Balancing Somatic Hypermutation and DNA Repair in Immunoglobulin Genes

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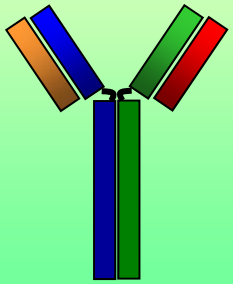
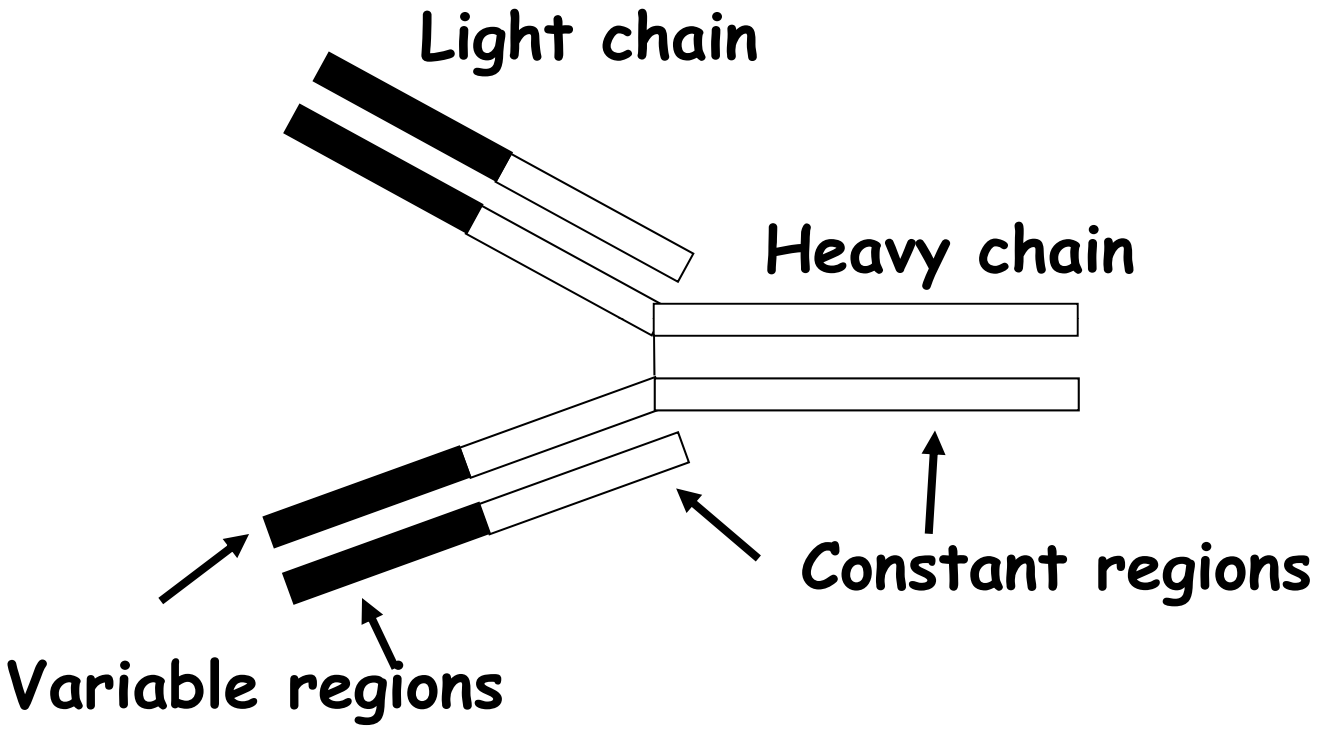


ANTIBODY (B cells)

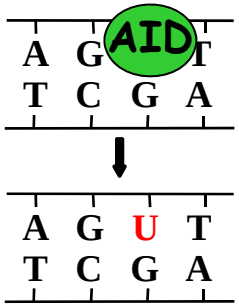


Bind pathogen;
hypermutation

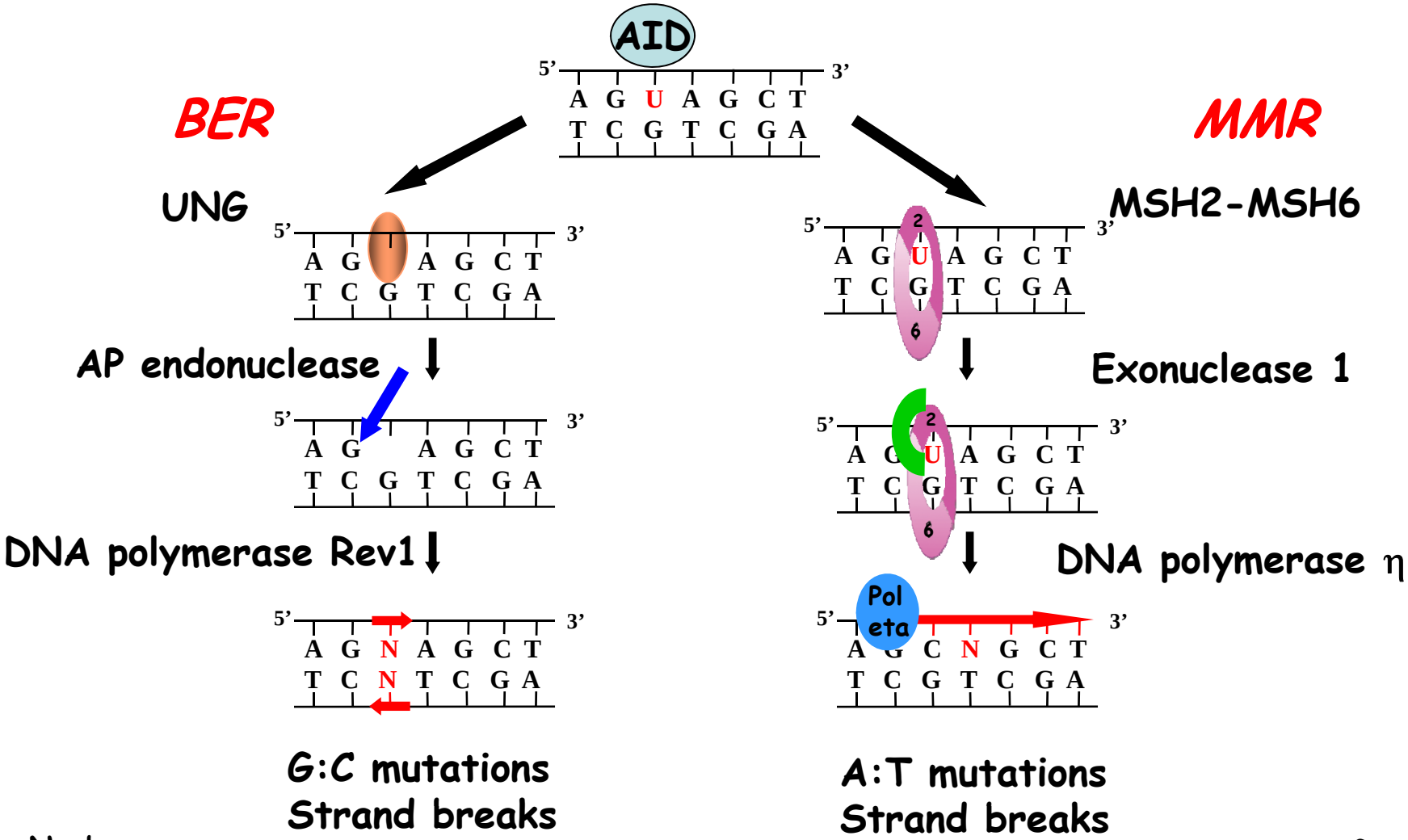
Eliminate pathogen;
switch recombination

Activation-Induced Deaminase (AID)

deaminates cytosine to uracil



U:G is mutagenic: strand breaks & mutations



Neuberger
Stavnezer
Jacobs

Gearhart
Scharff
Reynaud

I. The Master Catalyst

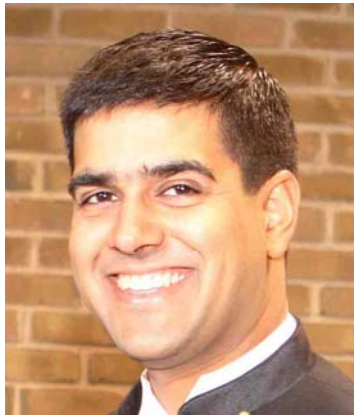
II. Going Rogue

III. The Targeting Enigma

I. AID - the Master Catalyst

Protein loop that recognizes the AID hot spot: WRC (W = A/T; R = A/G)

Rahul Kohli, JHU



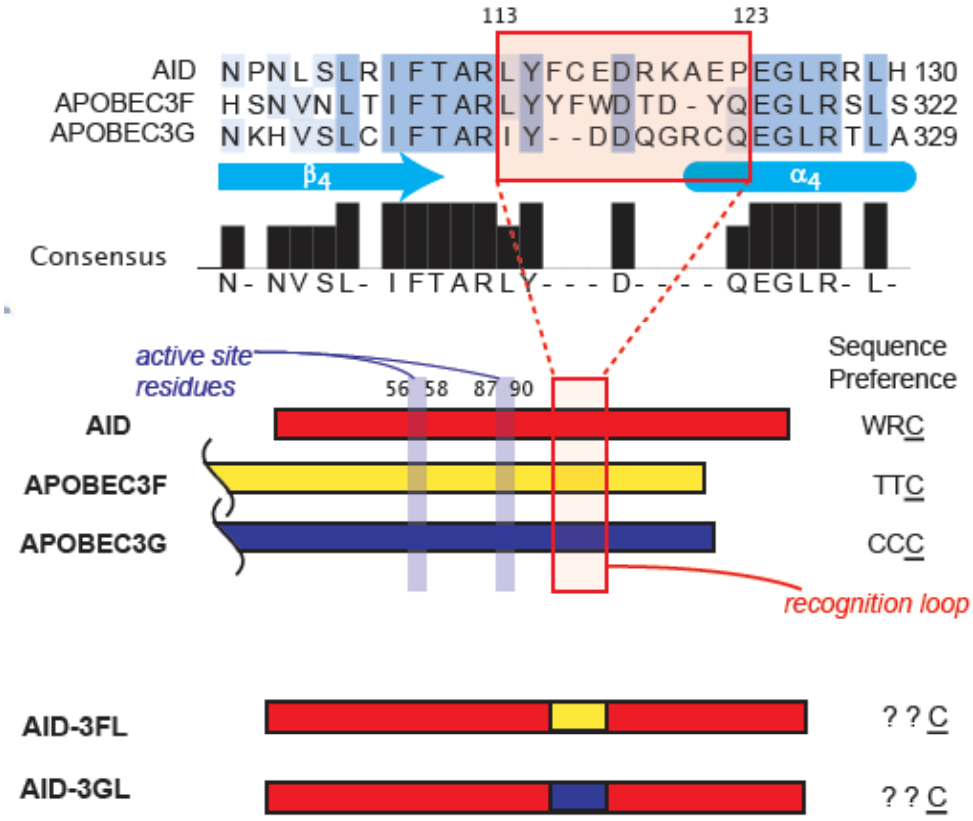
JBC 284:22898-04 (2009)

DNA cytosine deaminases

Antibody diversity:	AID	WR<u>C</u>
HIV retroviruses:	APOBEC 3F	TT<u>C</u>
	APOBEC 3G	CC<u>C</u>

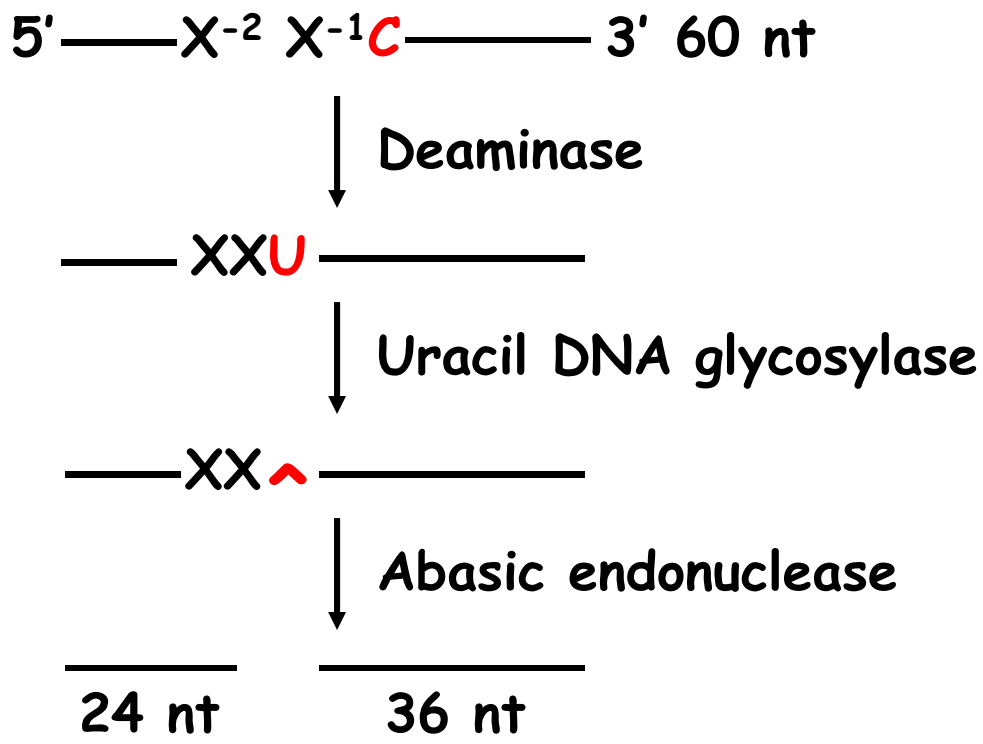
How do they recognize different motifs?

Structural alignment suggests a "hotspot recognition loop"



Hypothesis: Grafting loops from the APOBEC3 enzymes into AID will lead to predictable changes in specificity

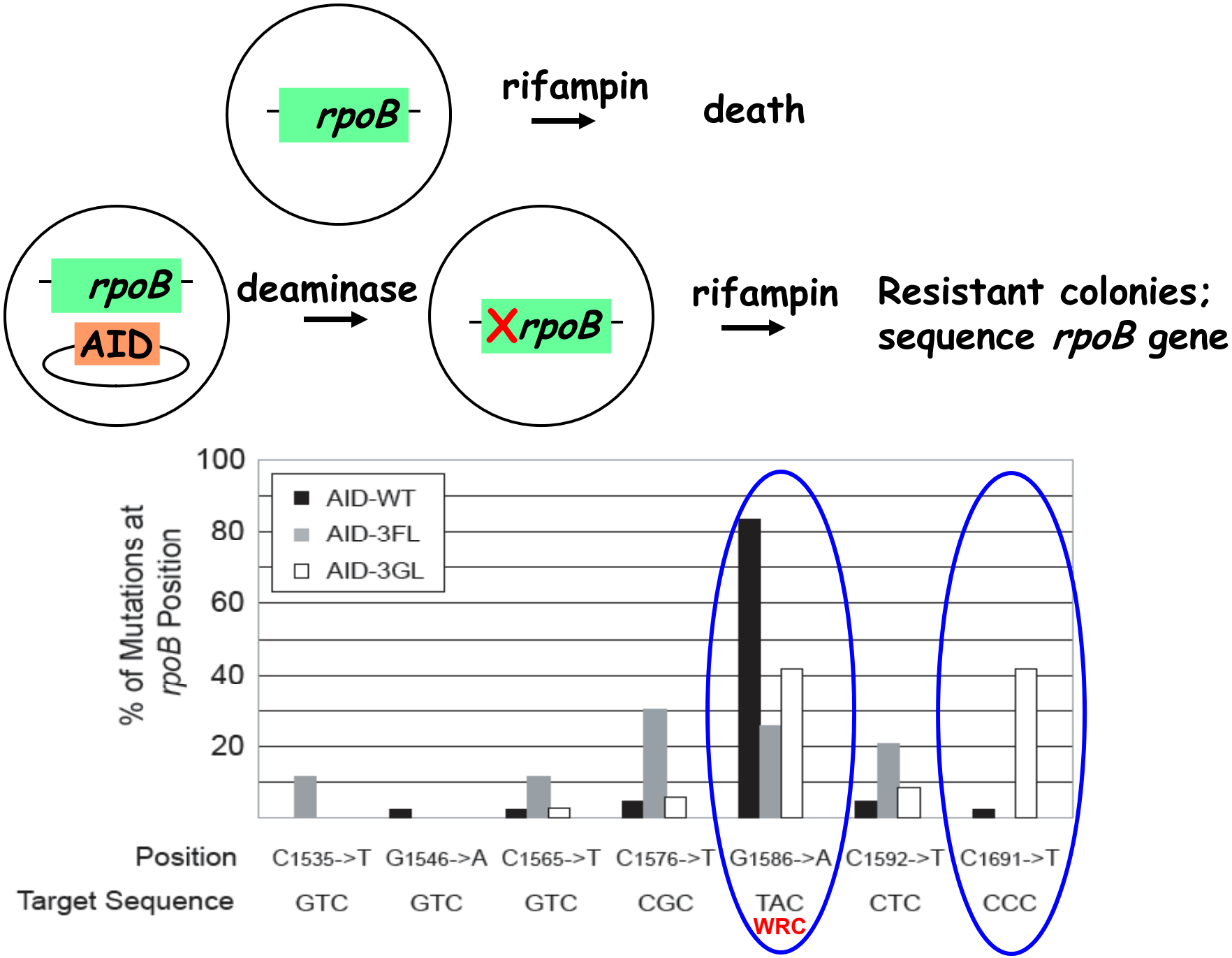
Purify variant deaminases from bacterial cultures. Incubate with oligonucleotides containing XXC (X = A, mC, G, or T).



AID/APOBEC loop graft variants change sequence motifs as predicted

AID	WRC
AID-3FL	TTC
AID-3GL	CCC

Are motifs altered in *E. coli*?


rpoB
rifampin
death
rpoB
AID
deaminase
XrpoB
rifampin
Resistant colonies;
sequence rpoB gene
% of Mutations at rpoB Position
100
80
60
40
20
AID-WT
AID-3FL
AID-3GL
Position
C1535->T
G1546->A
C1565->T
C1576->T
G1586->A
C1592->T
C1691->T
Target Sequence
GTC
GTC
GTC
CGC
TAC
CTC
CCC
WRC

9 amino acid loop in AID is responsible for recognizing hot spot motif WRC.

Similar loops in APOBEC3F/G can be transferred to change specificity of AID.

II. Going Rogue – Uracils in DNA

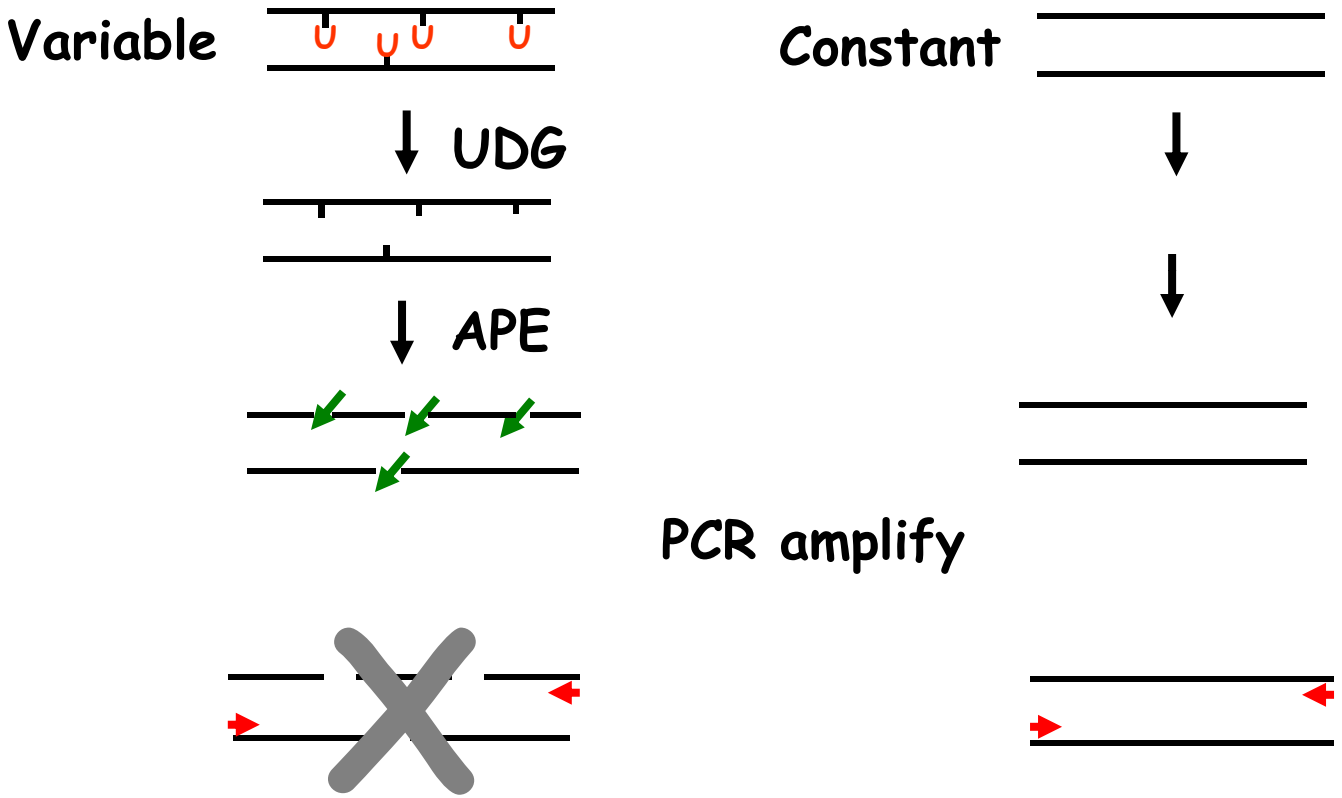
AID proposed to deaminate RNA, whereas genetic and biochemical data support DNA deamination

…but, no evidence for uracils in immunoglobulin loci.

Uracils in variable region DNA from B cells

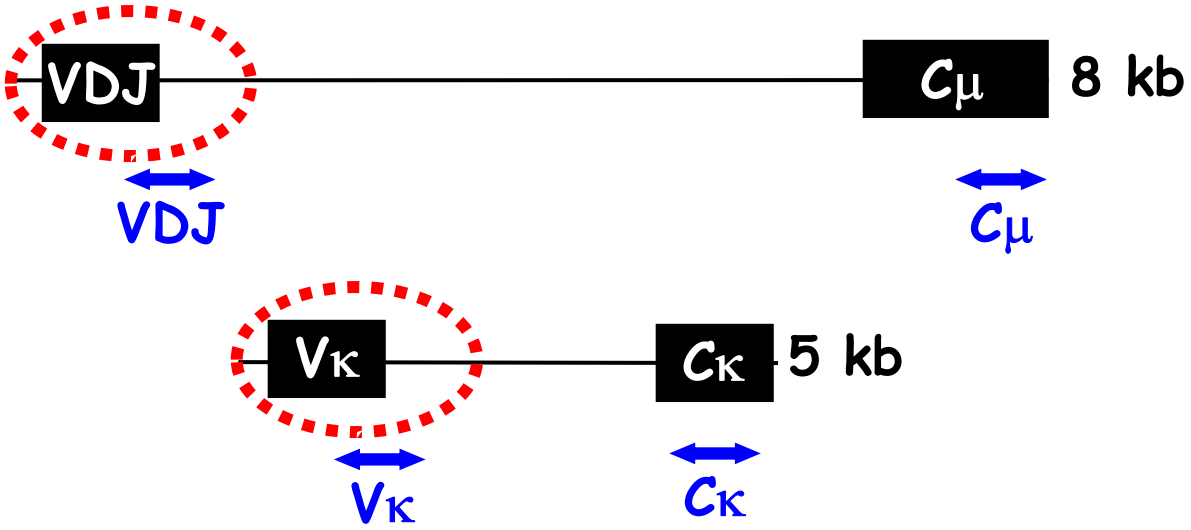
Rob Maul

Digest DNA (*Ung*$^{-/-}$ Peyer's patch B cells) with uracil glycosylase (UDG) and abasic endonuclease (APE)

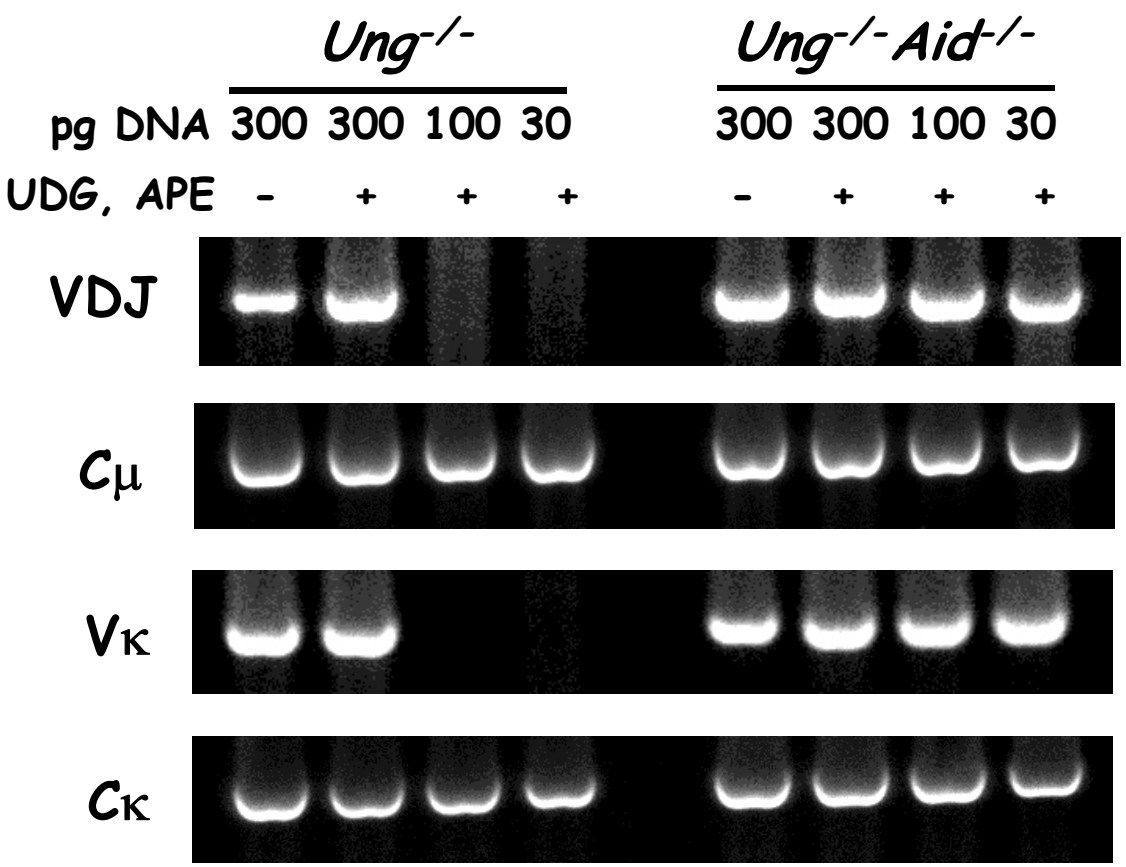


Hypothesis: more uracils = less amplification of V gene

Location of primers for nested PCR



PCR assay



More UDG-sensitive sites in V genes than C genes; AID dependent.

III. The Targeting Enigma

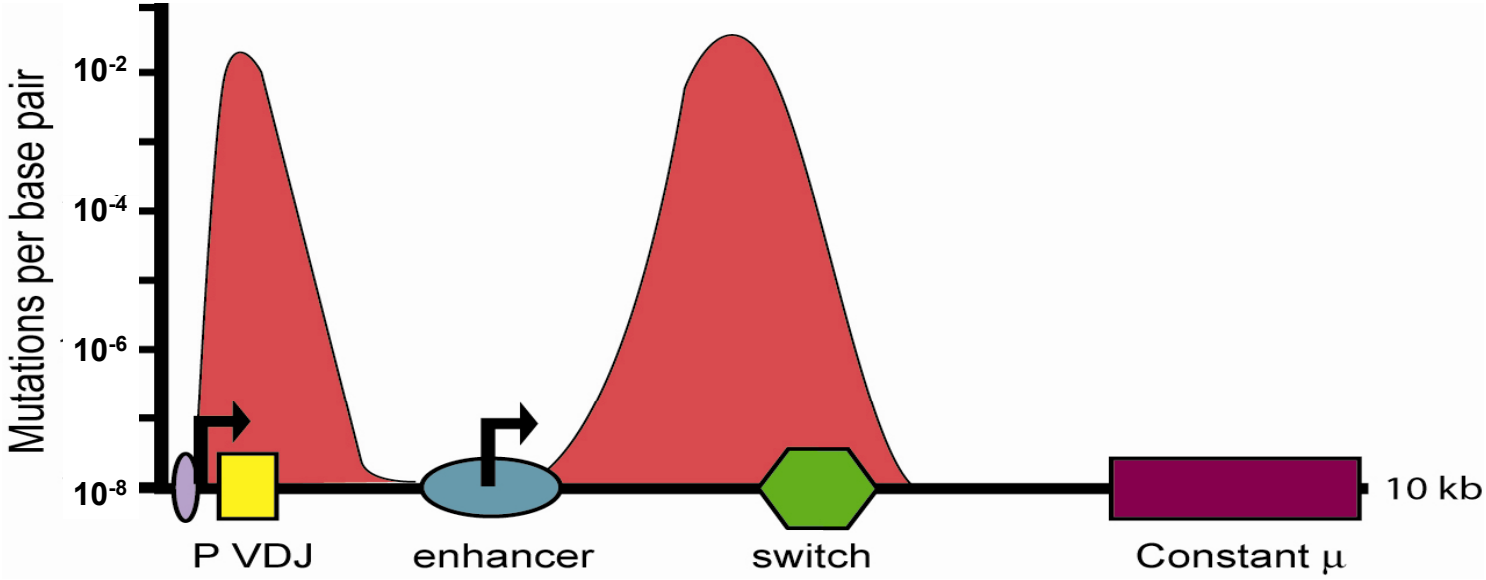
Setting up the Sμ locus for AID

Rob Maul

Deepa Rajagopal

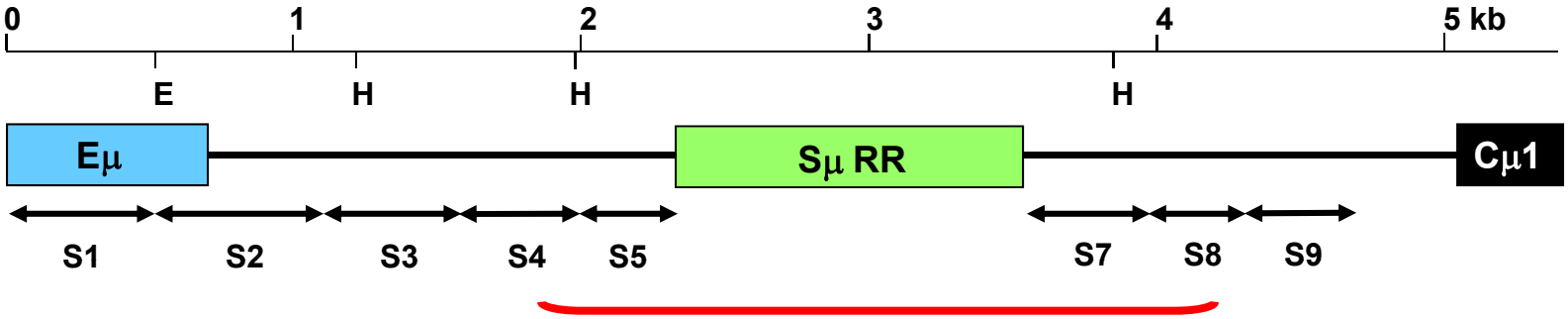
JEM 206:1237-44 (2009)

Peaks of hypermutation after V and Iμ promoters



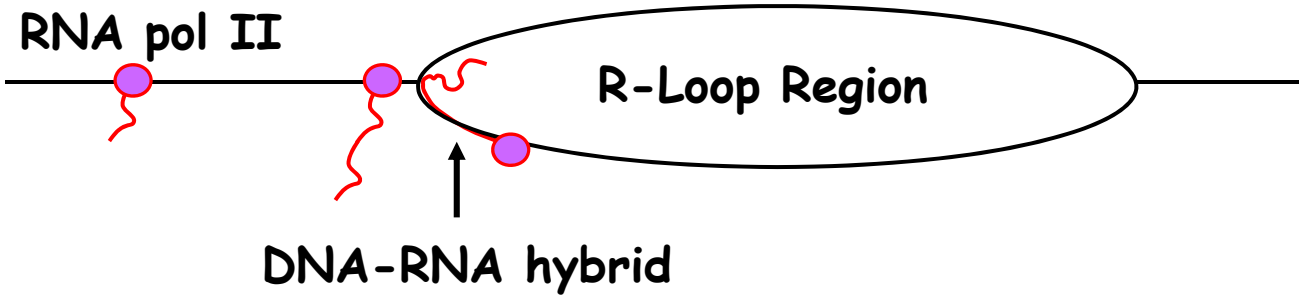
Does location of RNA pol II correlate with this pattern?

How does DNA structure in Sμ affect transcription?



GGGGAGCTGGGGAGCTGGG

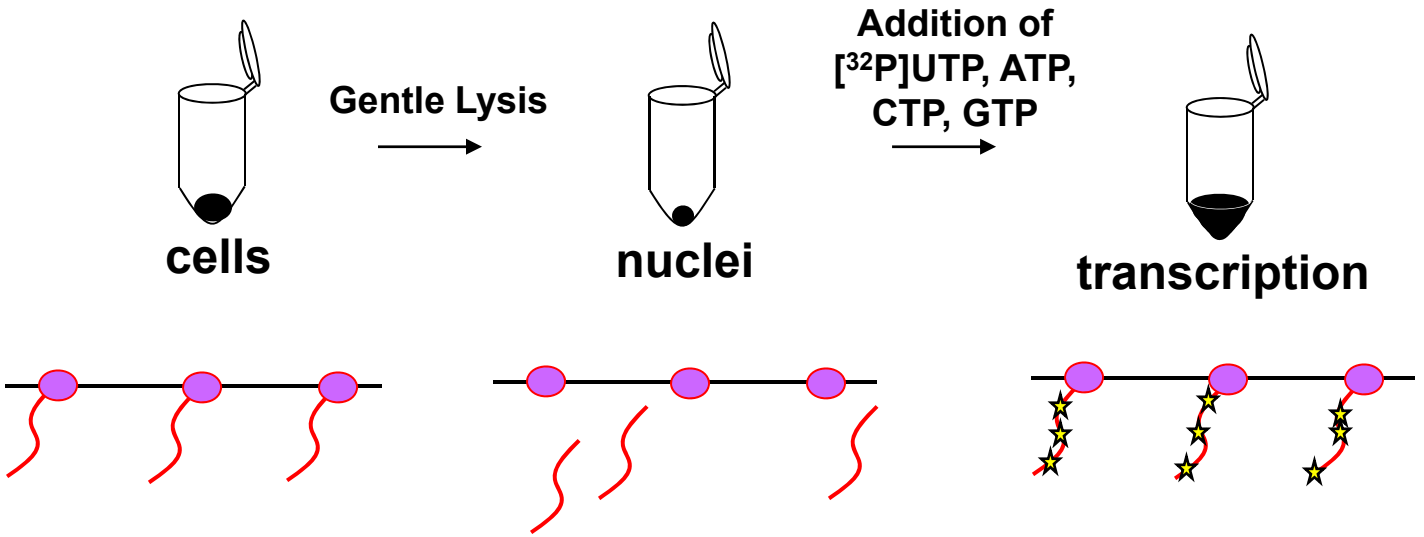
Clusters of G and AGCT hotspots for AID



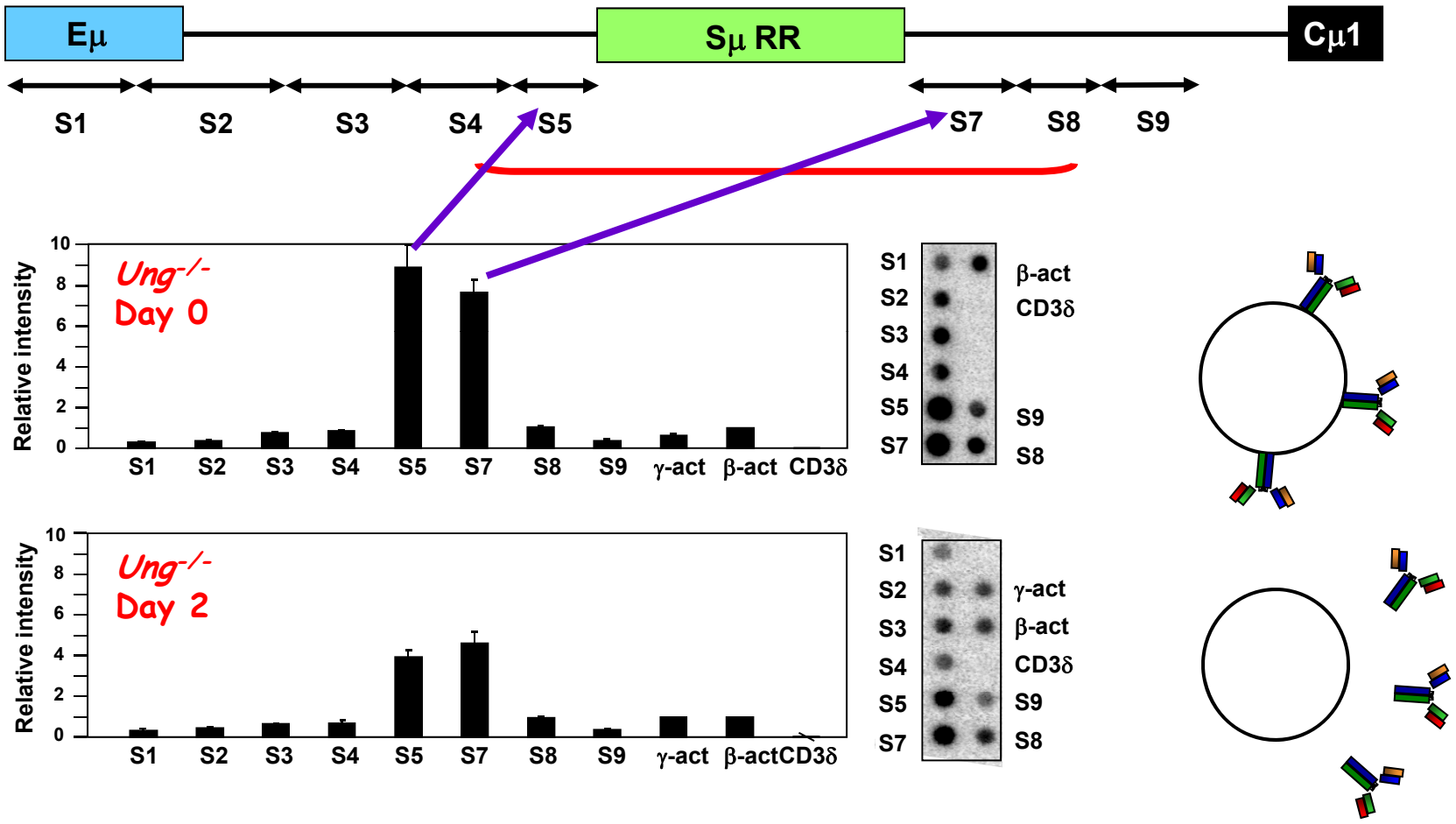
Lieber

Map the position of RNA pol II by nuclear run-on

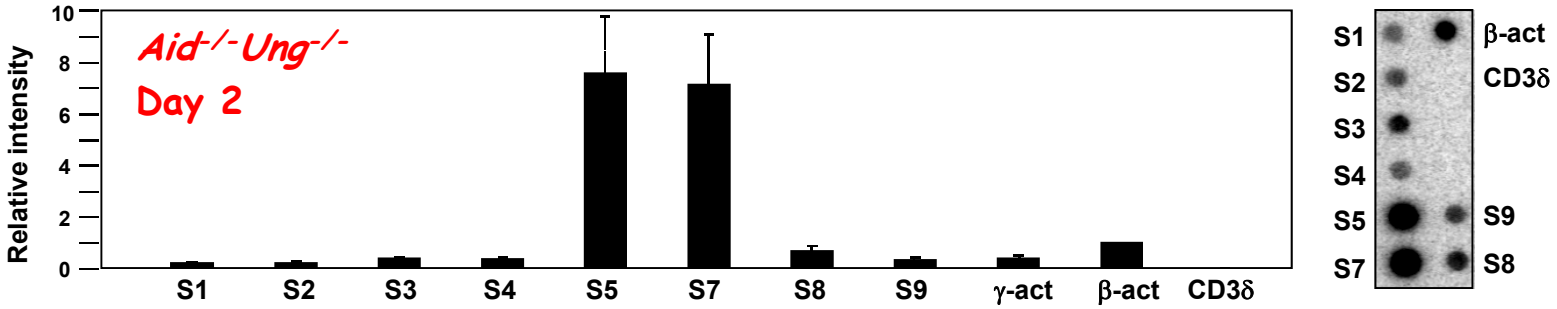
Stimulate *Ung*$^{-/-}$ splenic B cells with LPS + IL4. After 2 days, isolate nuclei.

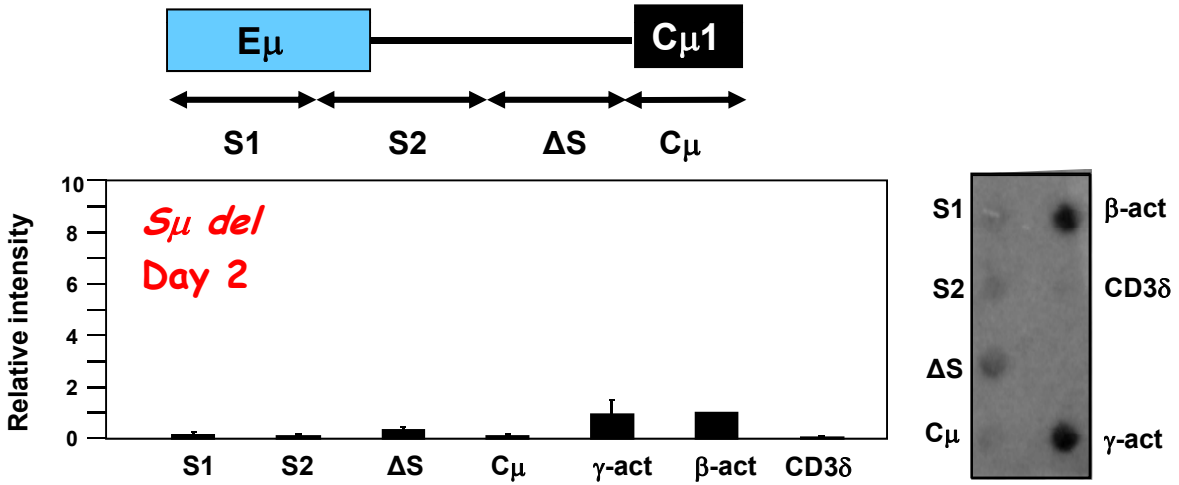


Position of RNA pol II molecules



Pileup of polymerases on either side of the repetitive region.
Does not depend on cell activation.

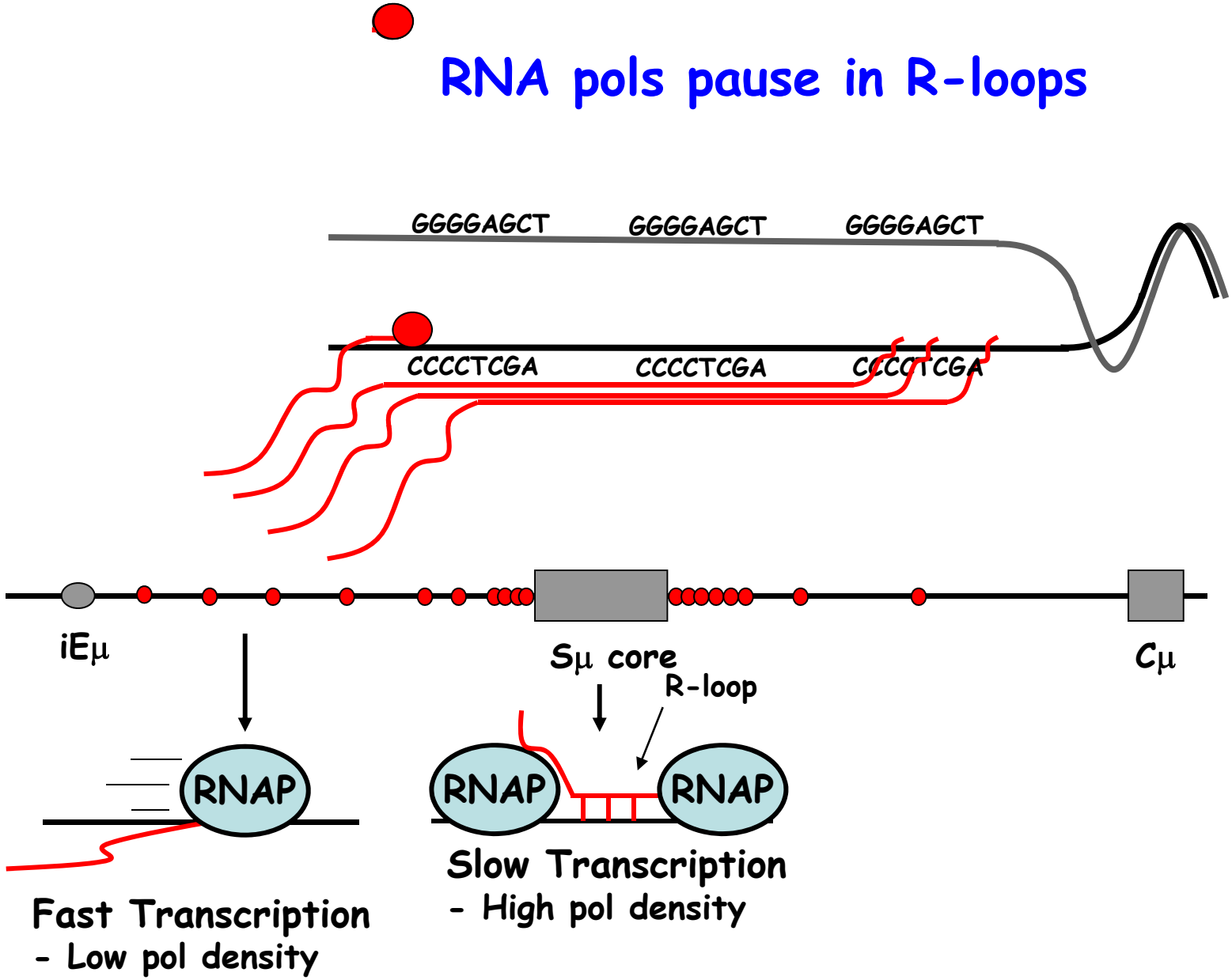




Pileup does not depend on AID. Depends on DNA sequence of Sμ.

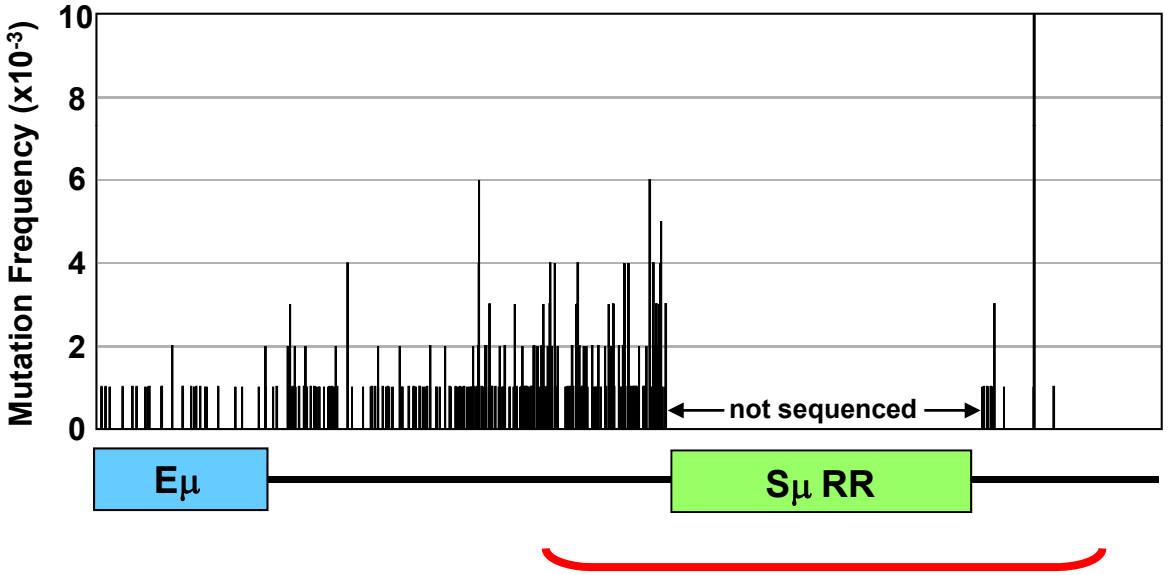
RNA pol II location confirmed by ChIP

RNA pols pause in R-loops



How does DNA structure affect somatic hypermutation?

Sequenced J_H4 intron DNA from immunized B cells from *Ung*$^{-/-}$ mice

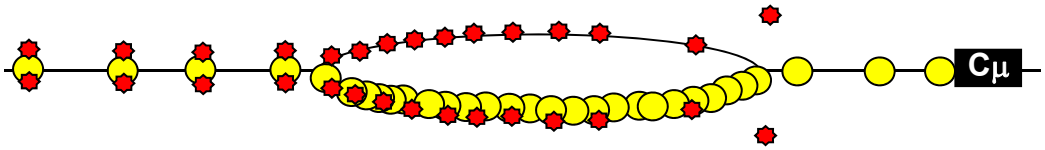


Mutations increase before the repetitive region and decrease after

DNA structure pauses RNA pol II and affects mutation

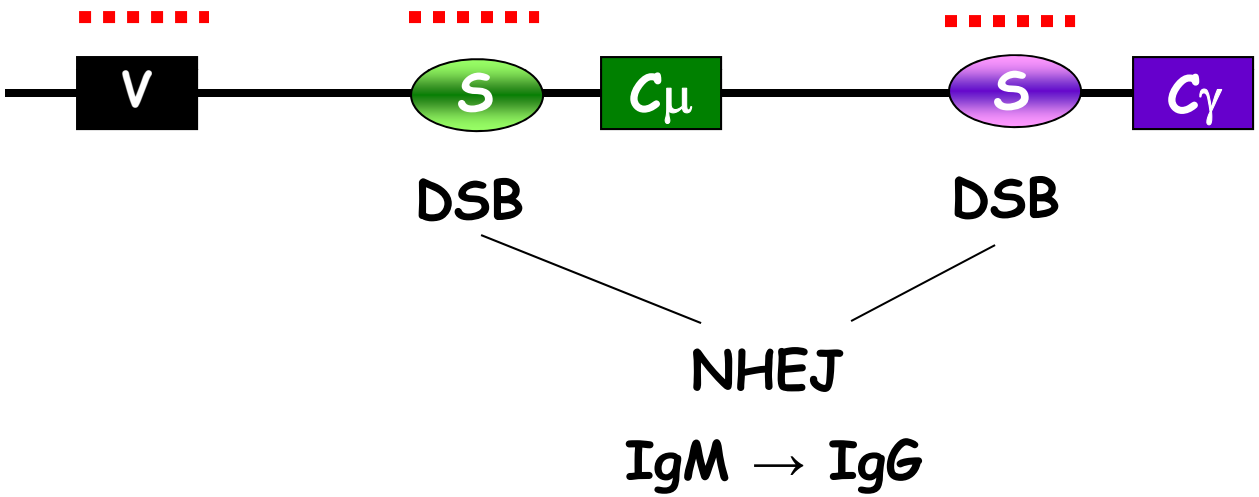
RNA pol II

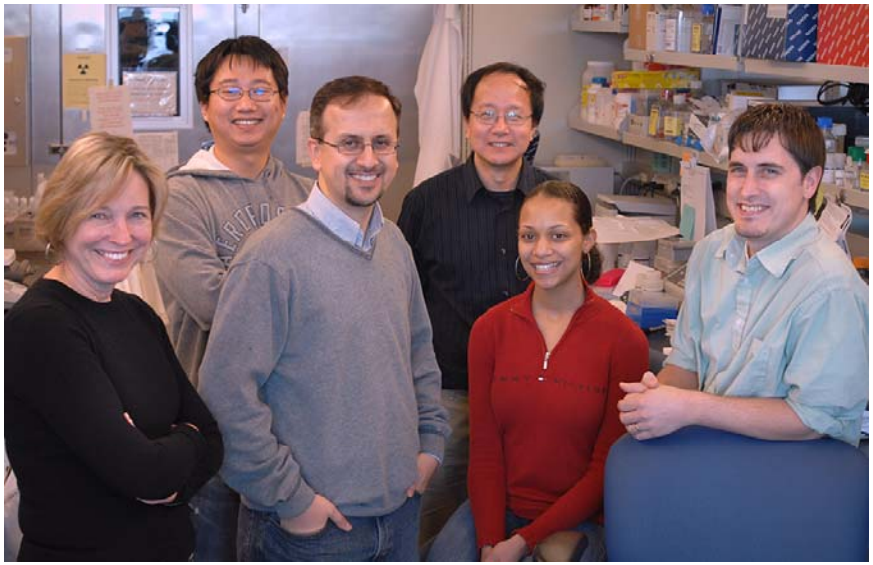
AID



AID may associate with RNA pol II. R-loop regions pause RNA polymerases, increasing AID binding to ssDNA and mutations. After core, AID falls off or is diluted out, and mutation stops.

What targets AID to V region?





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