RNA-driven DNA modifications from bacteria to human cells

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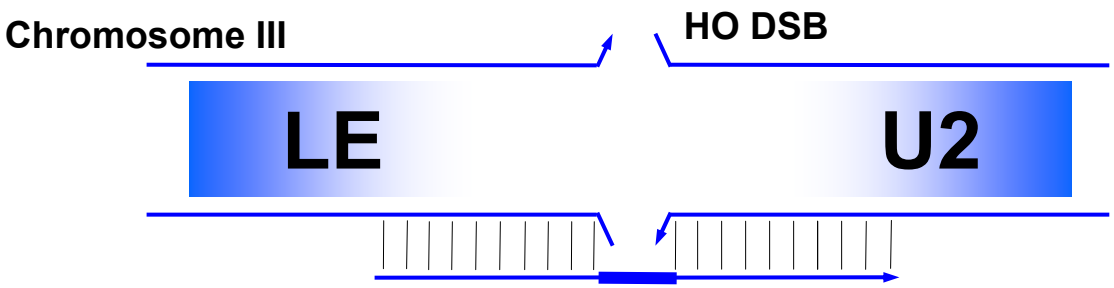
New functions of RNA

Central dogma of molecular biology

- **Template for repair of DNA double-strand break (DSB)**
- **Template for genetic modification**

RNA-templated DSB repair in yeast



Repair frequency (Leu$^+$) x 10^{-7}

Oligonucleotides 80-mers

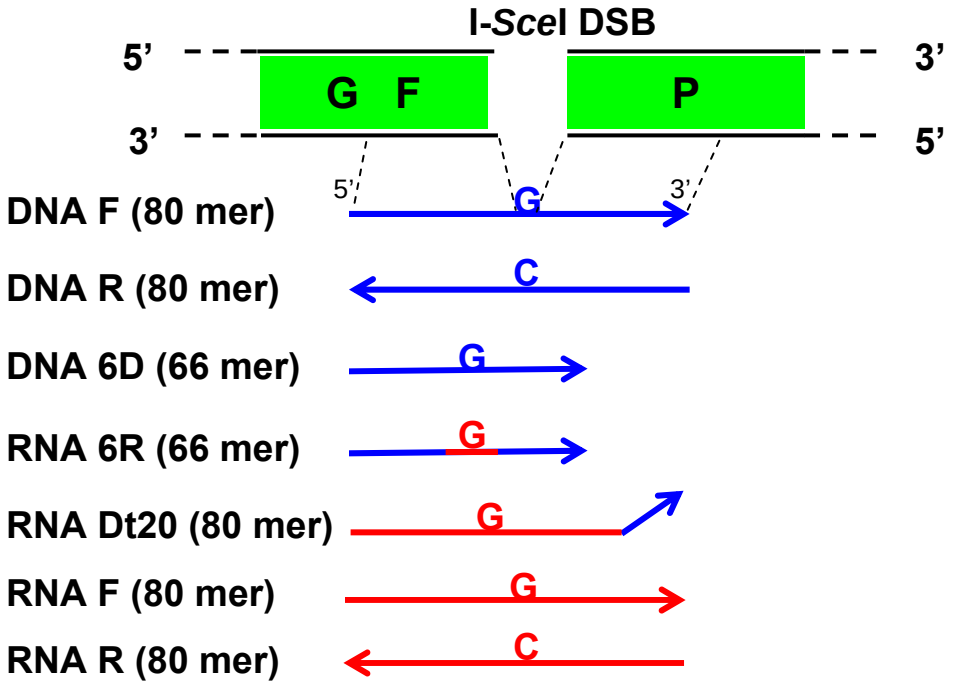
	Repair frequency (Leu$^+$) x 10^{-7}	
[D 34]-ins::D 12-[D 34]	220,000	
[D 34]-ins::D 4,R 4,D 4-[D 34]	66,000	
[D 34]-ins::R 6,D 6-[D 34]	45,000	
[D 34]-ins::R 12-[D 34]	4,100	16/18 correct sequence
[R 38]-R 2-[R 40]	5.2	
[R 40]-R 2-[R 38]		
[R 28]-R 2-[R 30]-D 20 tail	420	16/16
no oligo	<0.1	0/16 (only ins/del)

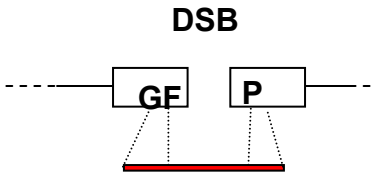
Storici et al., Nature 2007

Storici, Curr. Opin. Mol. Ther. 2008

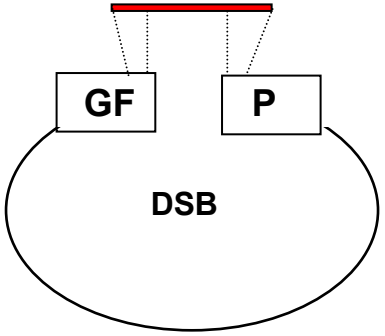
RNA can repair a DSB!
And directly transfer genetic information to yeast genomic DNA!

Assay to detect RNA-driven DNA repair in human cells



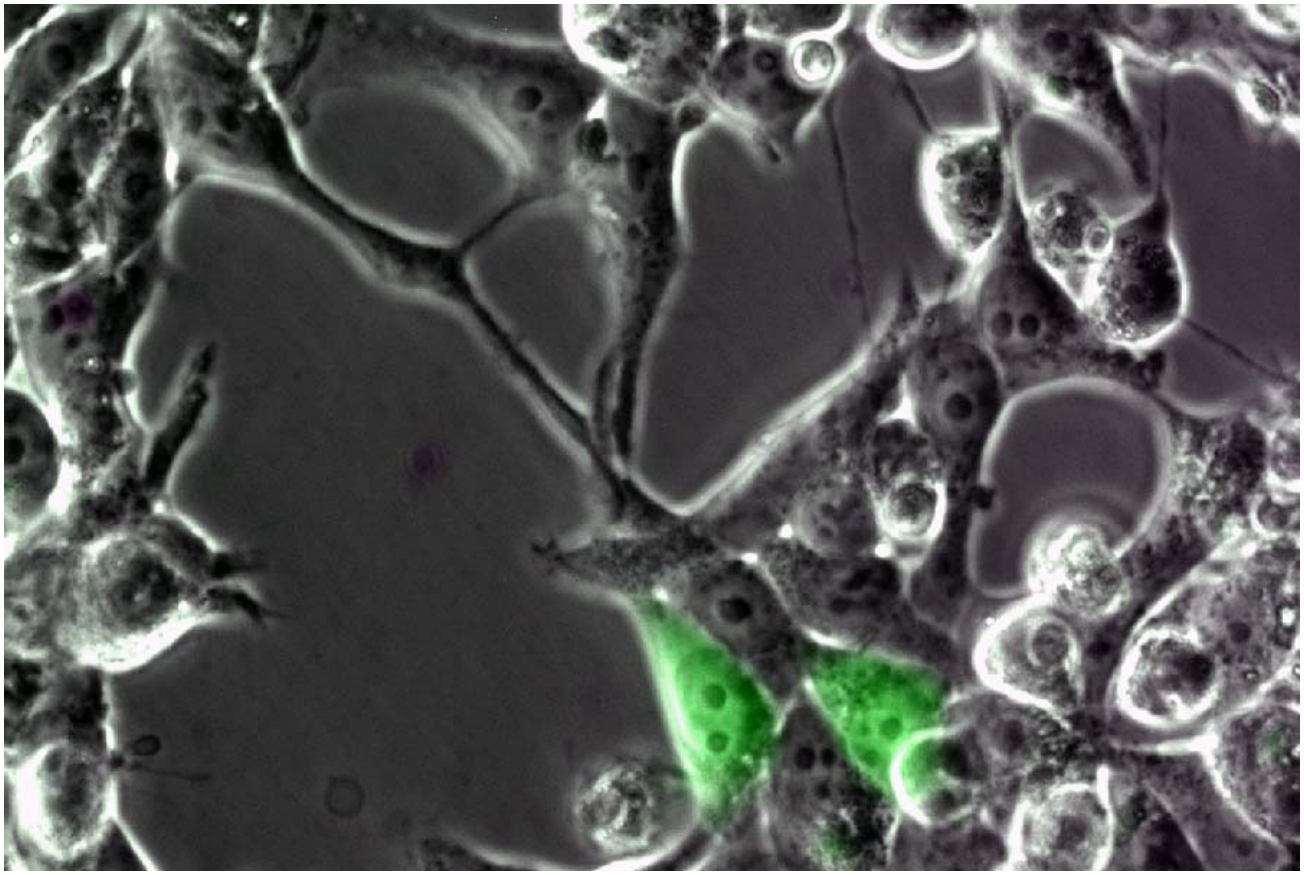


GFP on chromosome



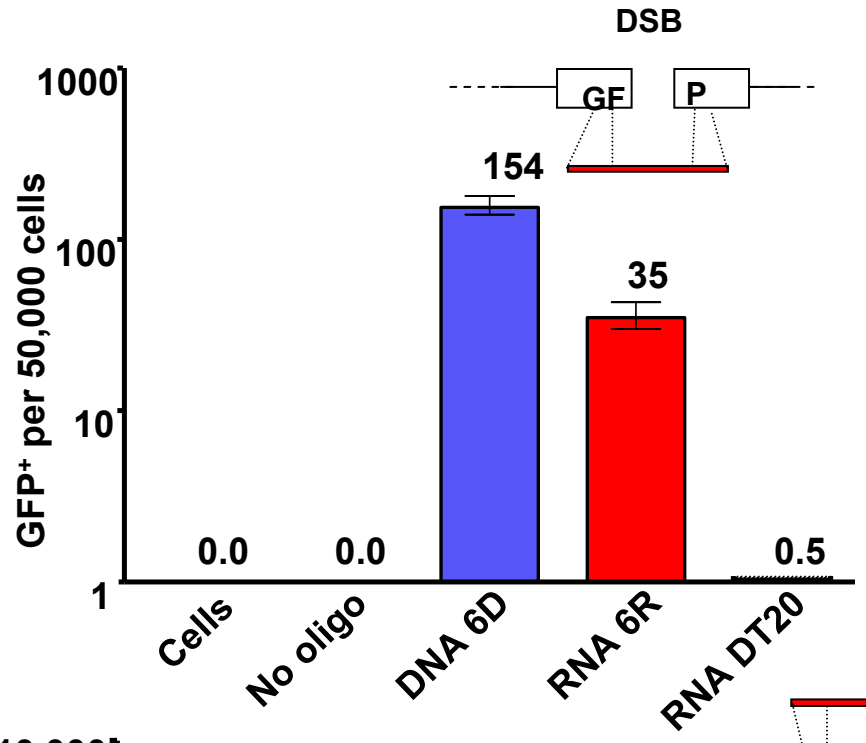
GFP on plasmid

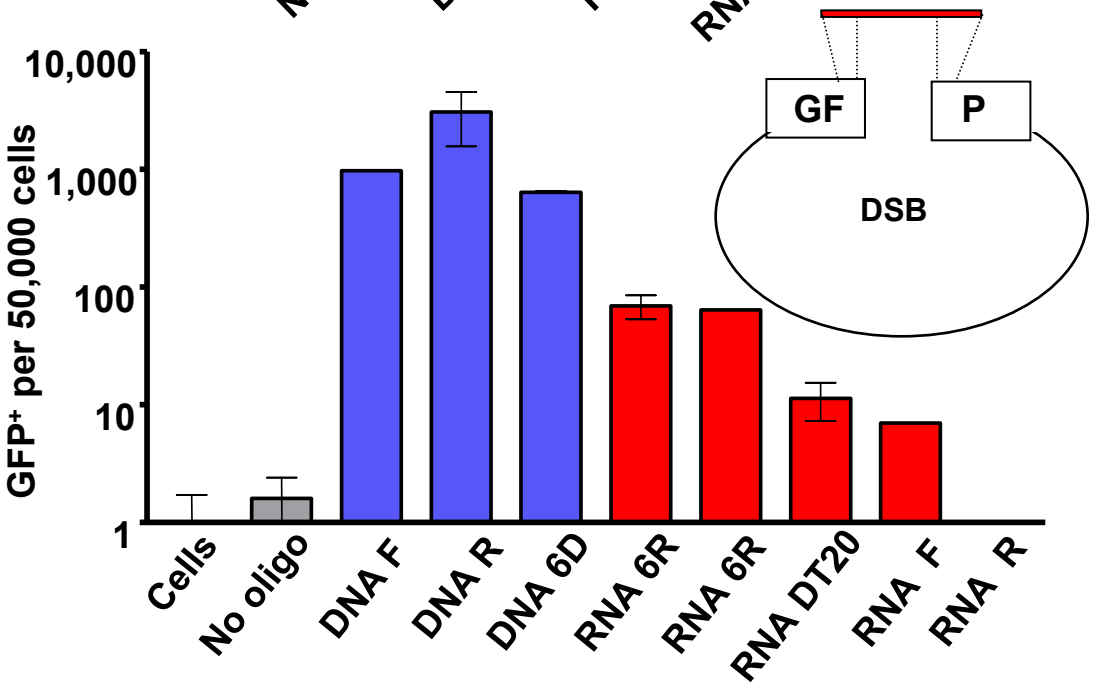
RNA-containing oligos can be used as template for plasmid and chromosomal DSB repair in human cells

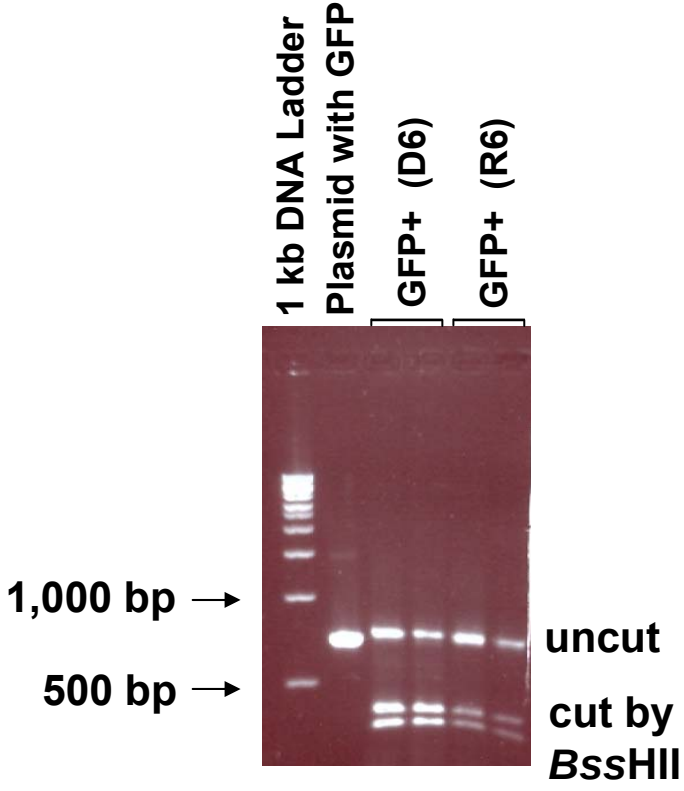


GFP positive (bright) human HEK-293 cells where oligos repaired the DSB

Frequencies of RNA–driven DNA repair and modification in human cells







The information flows from the RNA tract to human genomic DNA

RNA-driven DNA modification in *E. coli*

Chromosome
lacZ-minus …CATCT___TCGCTGGGGAATAAGTCAG…

DNA 65-mer
lacZ$^+$ …CATCTGGTCGCTGGGGAATGAGTCAG…

~ 1%

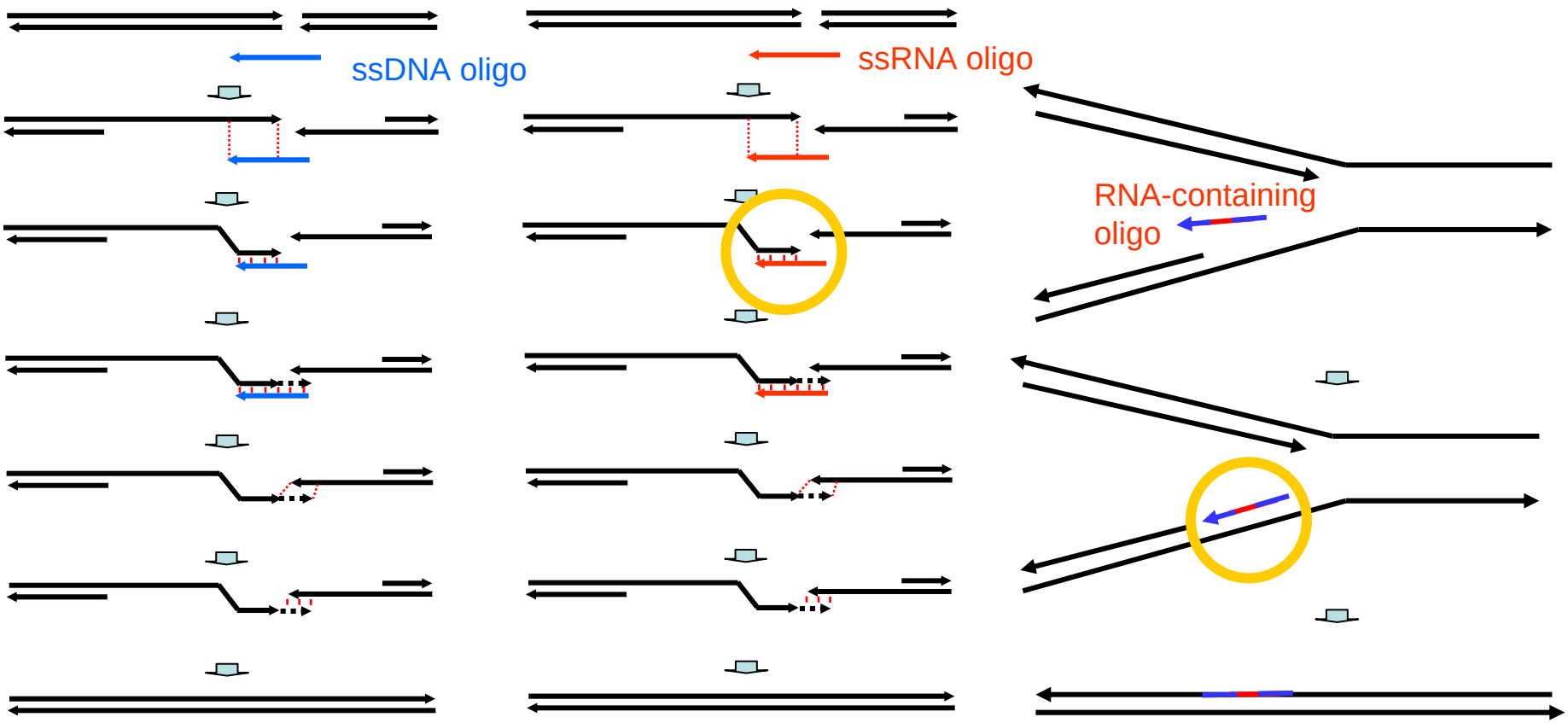
DNA-6R-DNA 65-mer
lacZ$^+$ …CATCUGGUCGCTGGGGAATGAGTCAG…

Genotype	Rel. transformation efficiency (%) [rN]$_6$RNA/DNA
Wild type	21

Two steps of single strand annealing

Hypothetical model for RNA-templated DSB repair

Hypothetical model for RNA-driven gene modification

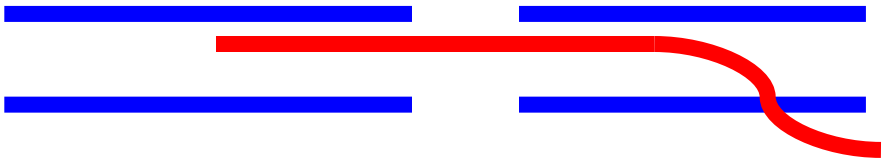


Storici, et al., MCB 2006

Mechanism of RNA-DNA hybrid stability ?

DNA/RNA hybrids
where information can flow from RNA to DNA

(i) RNA annealed with broken DNA



RNA-driven DNA repair

Storici et al, Nature, 2007

(ii) RNA embedded into DNA

Patel and Loeb, JBC, 2000

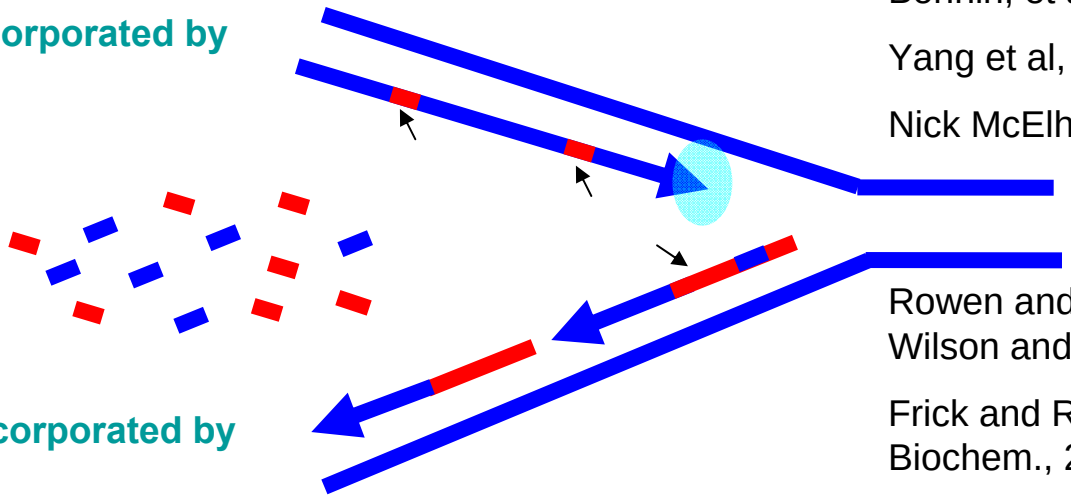
Astatke et al., PNAS, 1998

Bonnin, et al., JMB, 1999

Yang et al, Cell, 2002

Nick McElhinny et al., MCB, 2003

rNTPs incorporated by DNA Pols



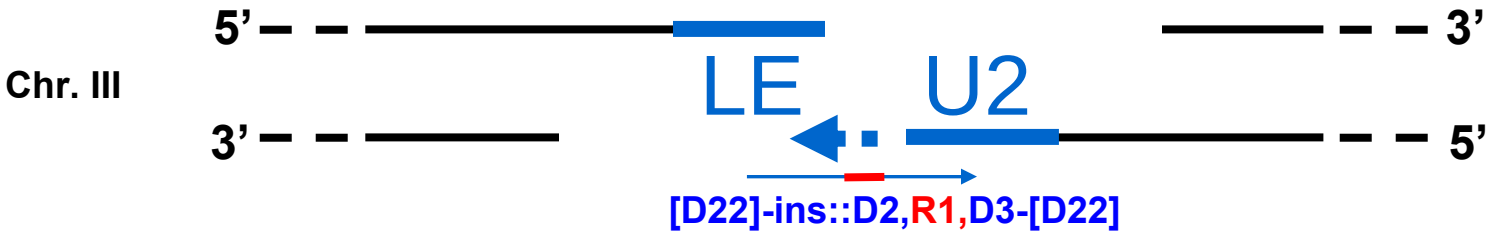
dNTPs incorporated by primase

Rowen and Kornberg, JBC, 1978
Wilson and Sugino, JBC, 1985

Frick and Richardson, Annu. Rev. Biochem., 2001

Kornberg, DNA Replication 2005

RNase H1 and H2 do not suppress DSB repair by a 1R-containing oligo in yeast

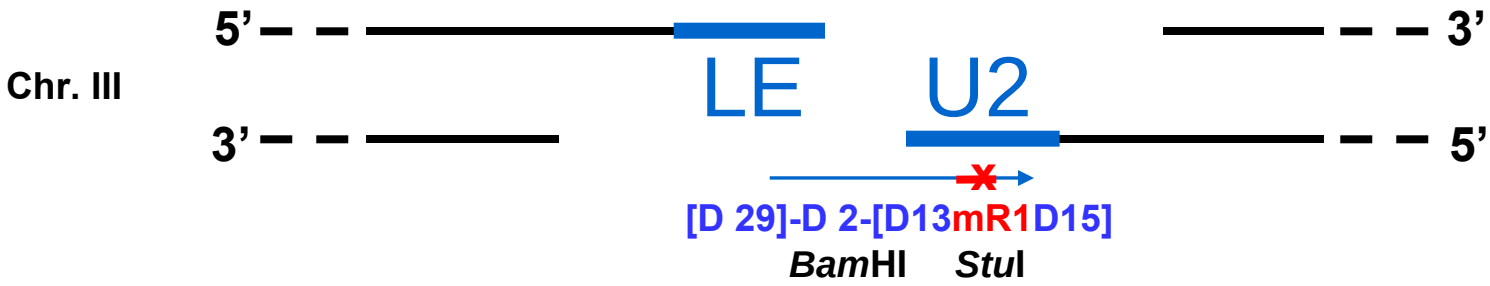


Deletion of RNase H1 and H2 genes

	Oligonucleotide	Transformation frequency (per 10^7 cells)	Rel. freq. *mut* / WT
WT	[D22]-ins::D2,R1,D3-[D22]	2,873 (1,710-3,181)	1.0
	[D22]-ins::D6-[D22]	1,349 (741-1,452)	1.0
rnh1 rnh35	[D22]-ins::D2,R1,D3-[D22]	2,268 (1,326-3,023)	0.8
	[D22]-ins::D6-[D22]	1,306 (928-1,814)	1.0

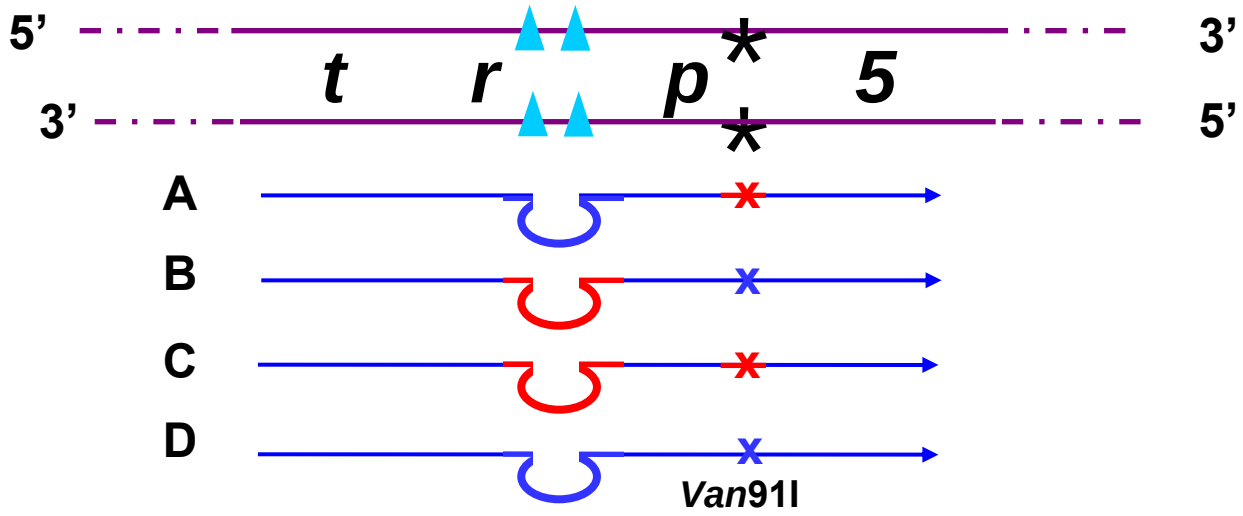
1R in DNA does not appear to be a block for DNA replication

RNase H1 and H2 suppress RNA-driven gene correction by 1.5 fold in yeast



Oligonucleotides	Genotype	RE	#	Dig. #	Dig. frequency
[D 29]-D 2-[D13mR1D15]	WT	StuI BamHI	24 35 48 24	7 9 10 24	0.29 0.26 0.21 1.0
[D 29]-D 2-[D13mD1D15]	WT	StuI BamHI	24 47 24	18 29 24	0.75 0.62 1.0
[D 29]-D 2-[D13mR1D15]	*rnh1 rnh35*	StuI BamHI	24 48 47 24	9 21 15 24	0.38 0.44 0.32 1.0
[D 29]-D 2-[D13mD1D15]	*rnh1 rnh35*	StuI BamHI	24 47 24	15 28 24	0.63 0.60 1.0

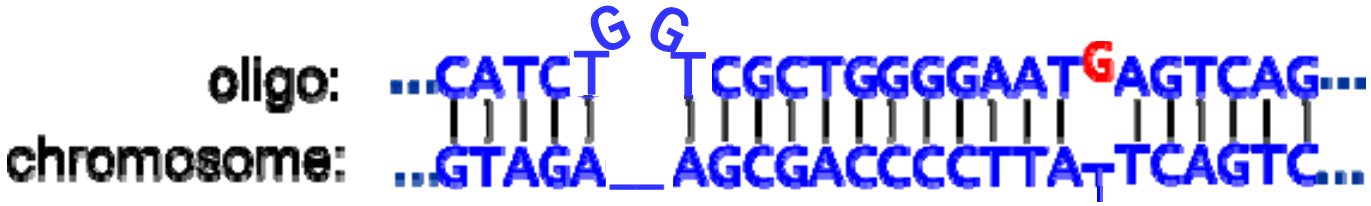
RNase H2 suppresses 2-base insertion and 1-base substitution by RNA-containing oligos in yeast



	Oligonucleotides	Genotype	#	Transformation frequency (per 10^7 cells)
A	[D 24]-ins::D2-[D14 mR1 D24]	WT	16	0.7 (0.4-1.5)
B	[D33 R2]-ins::R2-[R2 D33]		8	0 (0-0)
C	[D22 R2]-ins::R2-[R2 D12 mR1 D24]		8	0.7 (0-1.4)
D	[D 24]-ins::D2-[D14 mD1 D24]		8	2.4 (1.4-4.3)
A	[D 24]-ins::D2-[D14 mR1 D24]	*rnh35*	16	2.4 (1.5-3.6)
B	[D33 R2]-ins::R2-[R2 D33]		8	4.0 (2.7-4.8)
C	[D22 R2]-ins::R2-[R2 D12 mR1 D24]		8	1.8 (0.6-4.7)
D	[D 24]-ins::D2-[D14 mD1 D24]		8	2.7 (1.2-3.5)

≥2.5 fold

Strong effect of RNaseH II on replication of a single mismatched ribonucleotide in *E. coli*



Genotype	Rel. transformation efficiency (%) $[rN]_1$RNA/DNA
Wild type	0.09 ± 0.04
Δ*rnhB*	65 ± 3.9

>600 fold!

Conclusions

- We have developed a series of assays to study mechanisms how RNA can modify genomic DNA

- RNA can have a direct role in DNA repair and modification from bacteria to mammalian cells

- RNA tracts in DNA are differentially cleaved by RNaseHI/1 and HII/2 enzymes *in vivo*

- DNA with embedded 1R to 6R can be replicated

- Stable transmission of information from RNA to DNA might contribute to both genome integrity and variation

Thank you!

Collaborators

- Bernard Weiss (Emory Univ.)
- Tom Kunkel (NIEHS)
- Kasia Bebenek

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Storici's lab

Posters:

- Increasing Gene Targeting in Human Cells – Patrick Ruff
- Differential effect of RNases H enzymes on gene correction by RNA-containing oligos in yeast and E. coli cells – Ying Shen
- Yeast and mammalian systems to characterize the potential of DNA nicks to promote recombination and gene targeting – Samantha Stuckey
- Development of different assays to study DNA amplification both within a cell population and at the single cell level in vivo in yeast Saccharomyces cerevisiae – Kuntal Mukherjee