in dual-target agents)

Antibiotic resistance on the rise: a global health problem

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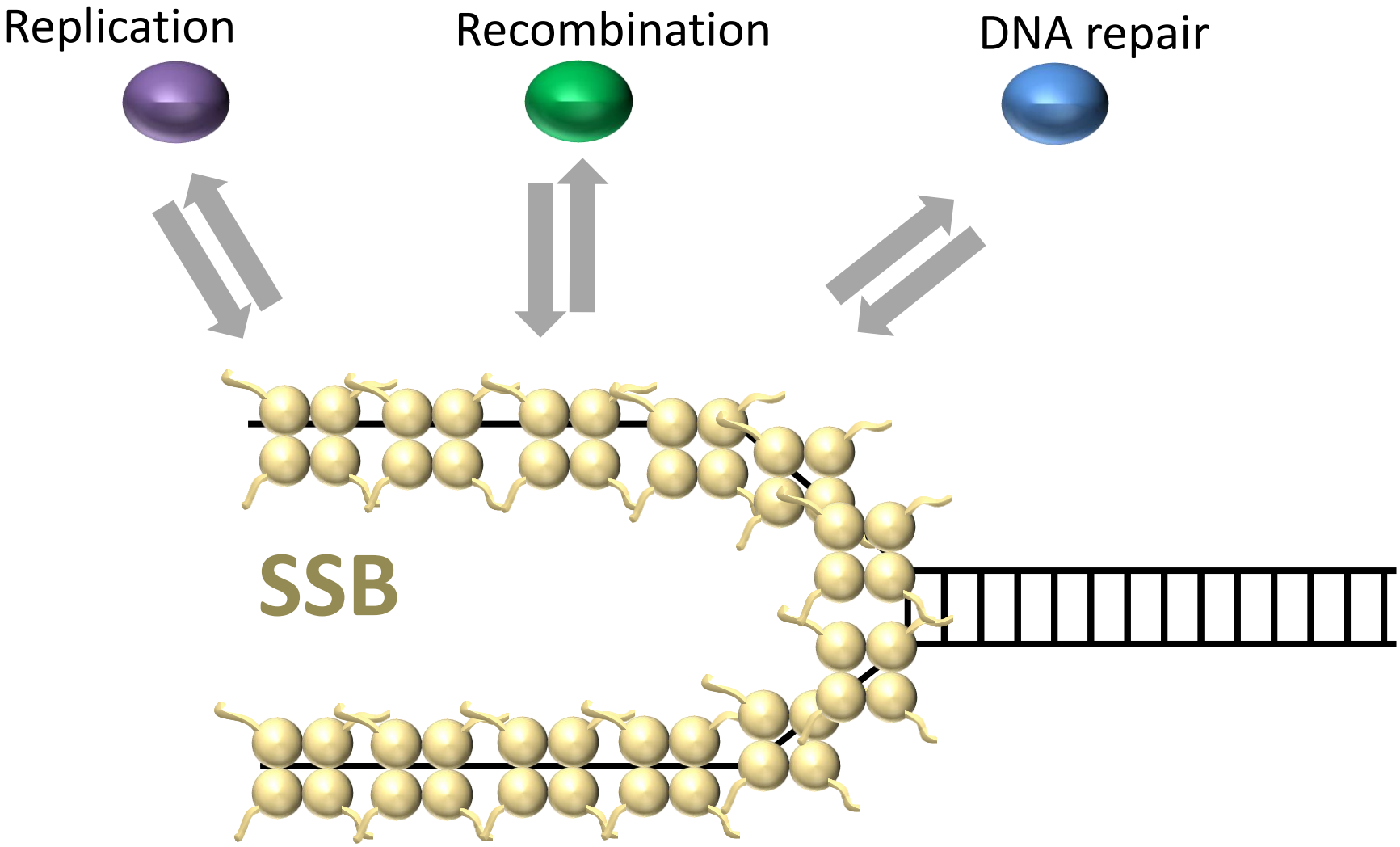
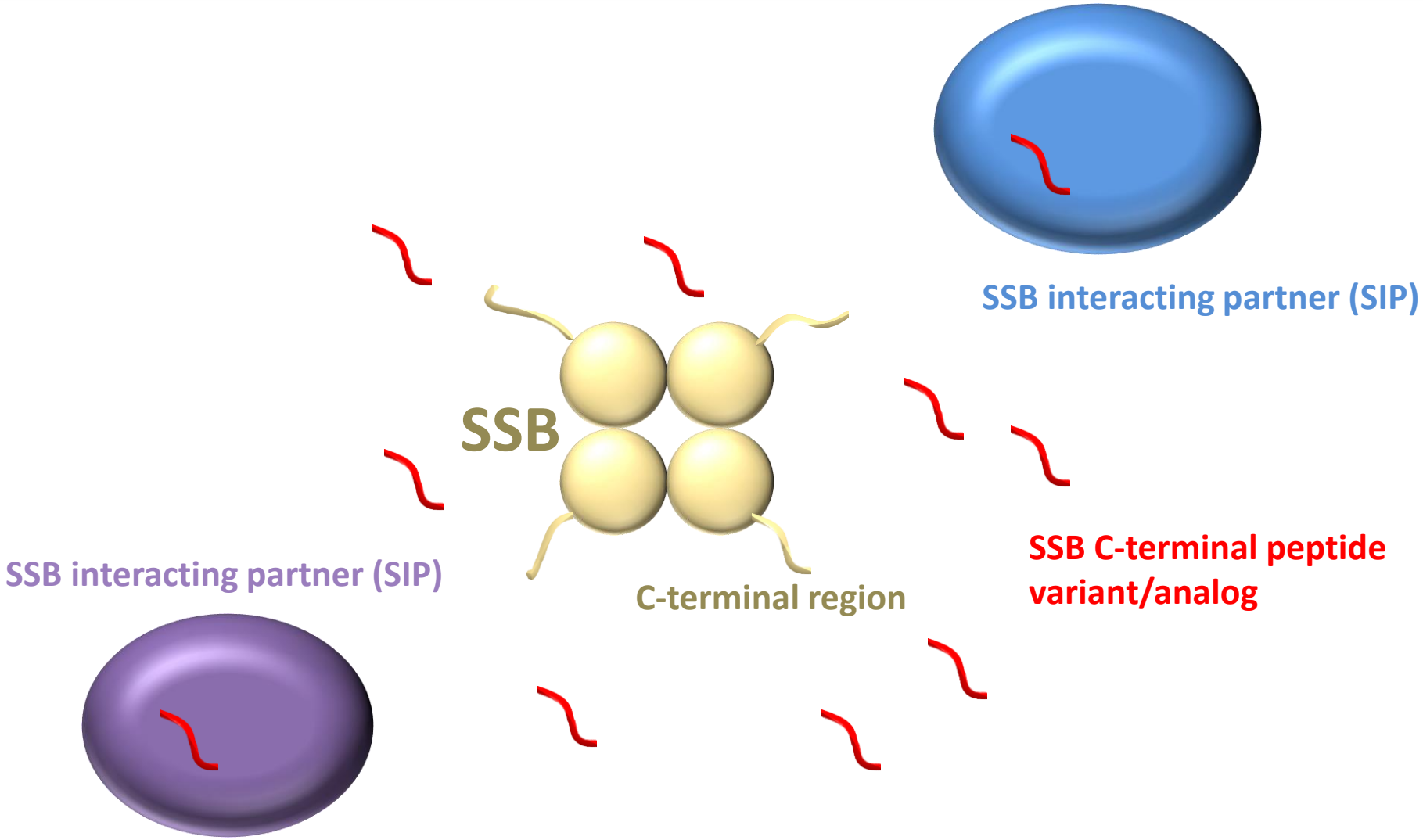
How about having many more targets, in a simultaneous and specific manner?

Target(s) in the cross-hairs: the SSB interactome

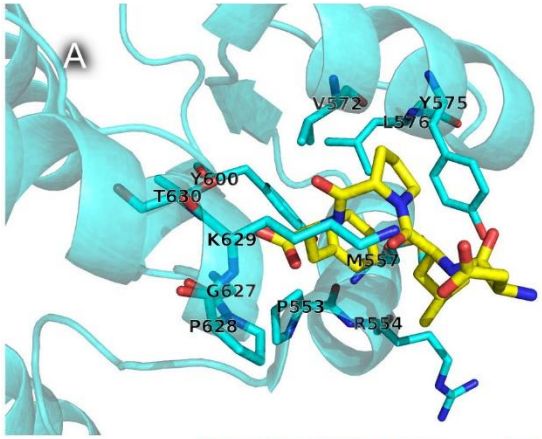


Intracellular competitive inhibition of SSB-SIP interactions

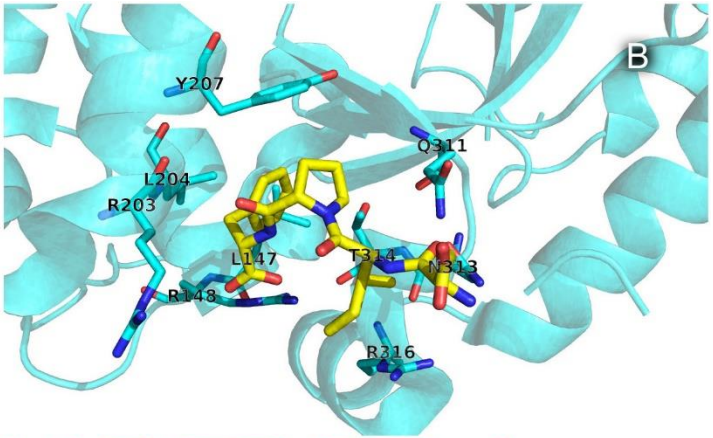


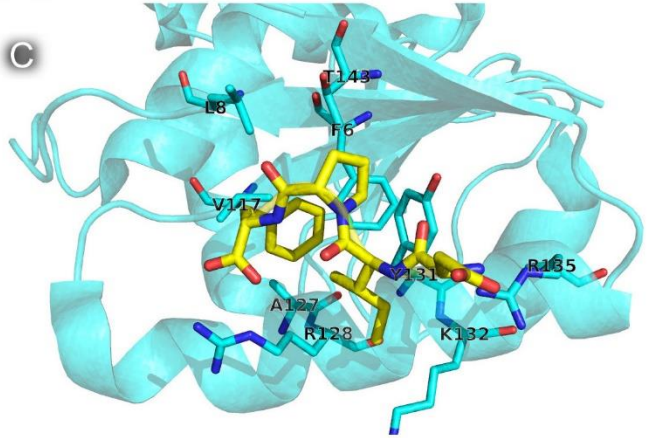
Convergent binding sites accommodate the SSB CTP

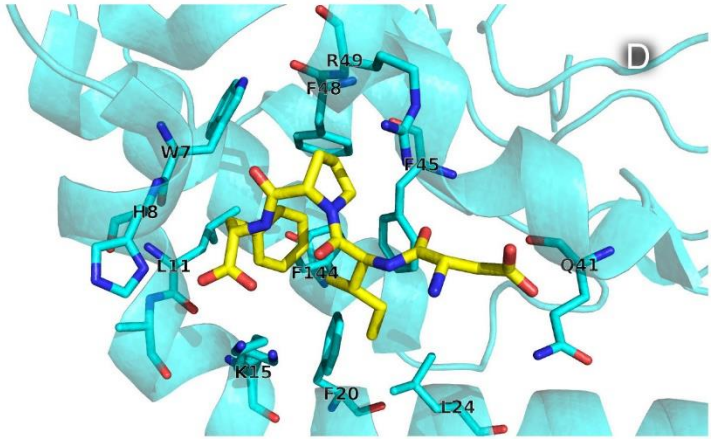
RecJ exonuclease



Exonuclease I





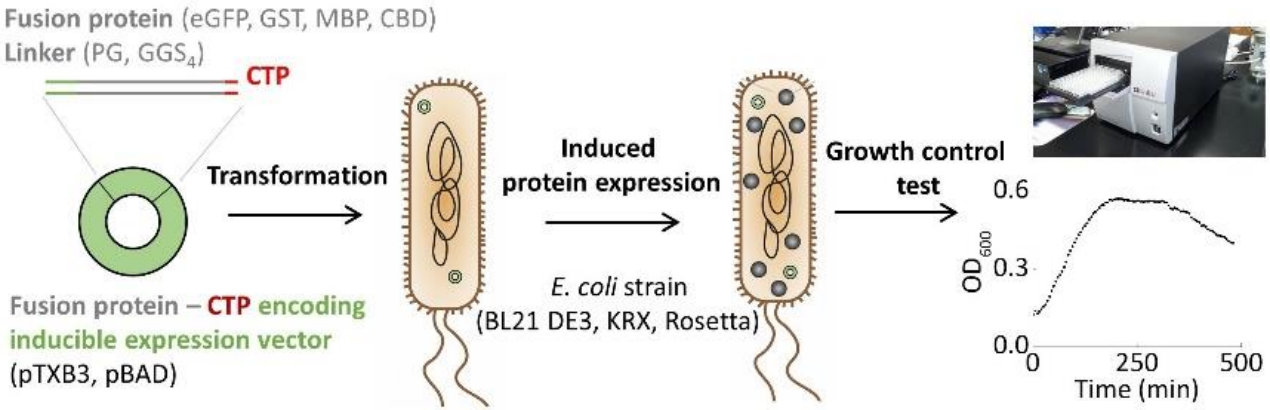


DNA polymerase III Chi subunit

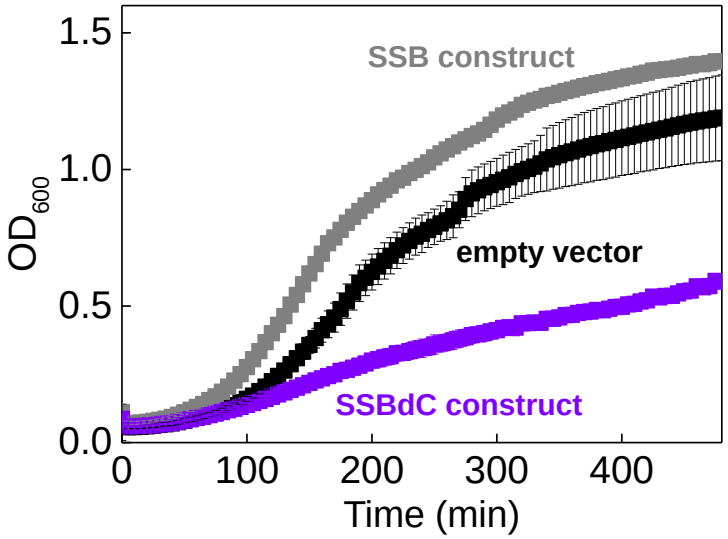
Uracil-DNA glycosylase

Intracellular competitive inhibition of SSB-SIP interactions

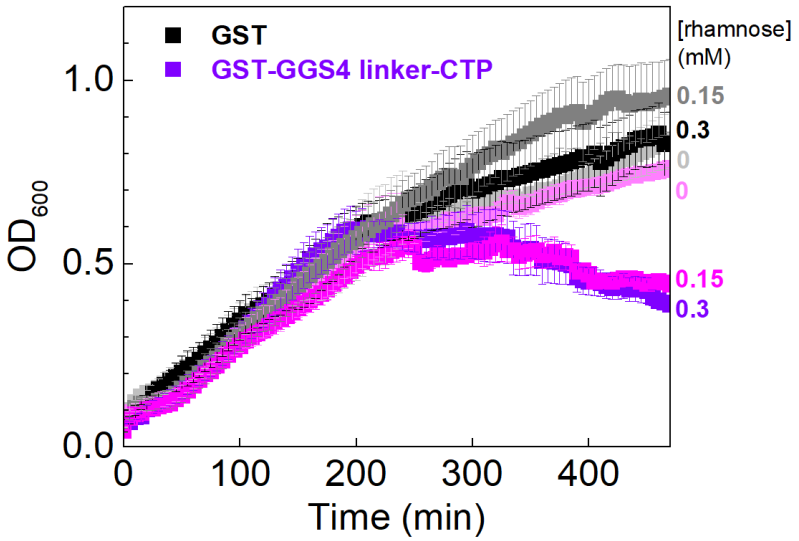
PINTA (Protein Interaction Network Targeting): Evolution-based high-throughput structure-activity approach to overcome bacterial drug resistance



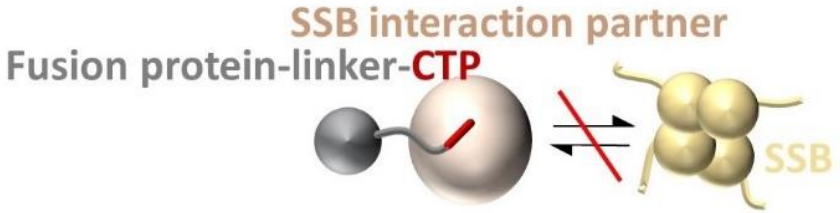
PINTA: Proof of concept
CTP-specific growth suppression



Bacterial growth suppression
***via* expression of CTP-less SSB variant**



Bacterial growth suppression
***via* expression of GST-linker-CTP fusion construct**



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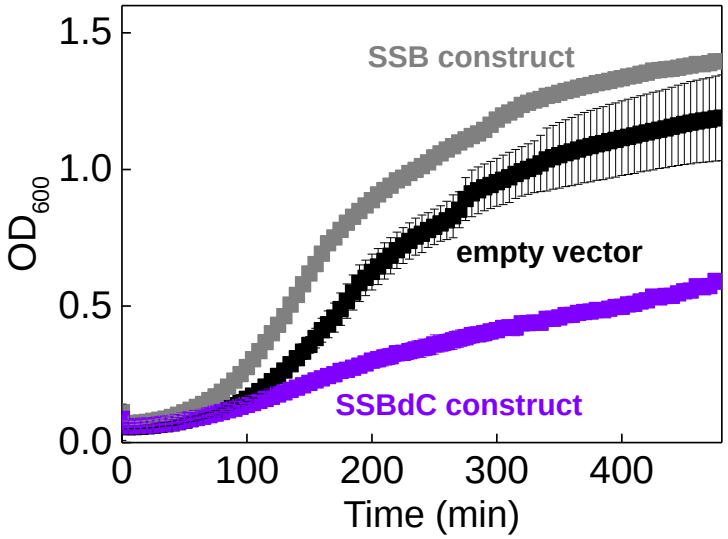

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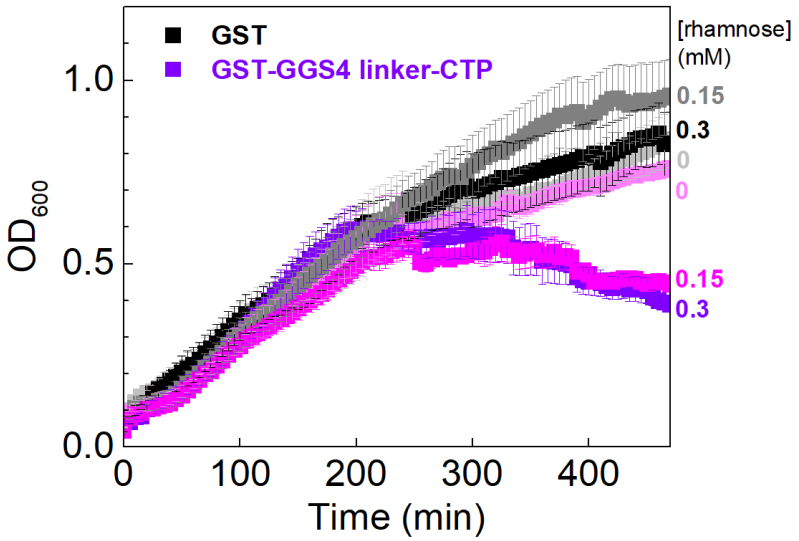

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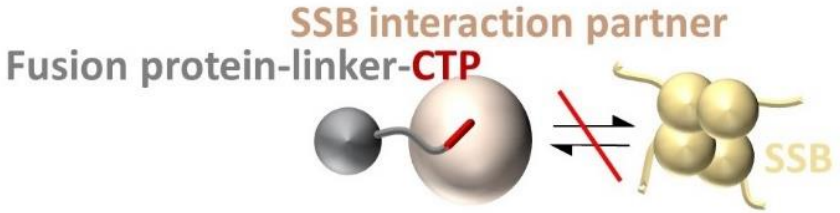
Useful dynamic range for **affinity potentiation**

PINTA: key pillars of concept

1. Targeting a **large PPI network**
- broadly conserved
- absent from eukaryotes



PINTA: key pillars of concept

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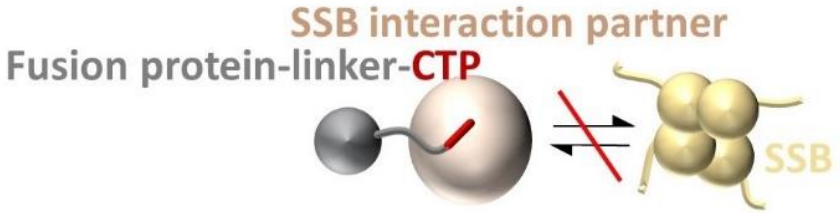
2. **Evolution-based high-throughput tests** for growth suppression

- high-throughput SAR screen (molecular interactions)



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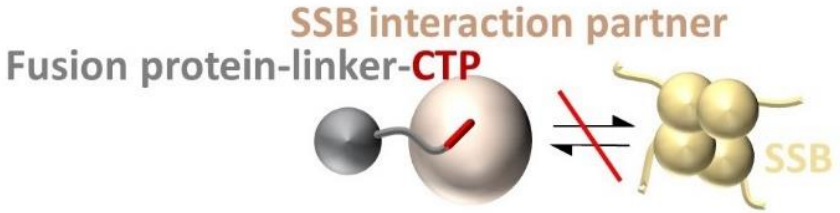

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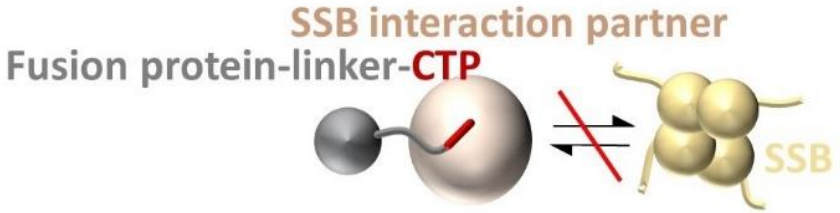
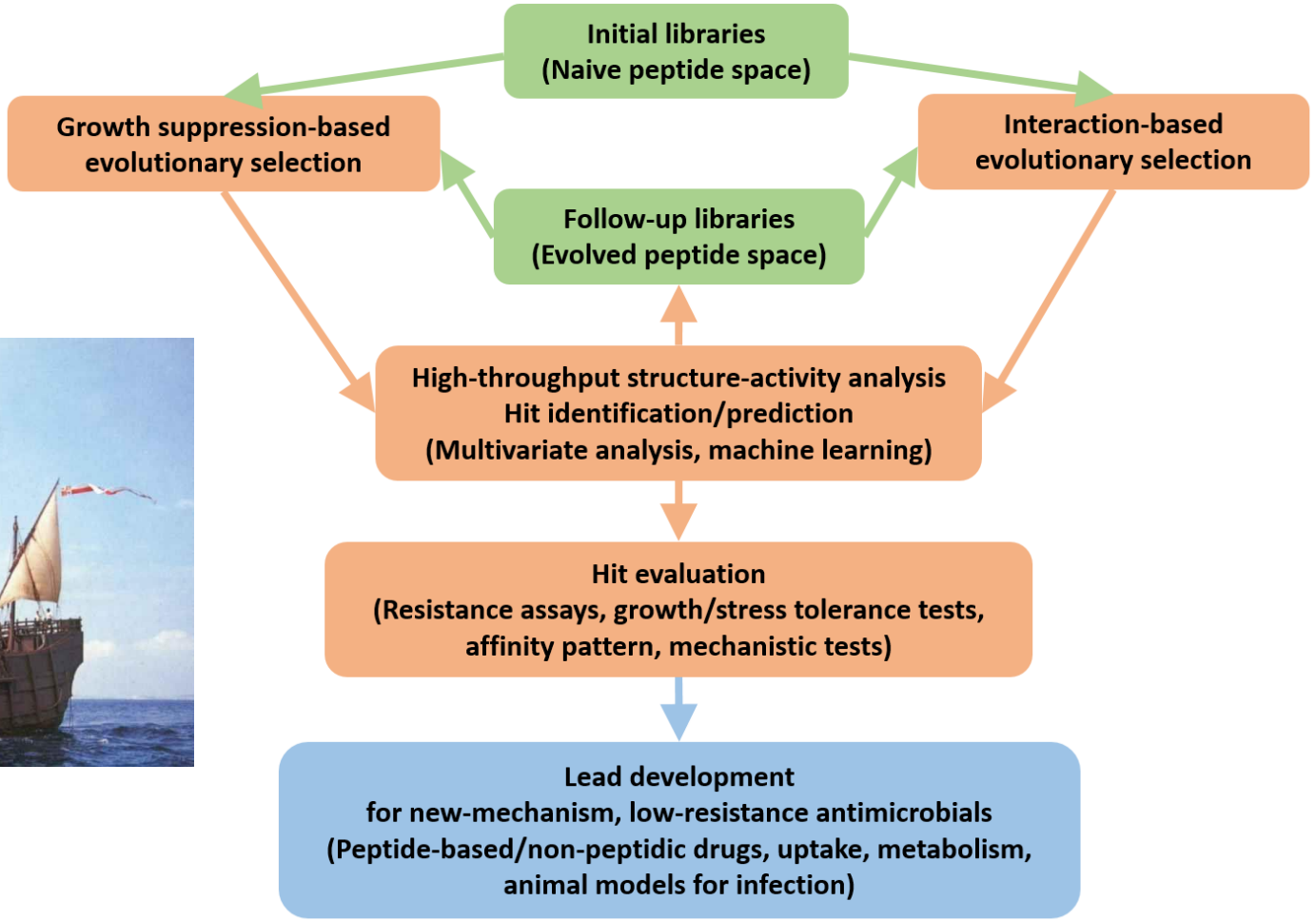
4. Data analysis: **machine learning**



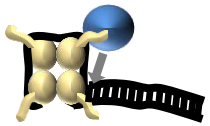
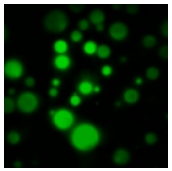
Advantages of the peptide-based approach

- Peptidic **natural ligand** of SIPs
- Evolutionary selection/screening - **vast chemical space** explored
- Rising potential of **peptide-based drug development**

PINTA: the flow



Action summary

	Discovered feature	Mechanistic determinant	Physiological function
	**Helicase shuttling** (local repetitive DNA unwinding)	**Mechanical tension** on displaced DNA strand	**HR quality control**
	**Oriented D-loop disruption**	**Geometry** of DNA strand junctions	
	**Non-linear pausing/stalling**	Local **base-pair energy** of DNA/mismatches	
	Access to DNA *via* **PPI-induced switch** in SSB-DNA binding mode	**Specific interaction** between SSB-CTP and SIP	**Organization/targeting of replication/repair complexes**
	**Liquid-liquid phase separation**	**Non-specific interaction** between SSB IDP regions **Specific interaction** between SSB-OB/CTP regions	**Rapid subcellular relocalization to genome replication/repair sites**

People who did it

ELTE-MTA „Momentum” Motor Enzymology Research Group
Department of Biochemistry, Eötvös Loránd University, Budapest, Hungary

Gábor Harami
Lili Farkas
Hajnalka Harami-Papp
Kristóf Jóna
Zoltán Kovács
János Pálinkás
Zsófi Turóczi

Alumni
Veronika Baráth (now at Univ. Copenhagen)
Anna Budai
Máté Gyimesi (UC Davis, now at Malnalab, ELTE)
Andries Napo Leemisa (now applying at Max Planck IMPRS-CMB)
Julianna Németh
Debasish Roy (now at Boston University)
Kata Sarlós (now at Univ. Copenhagen)

Collaborators
National Heart, Lung and Blood Institute, NIH, USA
Yeonee Seol
Keir C. Neuman
Research Centre for Natural Sciences
Rita Pancsa
ELTE
Krisztián Tárnok (ÉNB)
András Málnási-Csizmadia (BIK)

NEMZETI KUTATÁSI, FEJLESZTÉSI ÉS INNOVÁCIÓS HIVATAL

AZ INNOVÁCIÓ LENDÜLETE

AZ NKFI ALAPBÓL MEGVALÓSULÓ PROGRAM