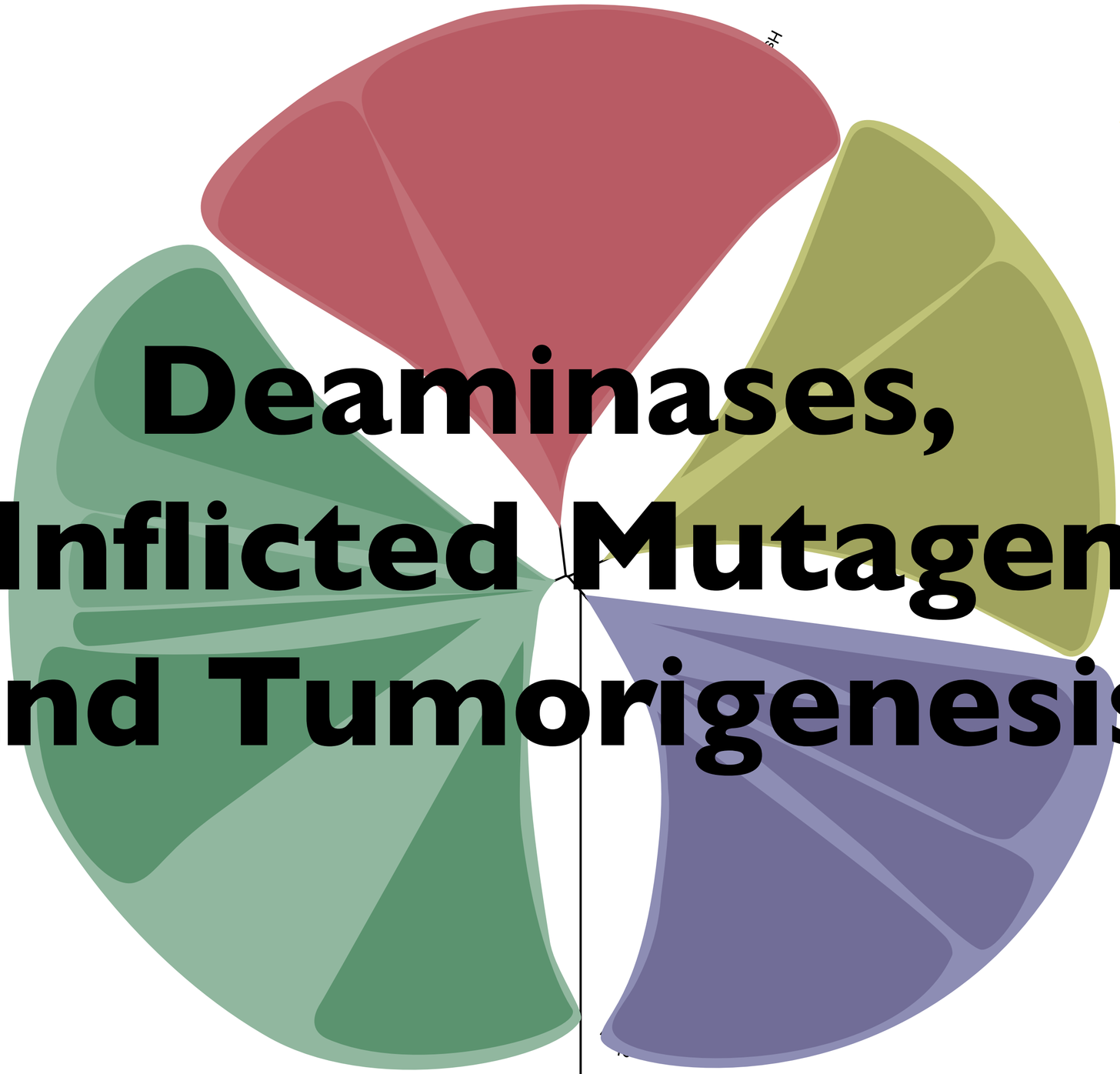
Core Research Laboratory
ITT
Istituto Toscano Tumori



Deaminases, Self-Inflicted Mutagenesis, and Tumorigenesis

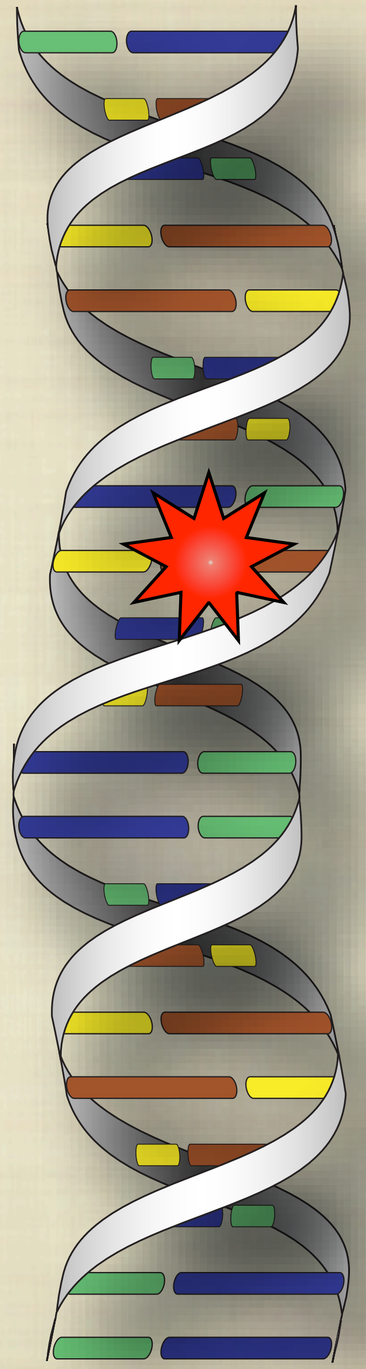
Silvo Conticello

Mutations...

* during replication/transcription

* environmental damage
  - Radiations
  - Chemicals
  - Spontaneous Breakdown

* DNA repair
  - Mismatch Repair
  - Excision Repair
  - End-Joining
  - Recombinational Repair

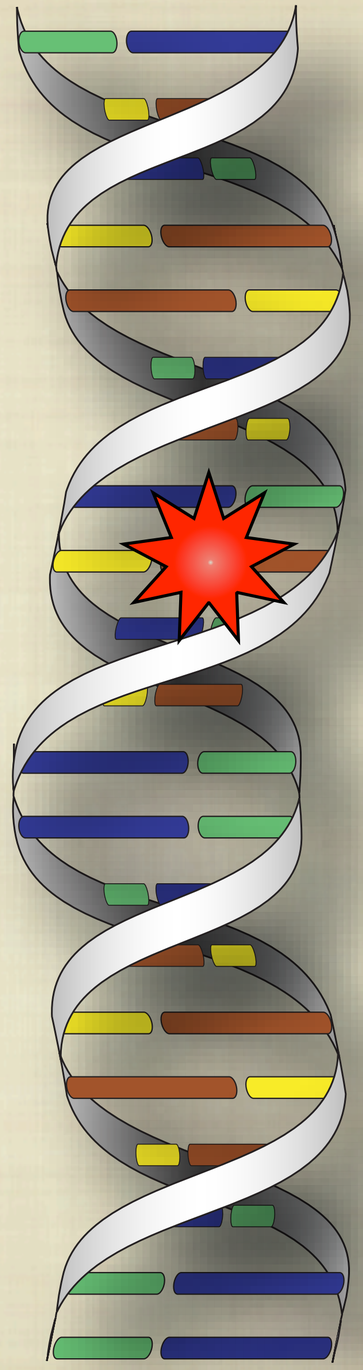


Mutations...

* during replication/transcription

* environmental damage
  - Radiations
  - Chemicals
  - Spontaneous Breakdown

* DNA repair
  - Mismatch Repair
  - Excision Repair
  - End-Joining
  - Recombinational Repair



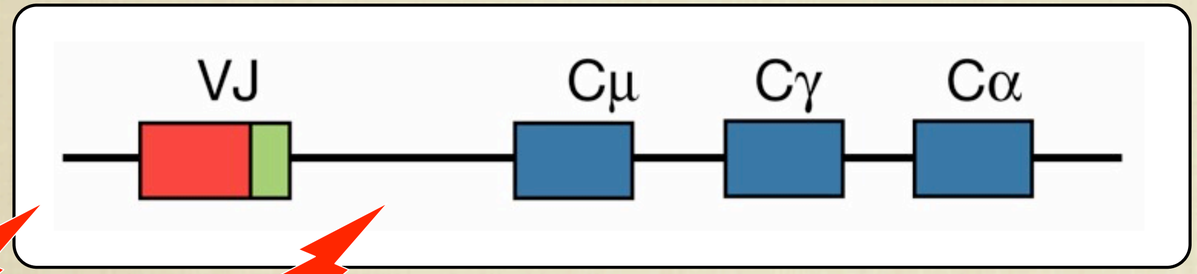
Stability

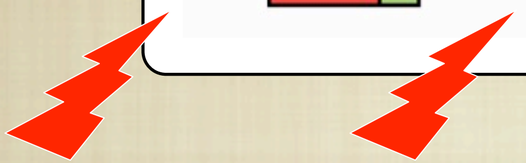
Evolution

Evolution - Live!

Antibody Diversification

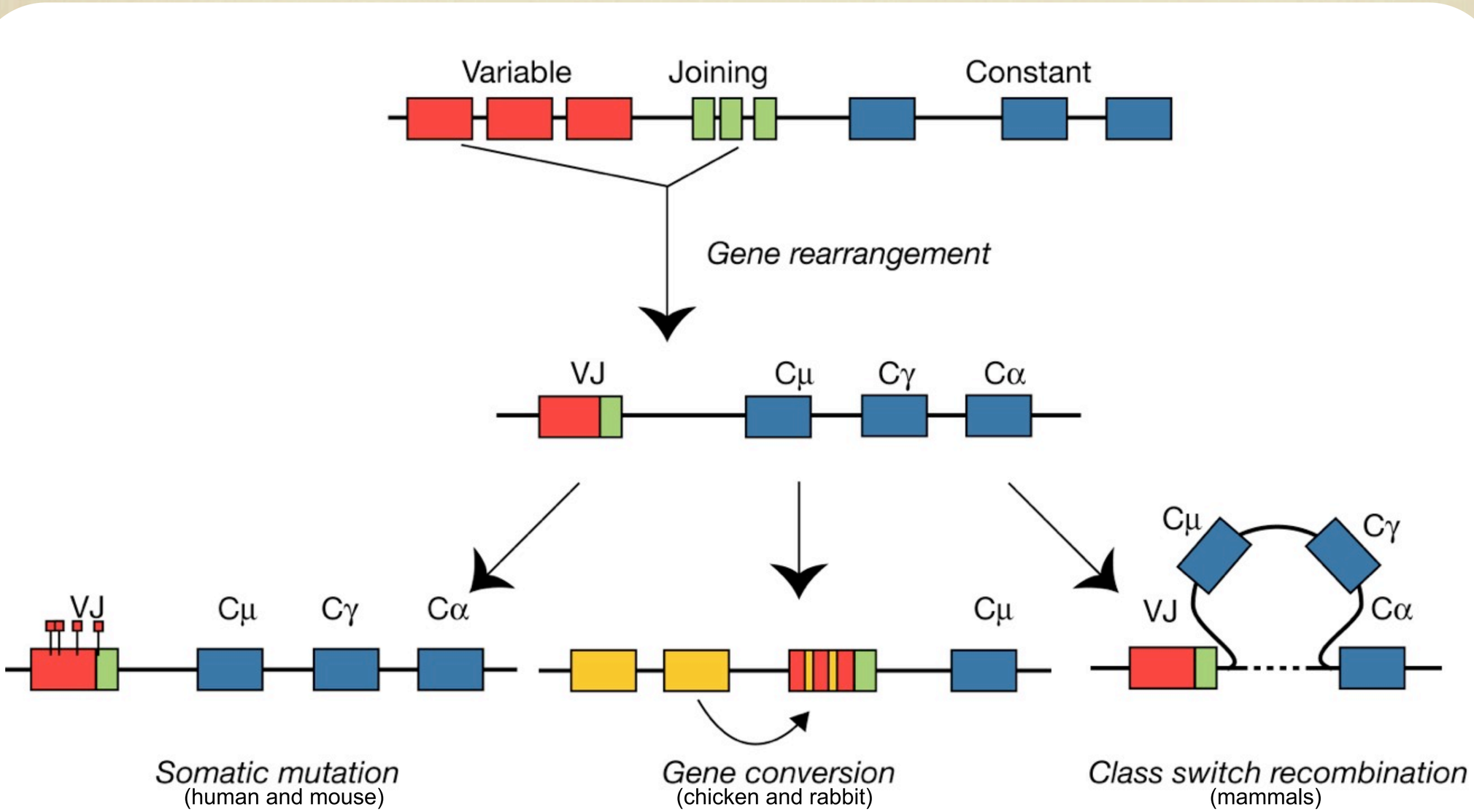
Immunoglobulin gene



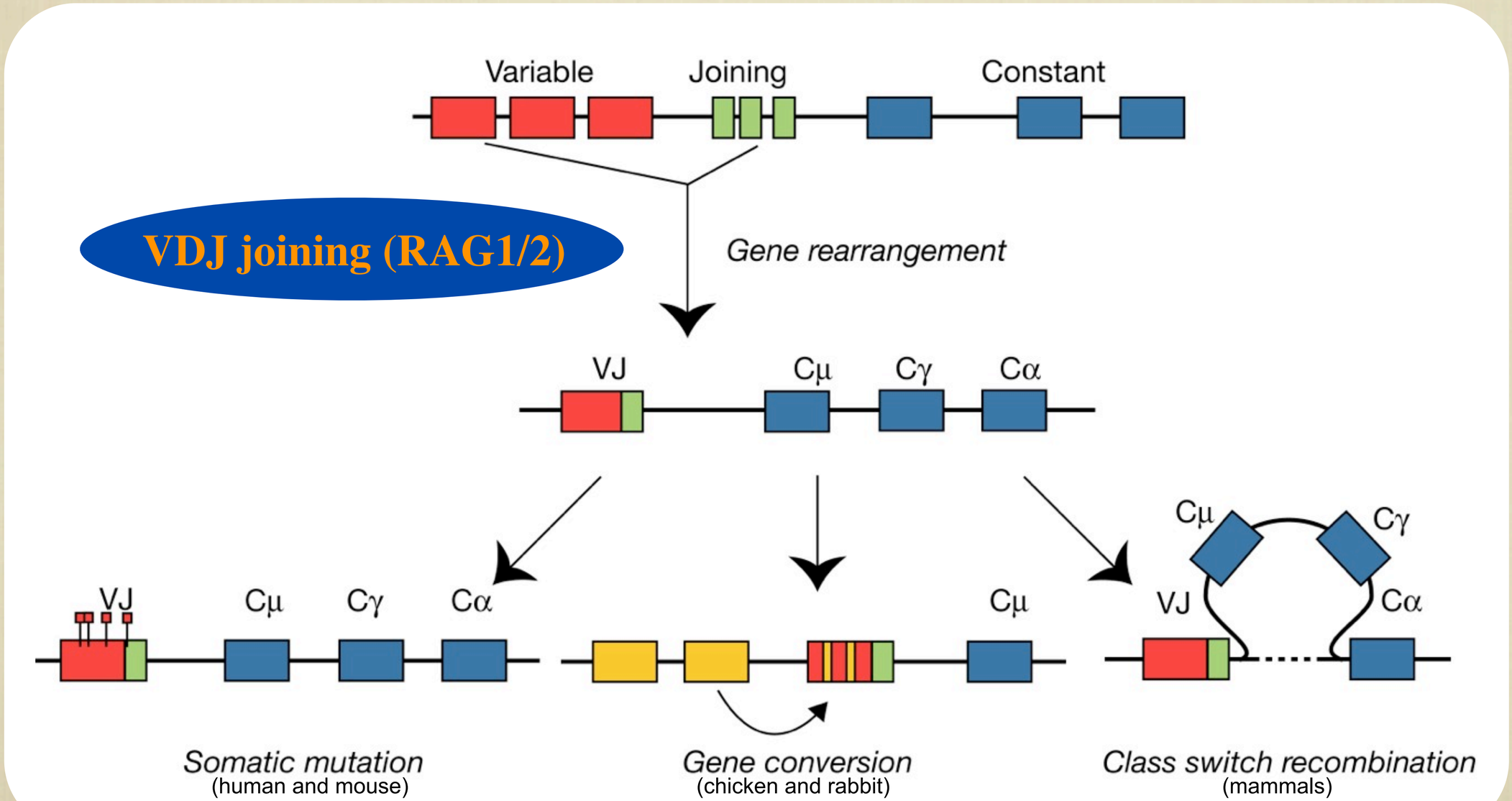


proteins

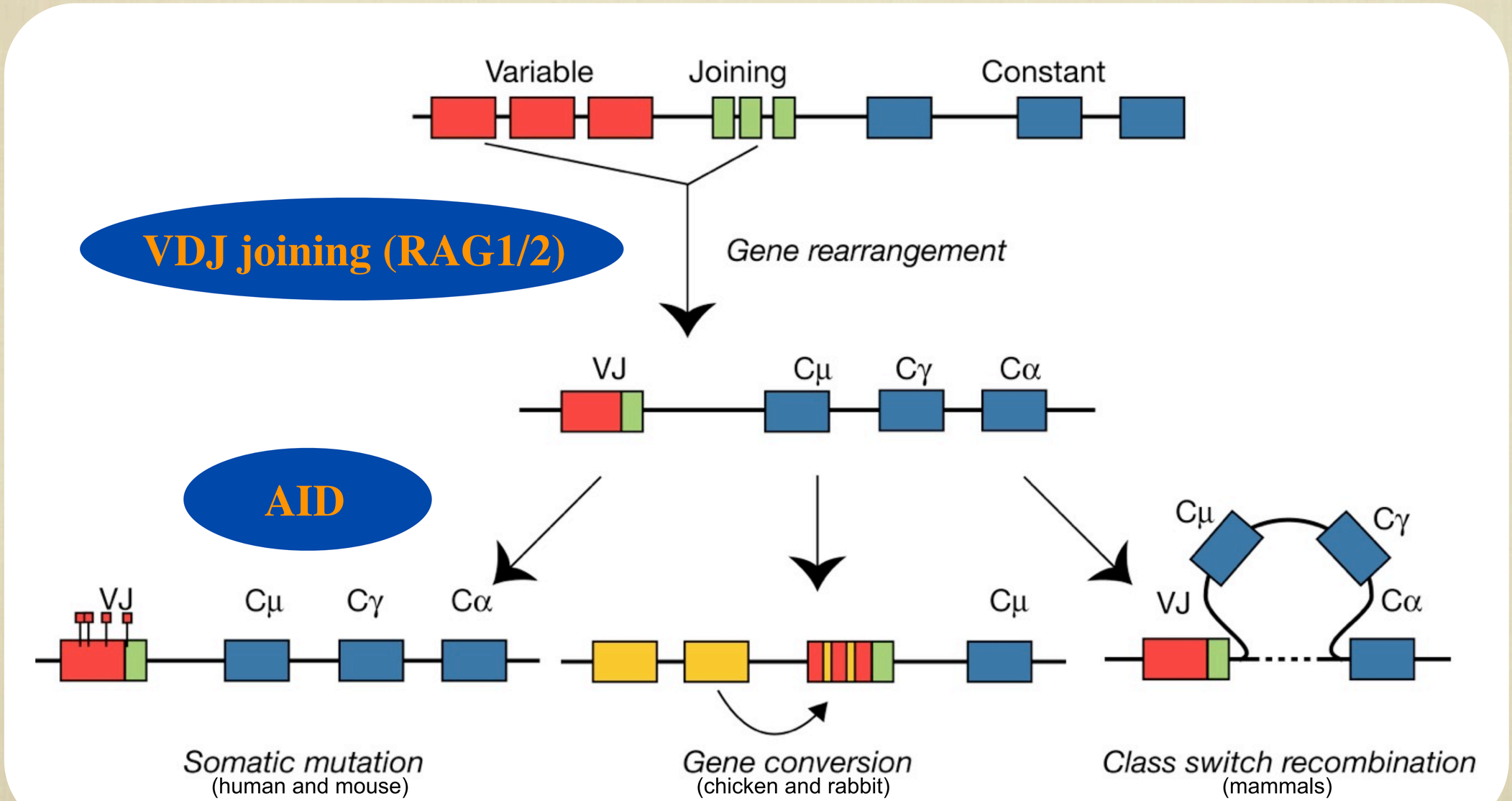
Gene Rearrangement and Antigen-Driven Diversification of the Ig Locus



Gene Rearrangement and Antigen-Driven Diversification of the Ig Locus



Gene Rearrangement and Antigen-Driven Diversification of the Ig Locus



AID is a DNA mutator

From

- **Expression of AID in E.coli gives an increased frequency of Rifampicin-resistant mutants**

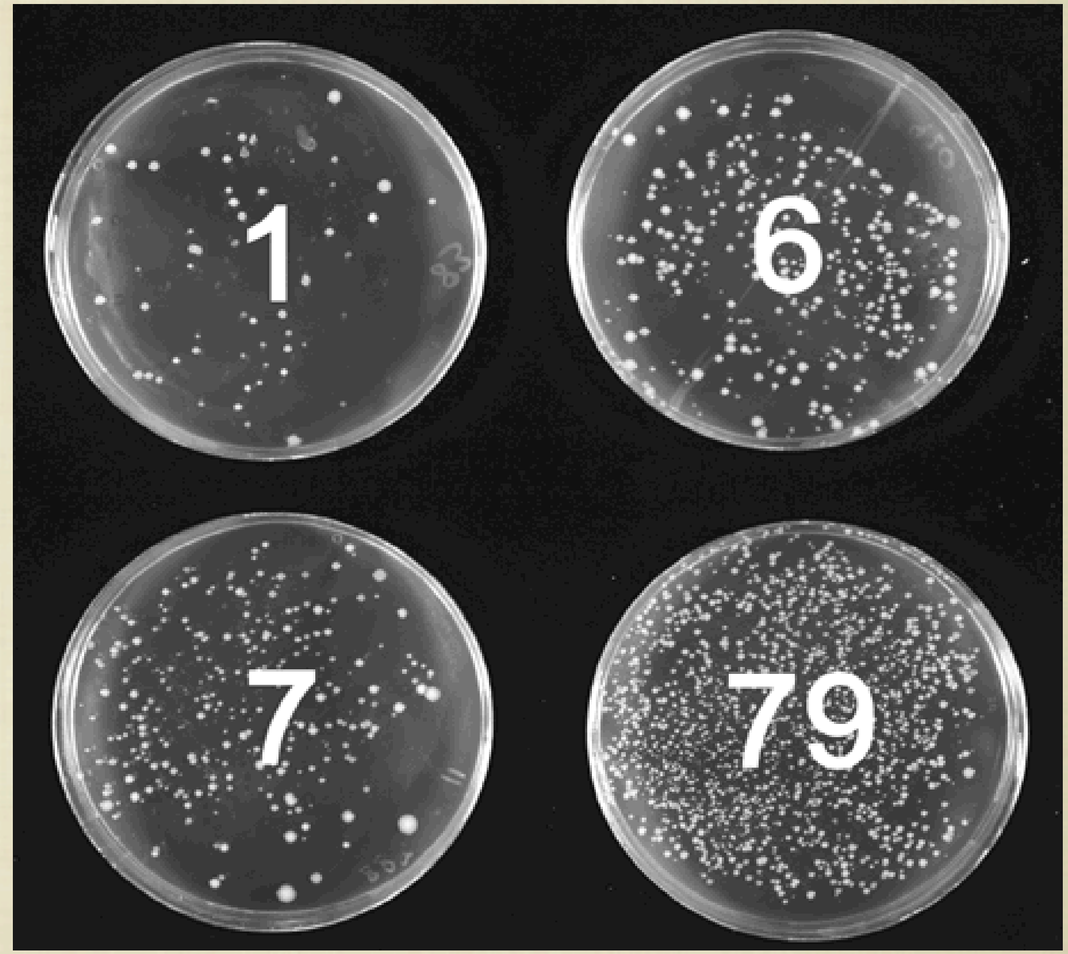
  Petersen-Mahrt et al., 2002

- **Expression of Ugi (UDG inhibitor) in DT40 cells abolishes all C>G mutations, leaving only the C>T originating from replication**

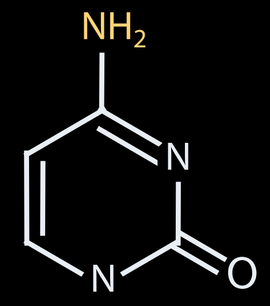
  Di Noia et al., 2002

- **Mutations in UNG-/- mice show the same pattern**

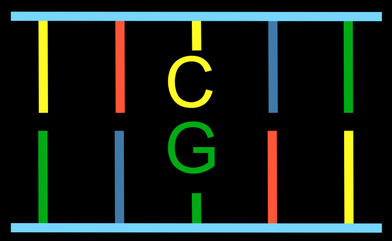
  Rada et al., 2002



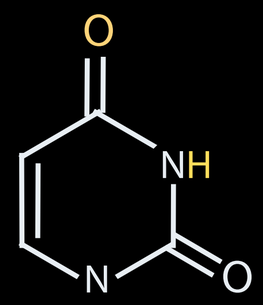
Diversification Through Deamination



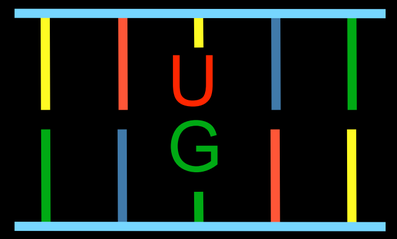
Cytosine



AID



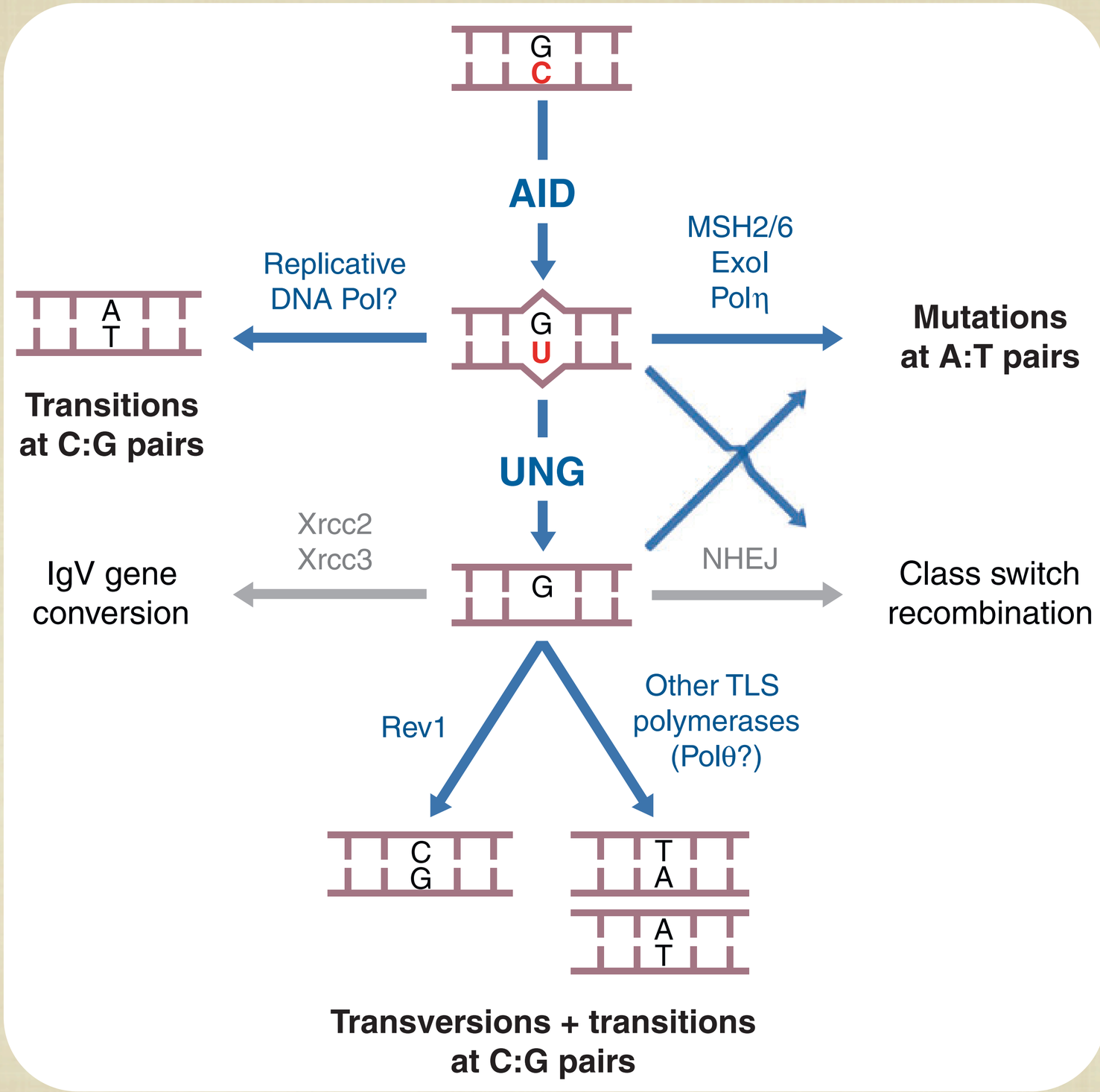
Uracil



Somatic Hypermutation

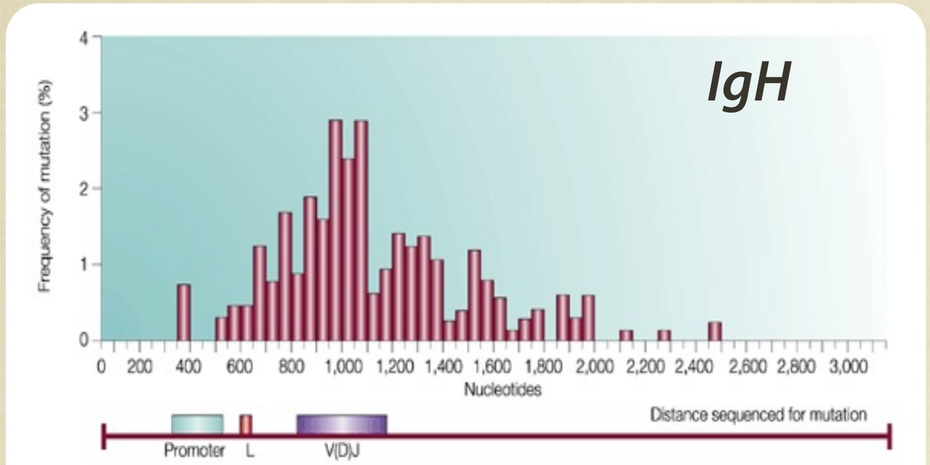
Gene Conversion

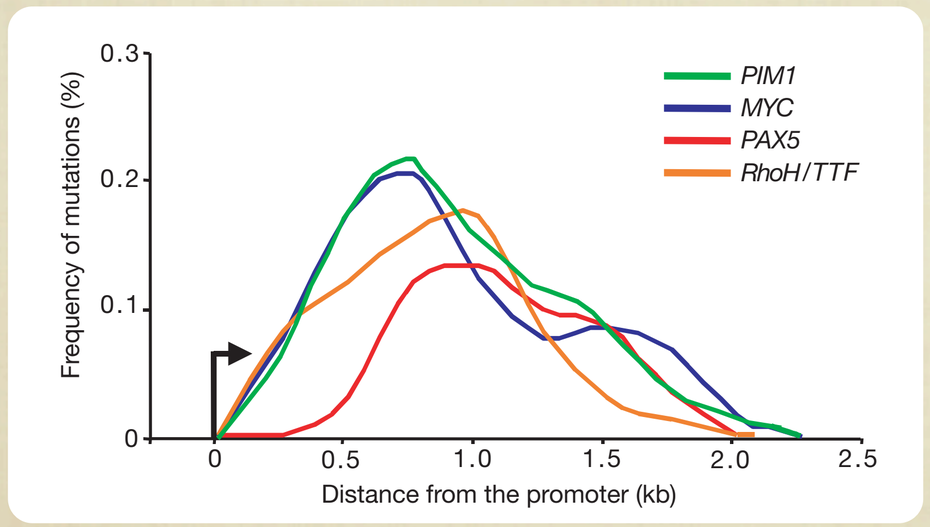
Class Switch Reconbination



Di Noia &
Neuberger, 2007

AID & Lymphomas: mutations





Pim1

	G	A	T	C
G				
A				
T	1	1		
C	4	4	45	

c-Myc

	G	A	T	C
G				
A	4			
T	5	3		
C	16	3	22	

RhoH/TTF

	G	A	T	C
G				
A	14			
T	3	4		
C	9	3	11	

Pax5

	G	A	T	C
G				
A	2			
T	1	2		
C	12	5	26	

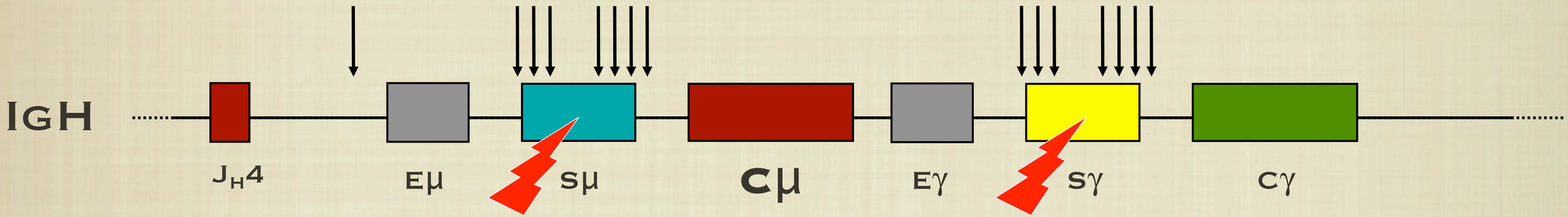
Bcl-6

	G	A	T	C
G				
A	14			
T	8	4		
C	12	3	19	

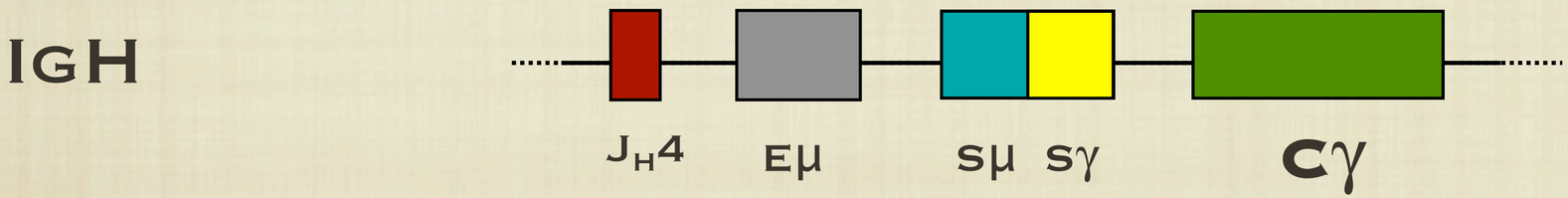
Dalla Favera Group

Physiology:

Class Switch Recombination

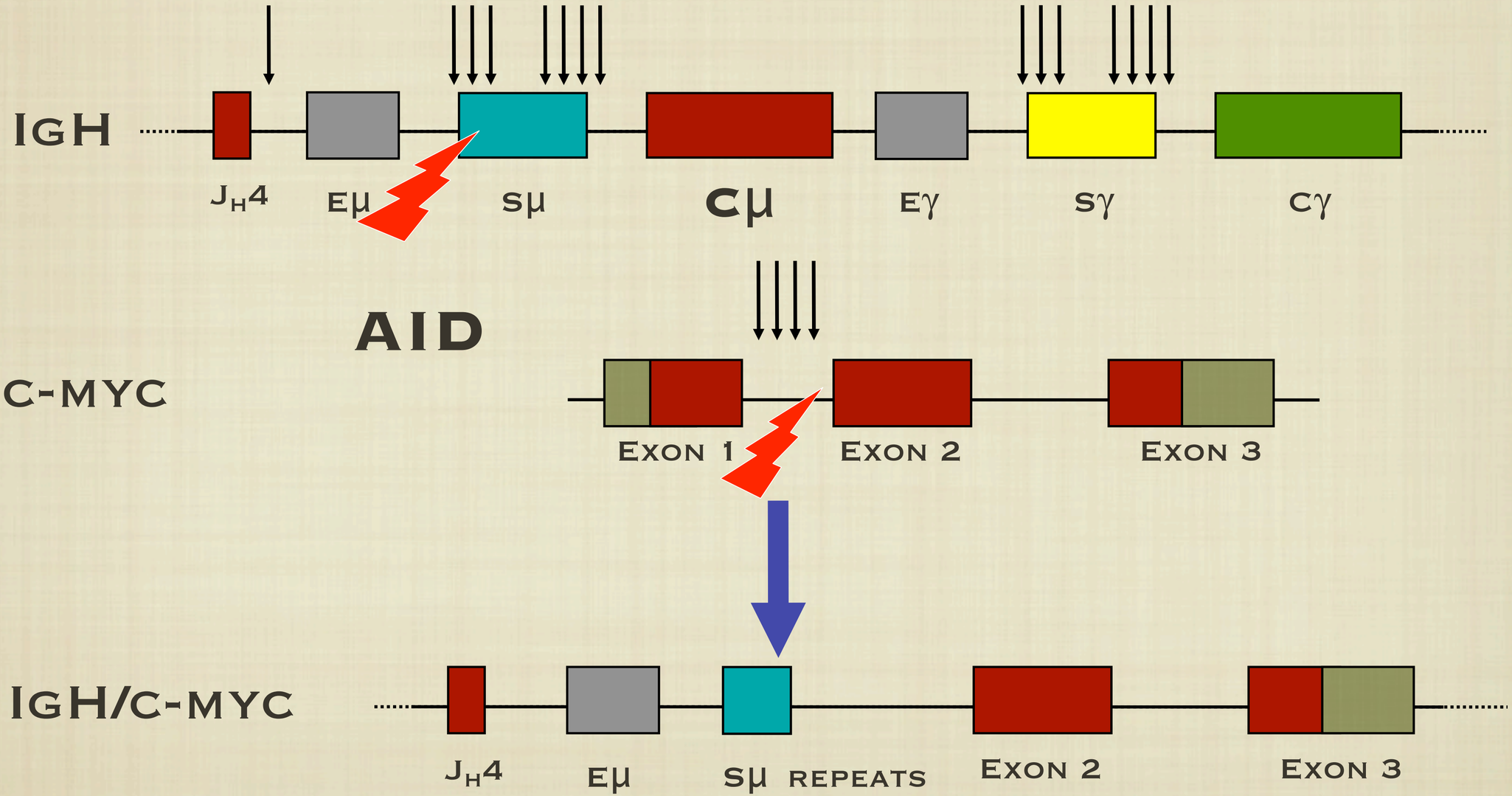


AID



Pathology:

Chromosomal Translocations



Ramiro et al. 2004; 2006

Robbiani et al. 2008; 2009

CSR-dependent Chromosomal Translocations

Chromosomal breakpoints at the Switch Regions

sporadic Burkitt's Lymphoma	***c-myc*, t(8;14)**
Diffuse Large B Cell Lymphoma	***bcl-6*, t(3;14)**
B-Chronic Lymphocytic Leukemia	***bcl-3*, t(14;19)**
Lymphoplasmacytoid Lymphoma	***Pax-5*, t(9;14)**
Diffuse Large Cell Lymphoma	***lyt-10*, t(10;14)**
Extranodal Lymphoma	***MUC-1*, t(1;14)**
Multiple Myeloma	***FGFR-3*, t(4;14);** ***c-maf*, t(14;16);** ***MUM1/IRF4*, t(6;14)**

AID & Cancer

AID transgenic Mice develop Tumors
(lung adenocarcinomas and T-cell lymphomas, other)

Okazaki et al. 2003; others

AID-deficient Cancer Prone Mice are unable to develop Mature B-cell Tumours

Pasqualucci et al. 2008

Humans

AID is expressed in lymphoproliferative disorders

Greeve, 2003; Oppezzo, 2003; many others

Expression of AID is triggered by a number of factors

Machida, 2004; Babbage, 2006; Matsumoto, 2007

AID is the trigger of Ig secondary diversification

What brings AID to the Ig locus?

Other physiological roles?

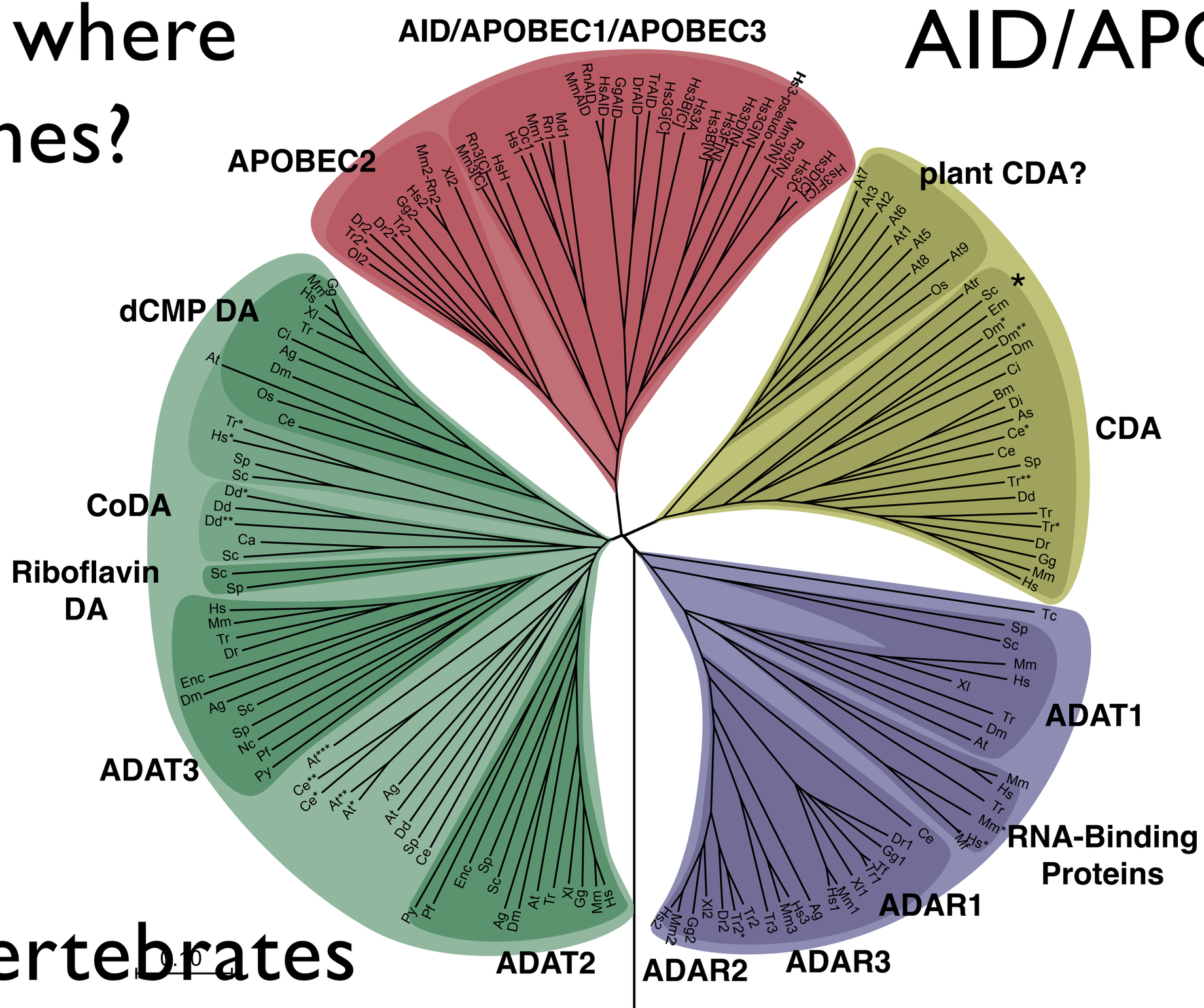
AID is a key player in mature B-cell Tumours

What makes AID fail and mistarget other genes?

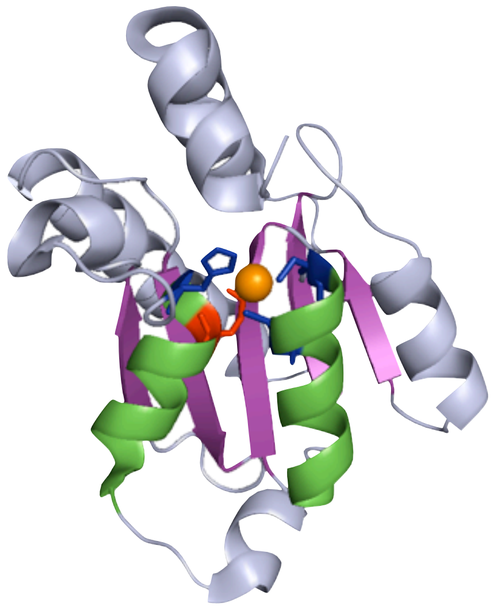
Is AID involved in tumorigenesis in other tissues?

From where it comes?

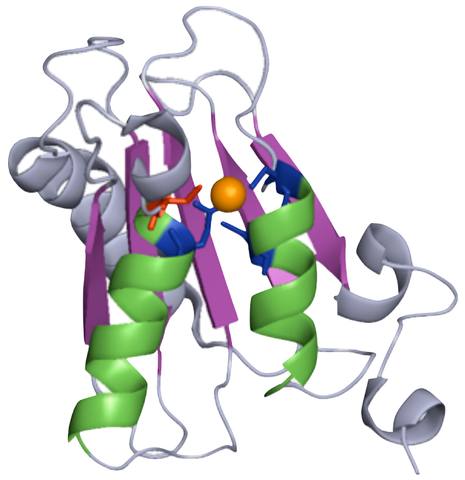
AID/APOBECs



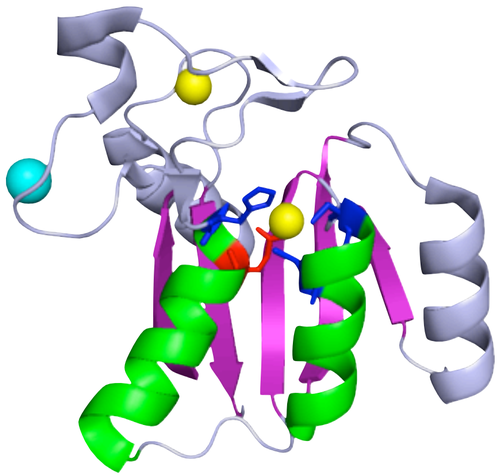
Just vertebrates



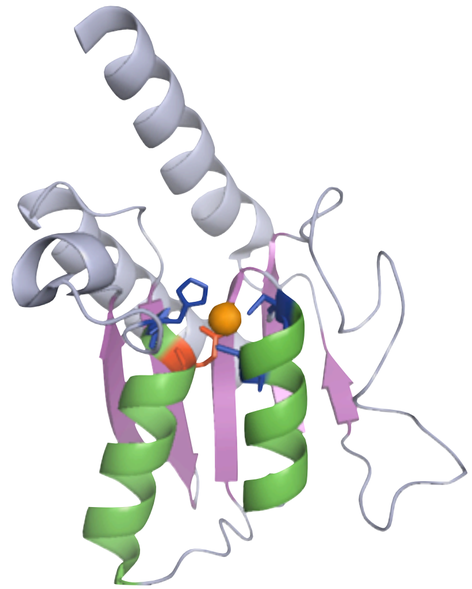
Cytosine DA



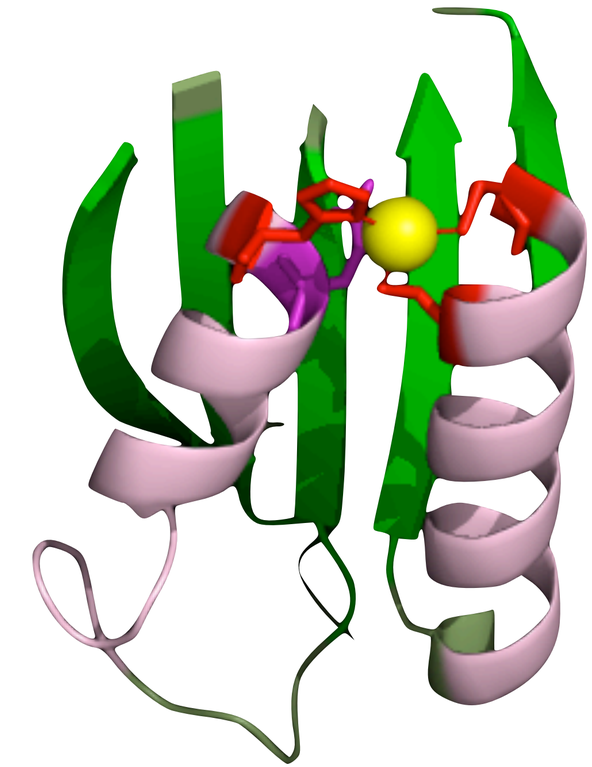
Cytidine DA

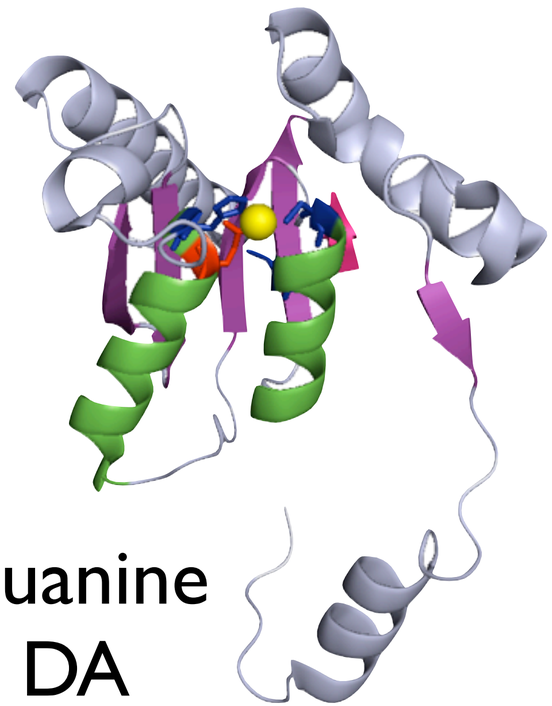


dCMP DA

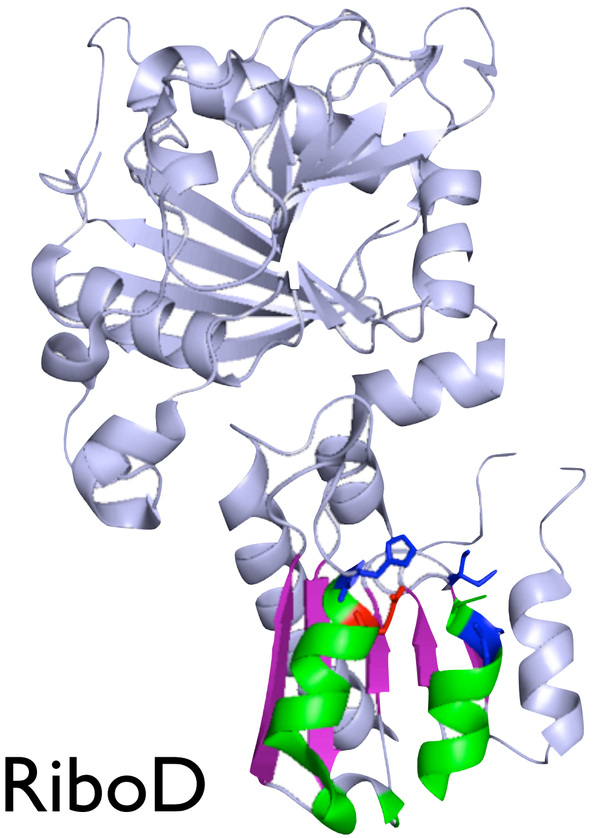


TadA/ADAT2

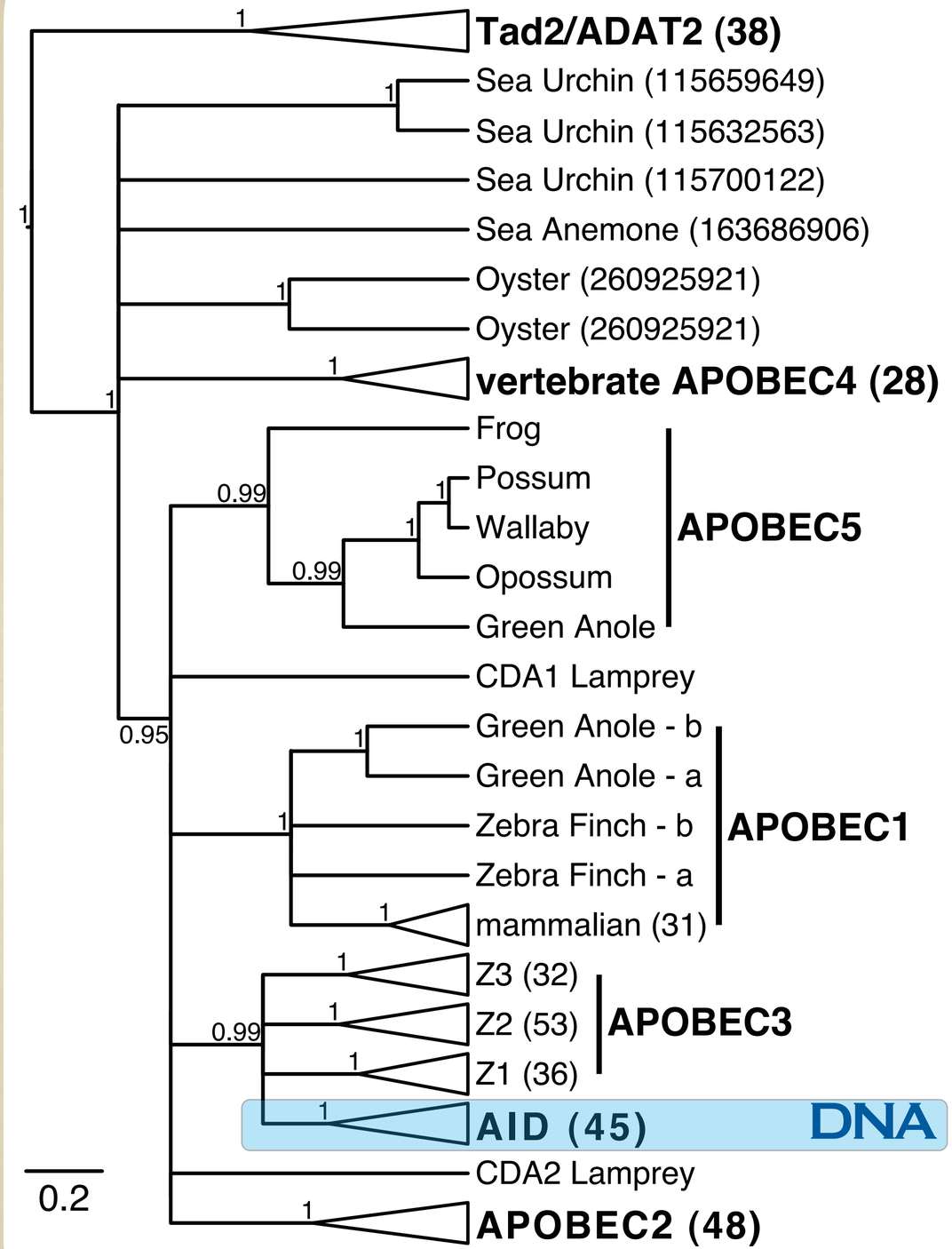




Guanine
DA



RiboD



Beyond AID...

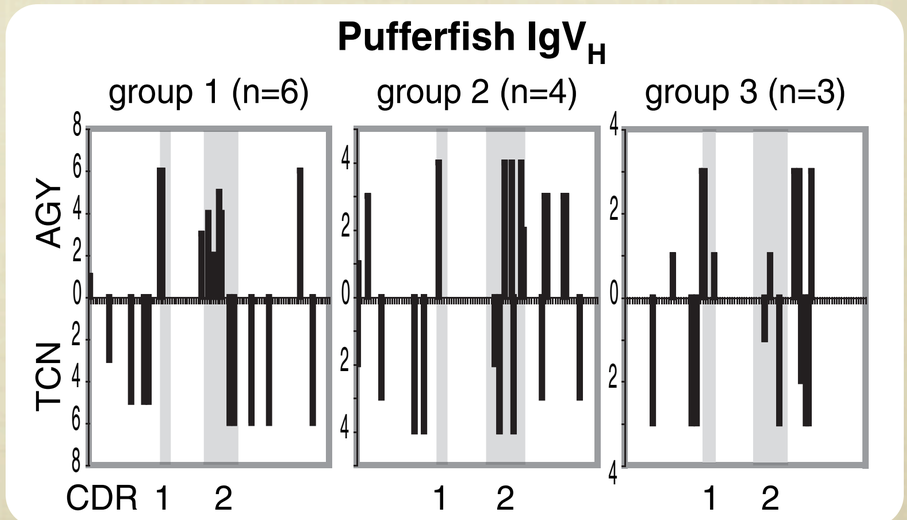
AID - Immune System

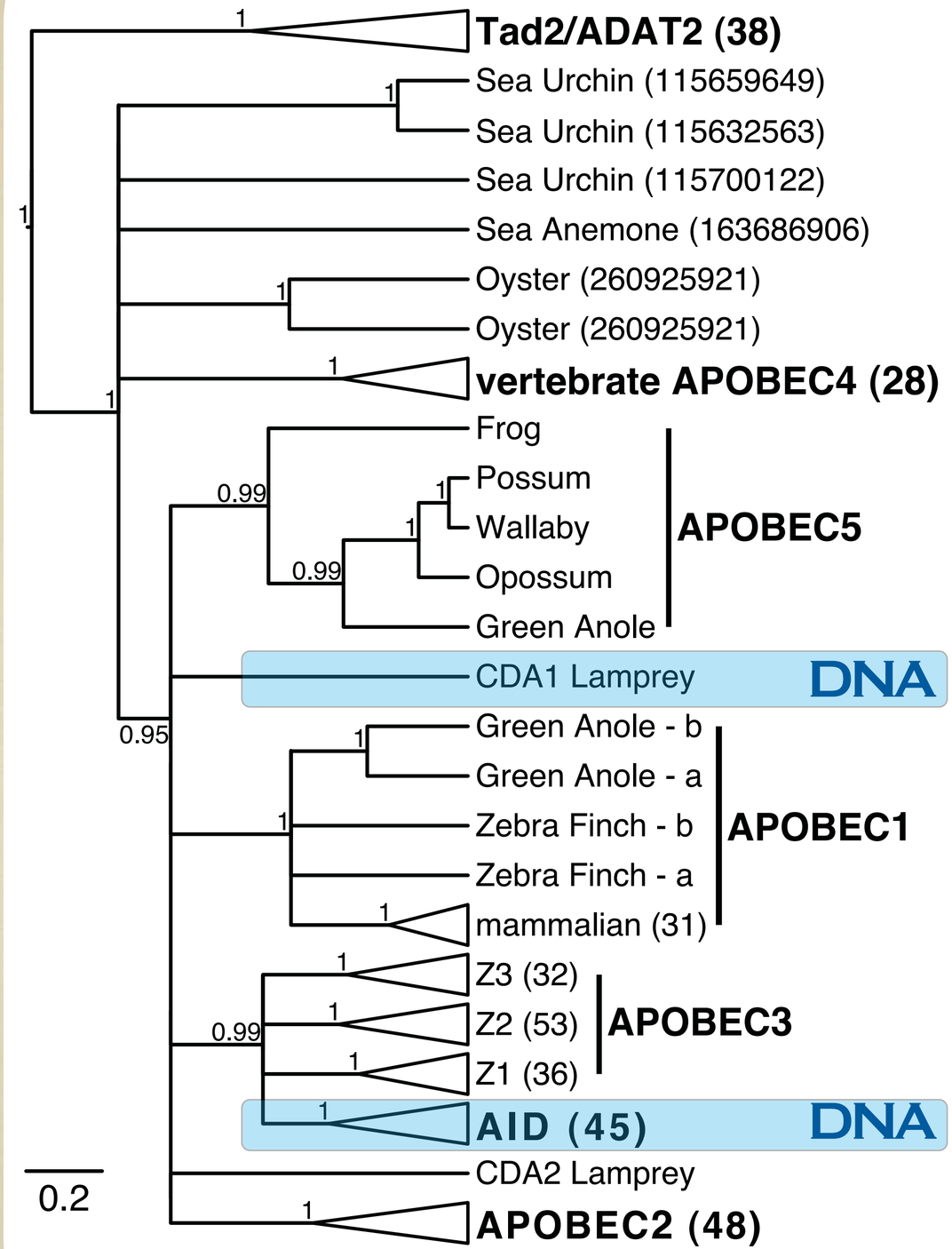
Bony Fish

Saunders & Magor 2004;
Conticello 2005; Zhao 2005

Cartilaginous Fish

Conticello 2005





Beyond AID...

AID - Immune System

CDA1 - Lamprey Immune System

Bony Fish

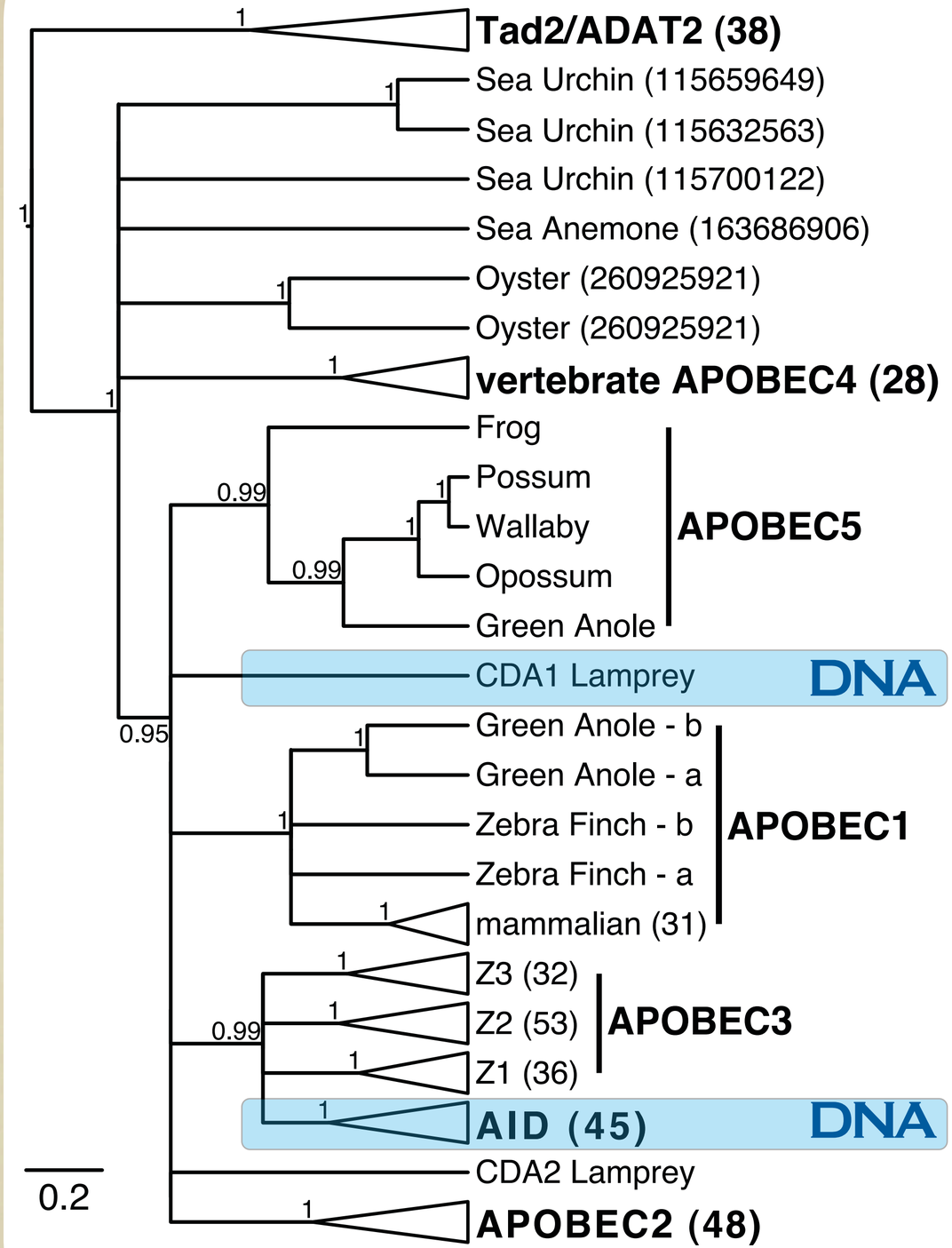
Saunders & Magor 2004;
Conticello 2005; Zhao 2005

Cartilaginous Fish

Conticello 2005

Jawless Fish

Rogozin 2007; Guo 2009



Beyond AID...

AID - Immune System

CDA1 - Lamprey Immune System

Bony Fish

Saunders & Magor 2004;
Conticello 2005; Zhao 2005

Cartilaginous Fish

Conticello 2005

Ig genes

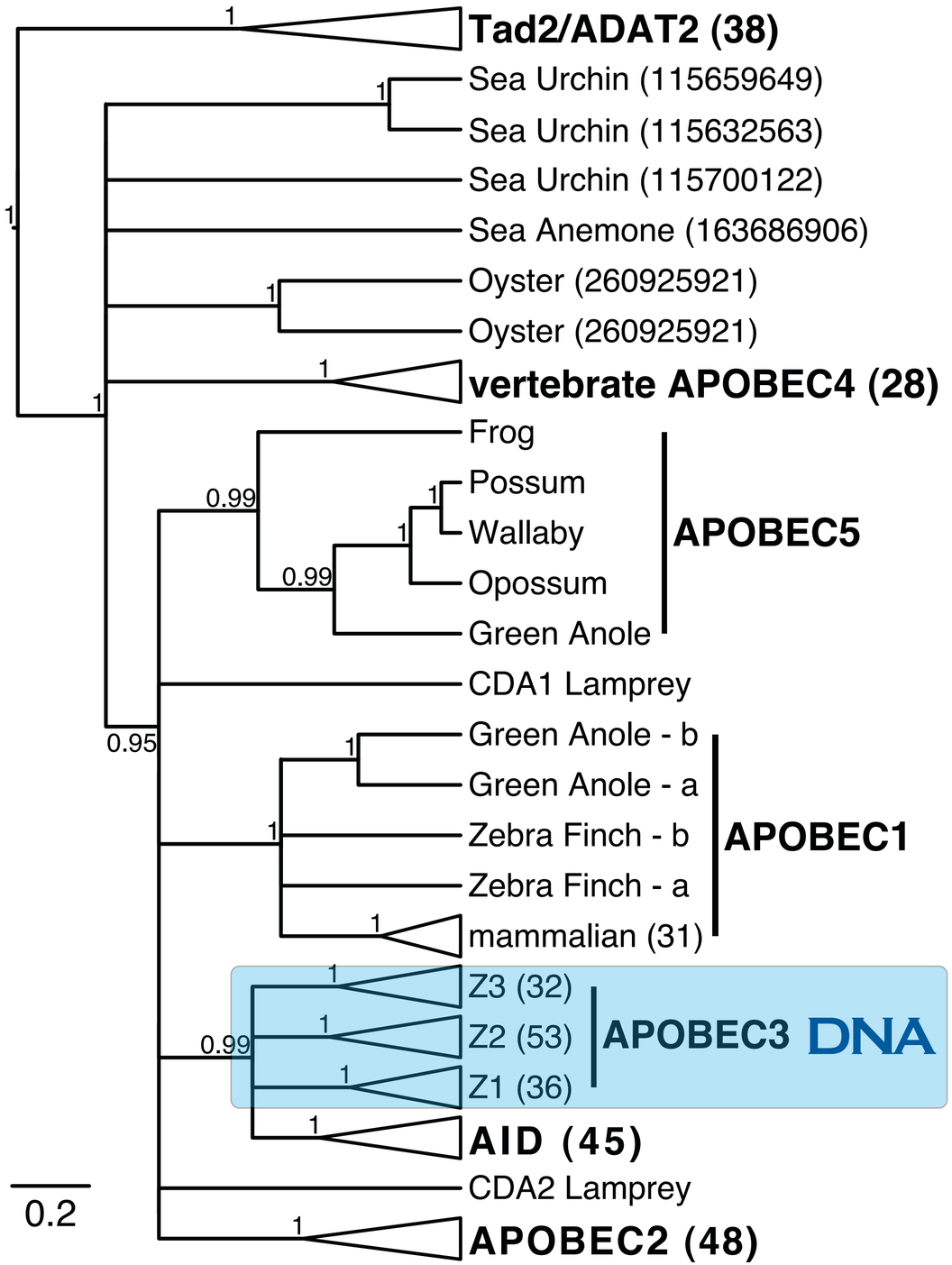
Jawless Fish

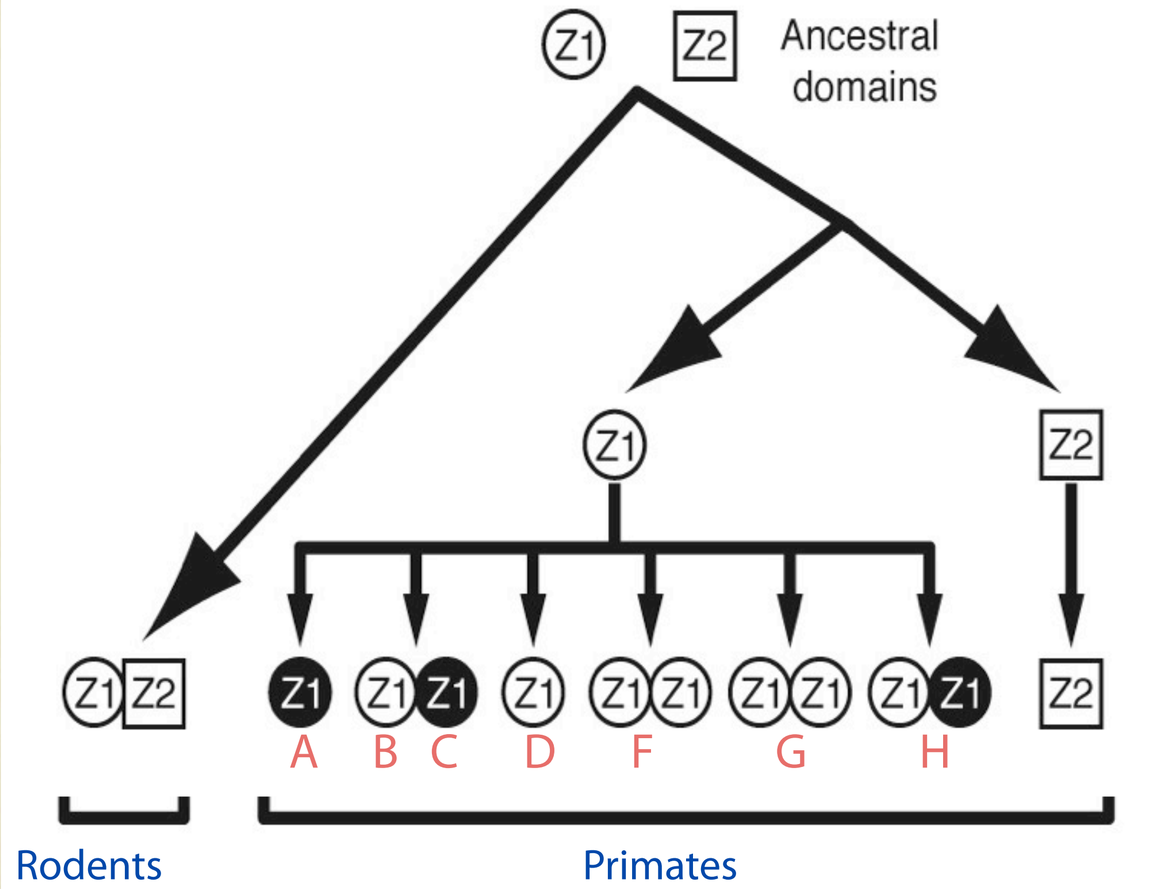
Rogozin 2007; Guo 2009

VLR genes

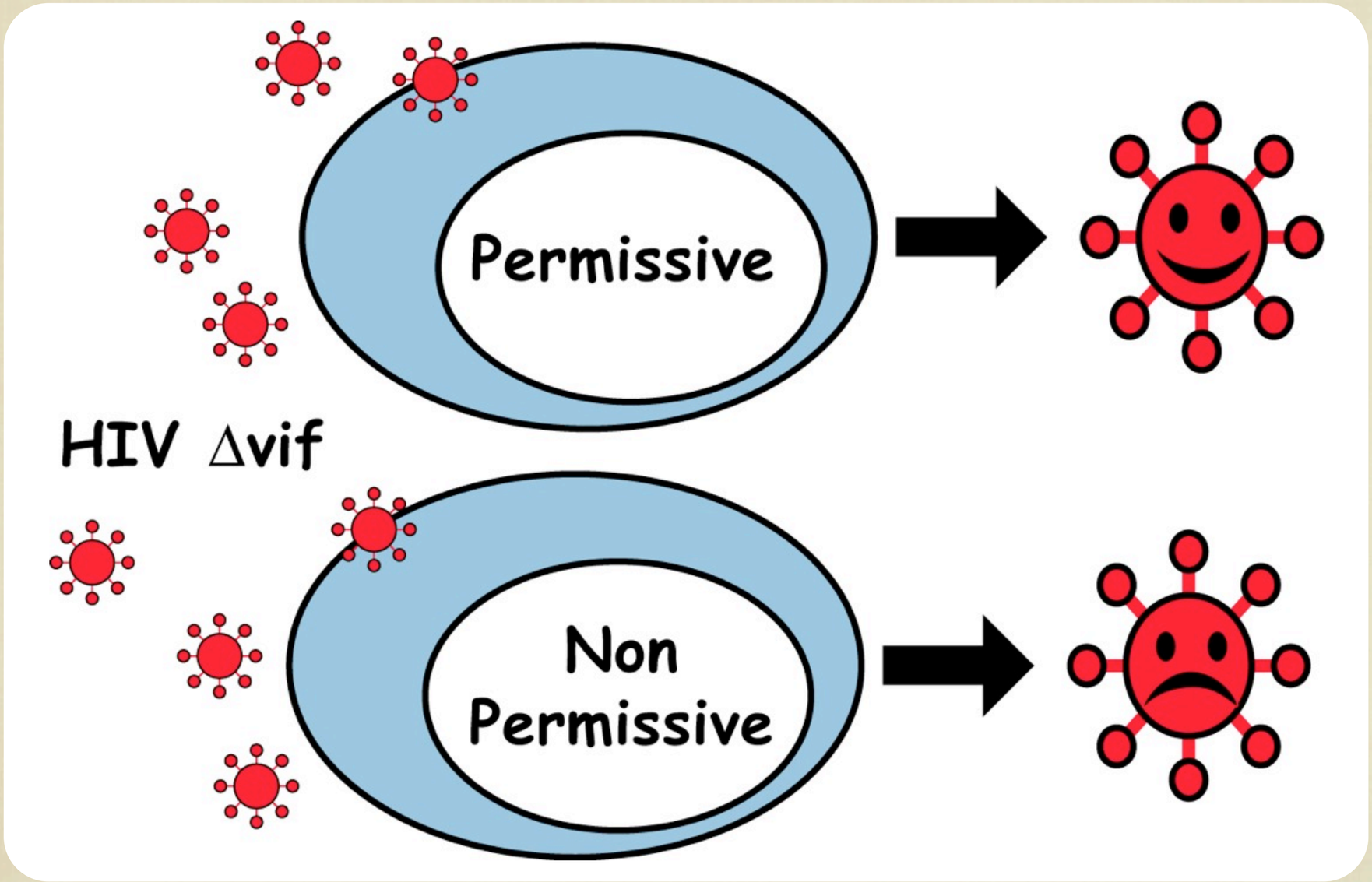
APOBEC3s

Restrict retroviruses, mobile elements, foreign DNA



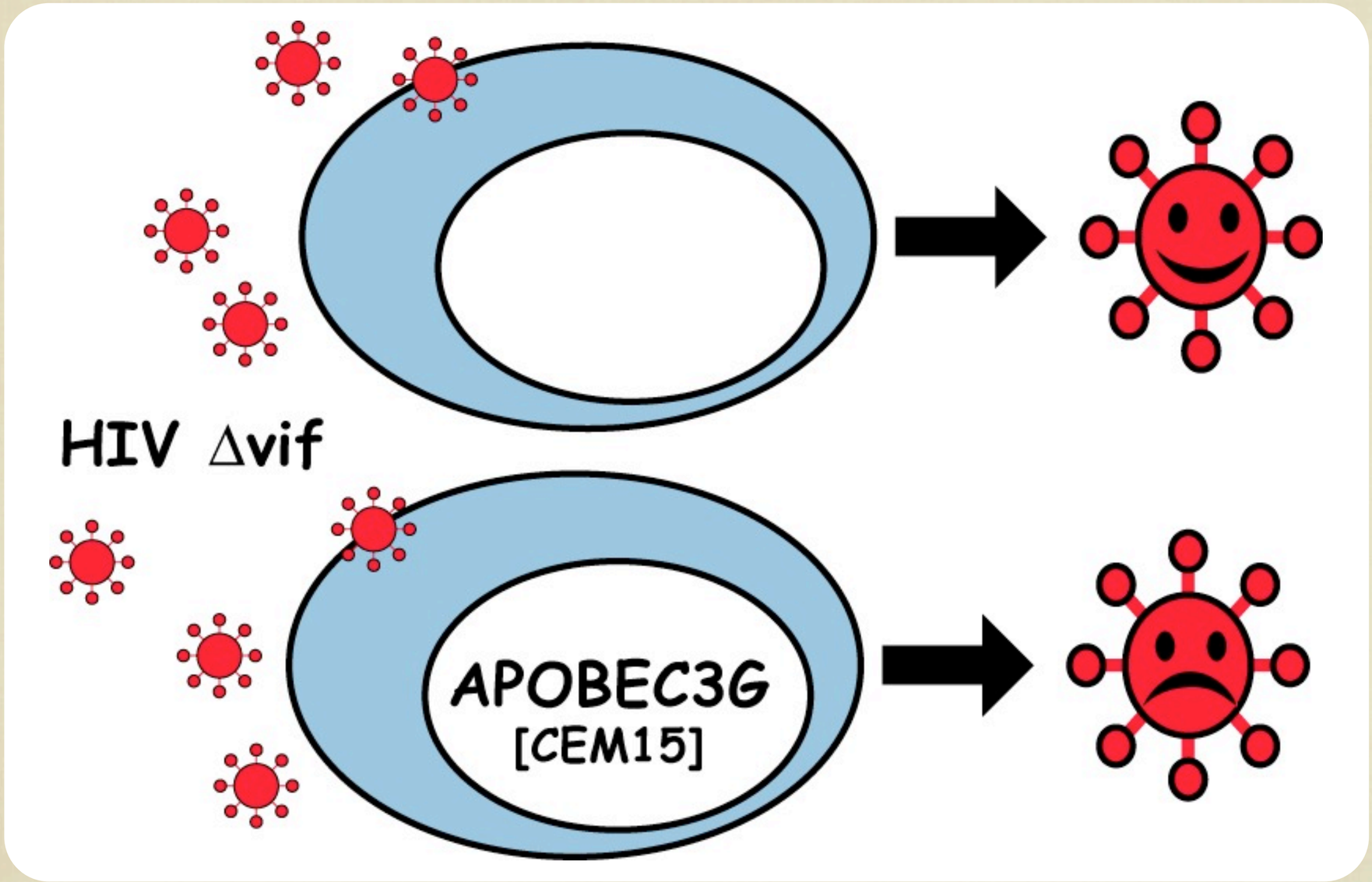


Vif
an "accessory" gene of HIV



Gabudza et al., 1992; von Schwedler et al., 1993; Madani and Kabat, 1998; Simon et al.,1998

APOBEC3G - INNATE IMMUNITY AGAINST RETROVIRUSES



Sheehy et al. 2002

APOBEC3s

IMMUNITY AGAINST...

- **APOBEC3G is able to restrict HIV infection (under specific conditions)**

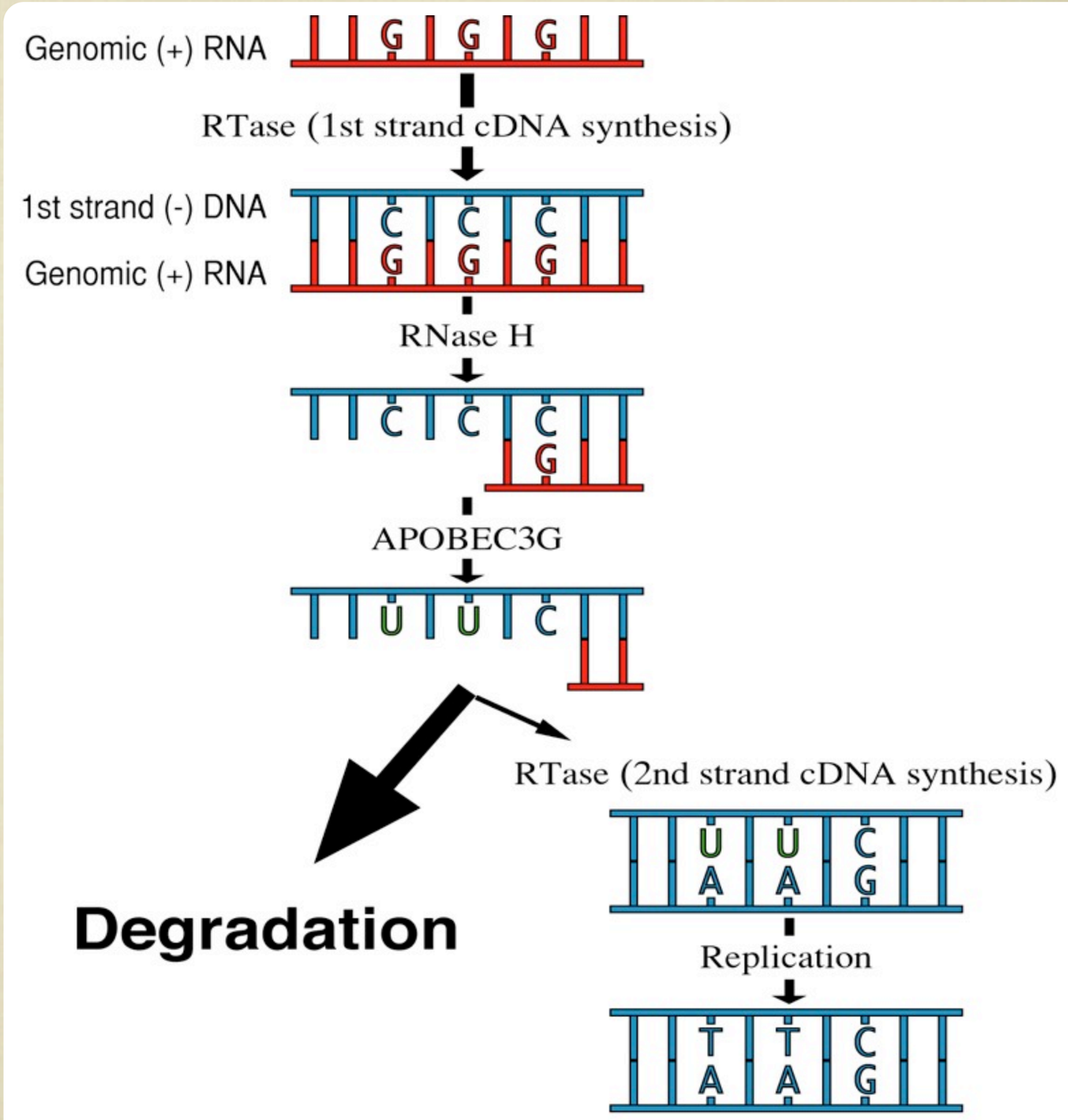
  Sheehy, 2002

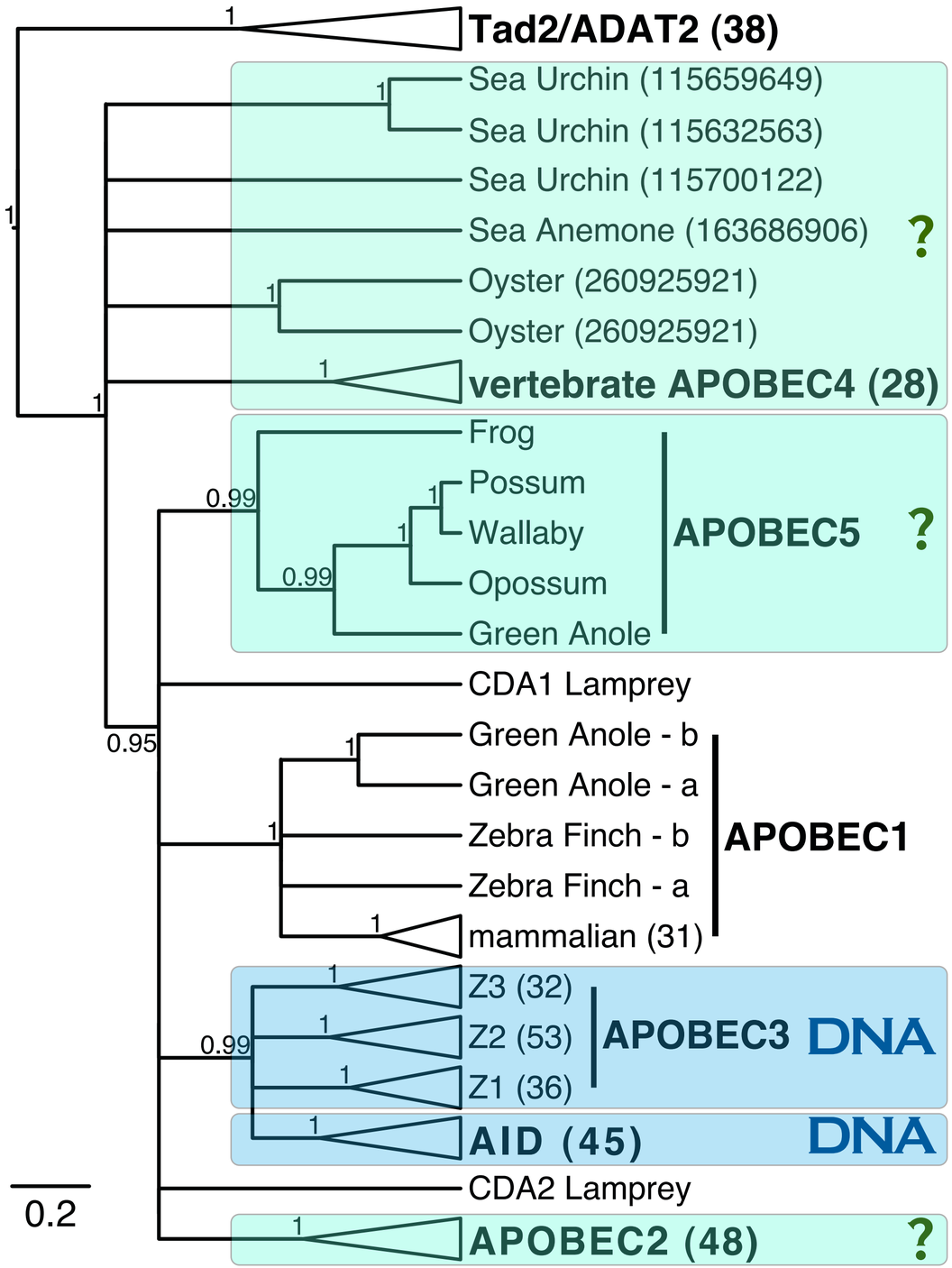
- **APOBEC3G restricts viral infectivity through DNA deamination**

  Harris, 2003; Mangeat, 2003; Lecossier, 2003; Mariani, 2003; Zhang, 2003

- **Most of the other APOBEC3s have some sort of anti-retroviral activity**

- **Many are active against mobile elements and foreign DNA**

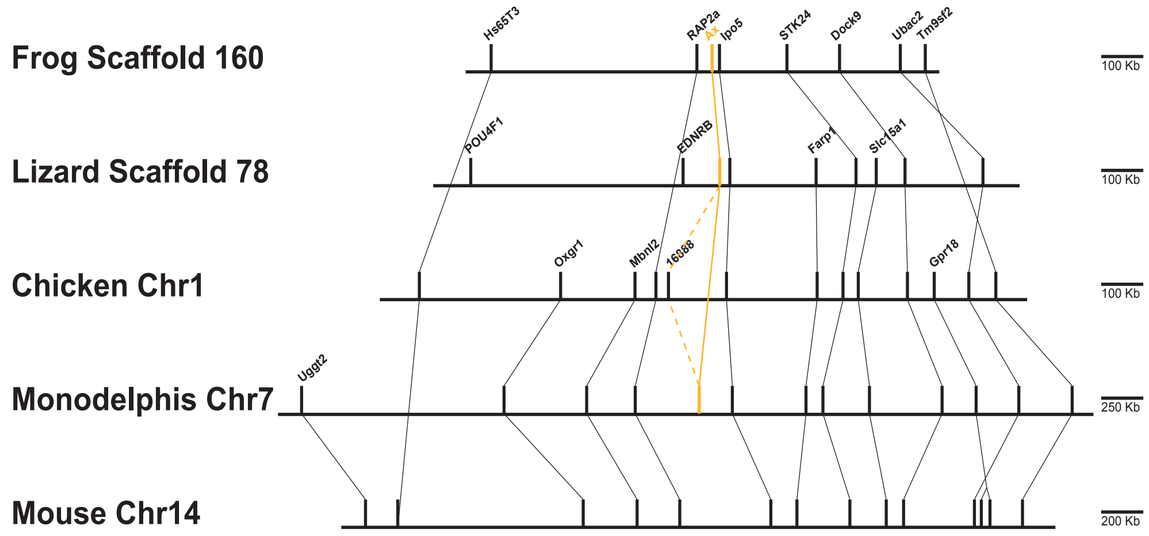


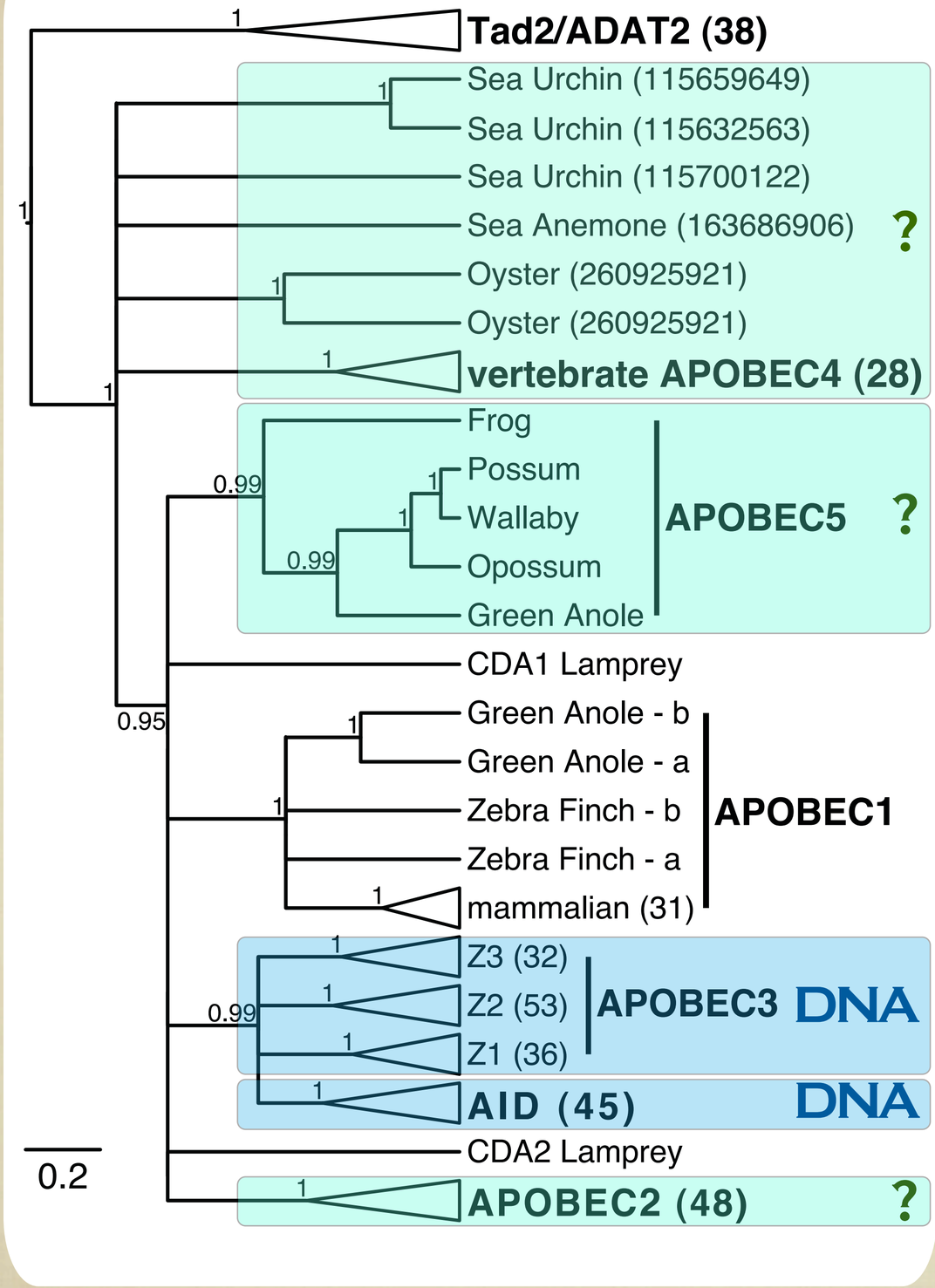


Beyond AID...

APOBEC4 - ...

APOBEC5 - same as APOBEC3s?



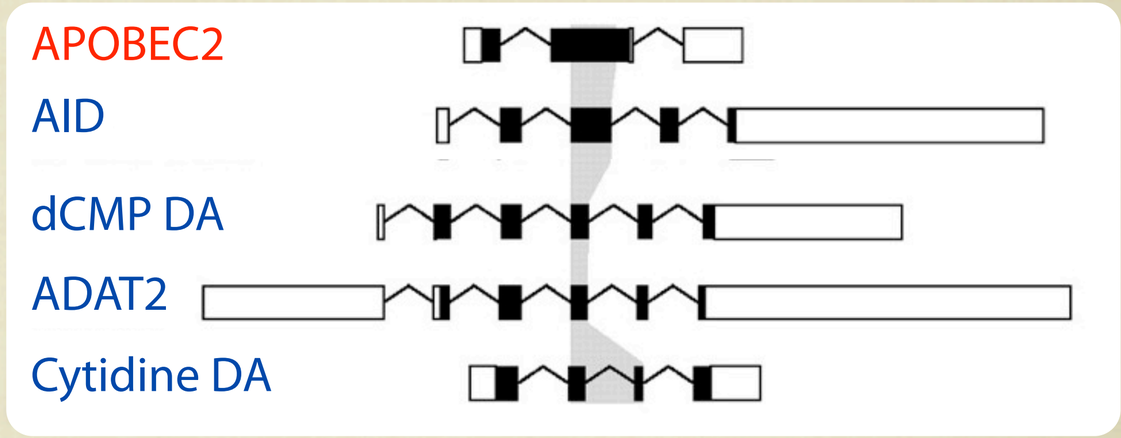


Beyond AID...

APOBEC4 - ...

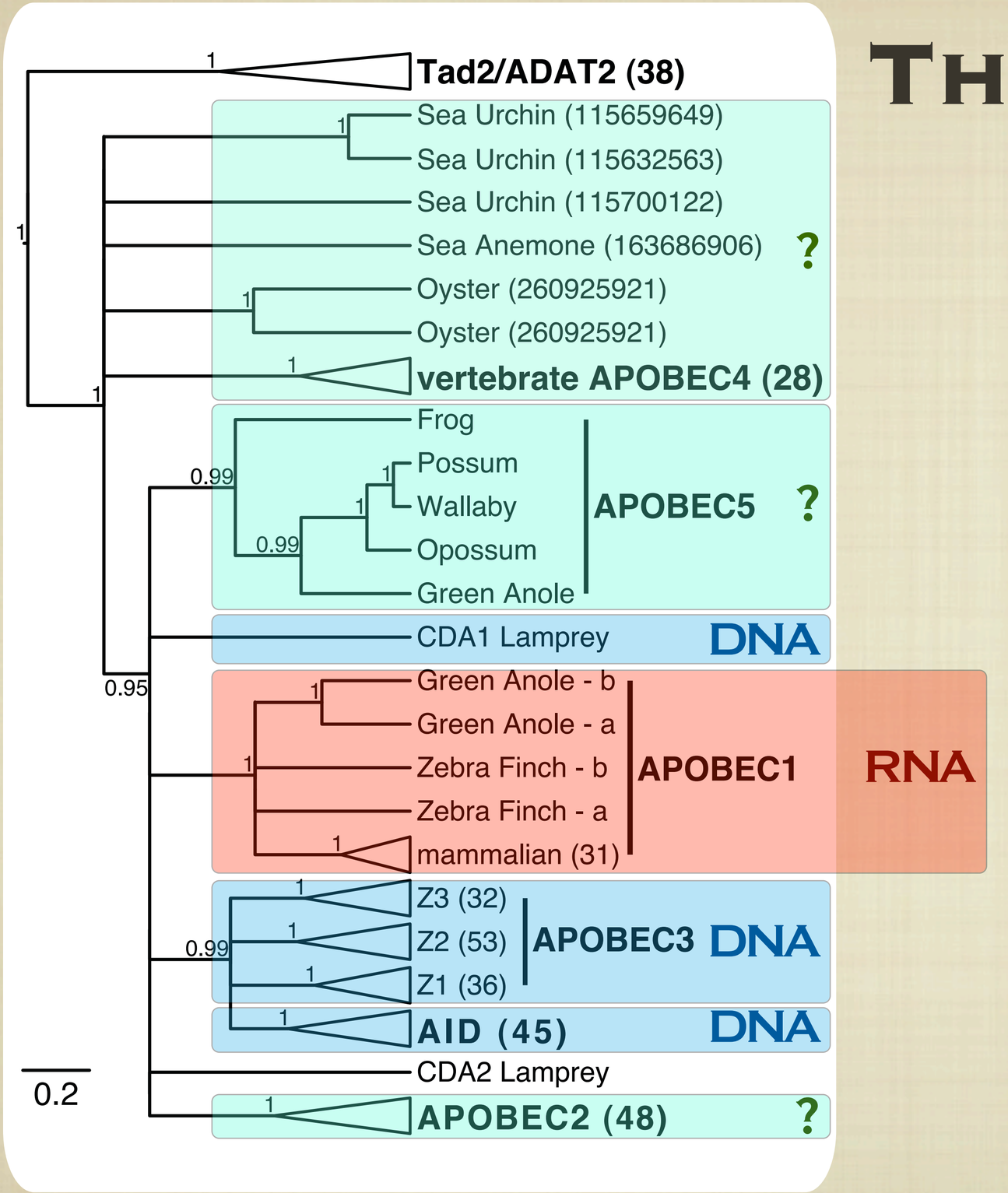
APOBEC5 - same as APOBEC3s?

APOBEC2 - muscle development



ancient retrotranscriptional event?

The Odd one


Tad2/ADAT2 (38)
Sea Urchin (115659649)
Sea Urchin (115632563)
Sea Urchin (115700122)
Sea Anemone (163686906)
?
Oyster (260925921)
Oyster (260925921)
vertebrate APOBEC4 (28)
Frog
Possum
Wallaby
Opossum
Green Anole
APOBEC5
?
CDA1 Lamprey
DNA
Green Anole - b
Green Anole - a
Zebra Finch - b
Zebra Finch - a
mammalian (31)
APOBEC1
RNA
Z3 (32)
Z2 (53)
Z1 (36)
APOBEC3
DNA
AID (45)
DNA
CDA2 Lamprey
APOBEC2 (48)
?
1
0.99
0.95
0.2

APOBEC1
AN RNA EDITING ENZYME

- **catalyzes the C>U deamination of the ApolipoproteinB mRNA**

  Scott, 1987; Davidson, 1993

- **Other mRNA targets**

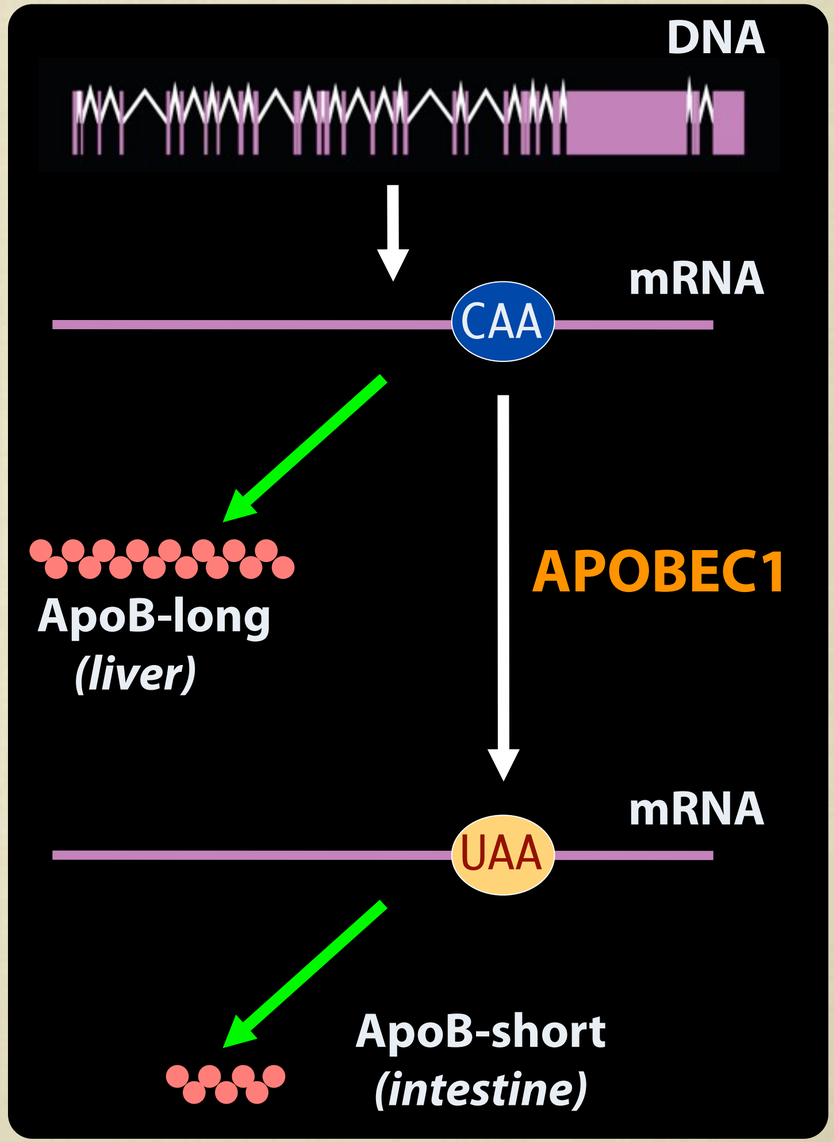
  Skuse, 1996; Anant 2000; Rosenberg 2011

- **APOBEC1 deficient mice do not present any characteristic phenotype, aside the lack of ApoB editing**

  Morrison, 1996; Nakamuta, 1996; Hirano, 1996

- **It is able to deaminate DNA**

  Harris, 2002; Cervantes Gonzalez, 2009; Petit, 2009



WHAT ABOUT CANCER?

APOBEC1 transgenes develop Tumors

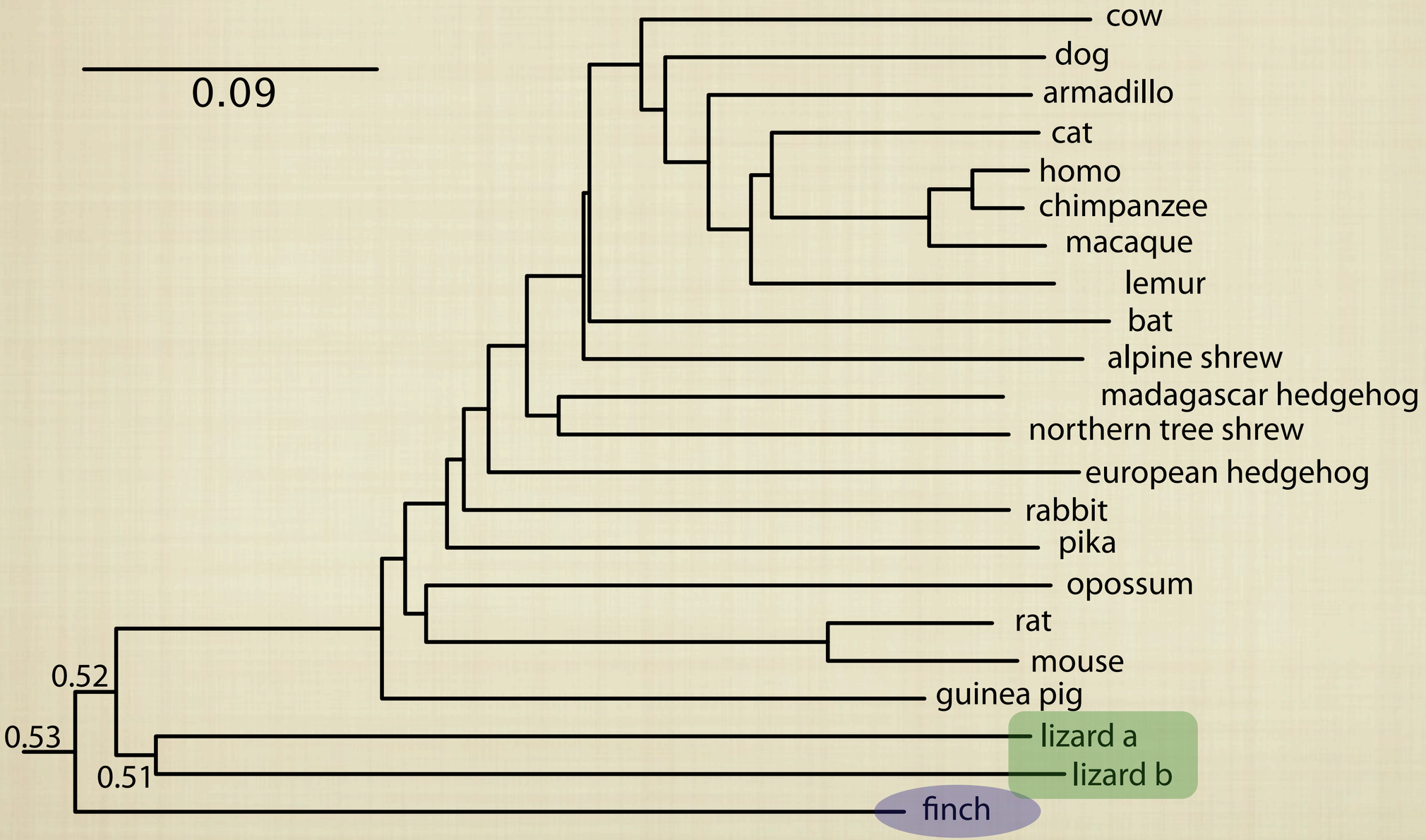
(hepatocellular carcinomas)

Yamanaka et al. 1995

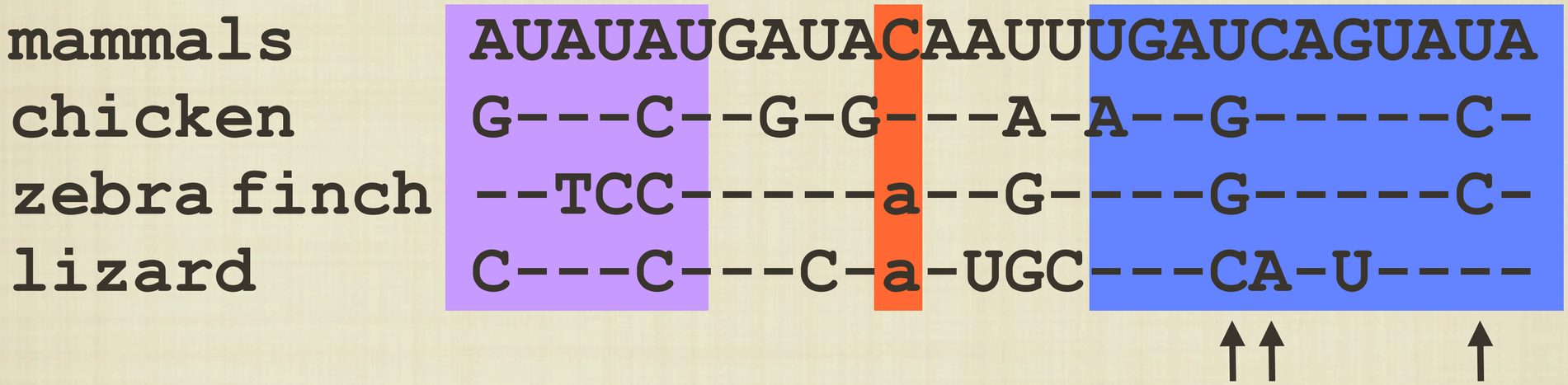
Cancer prone (Apc^{-}) mice deficient in APOBEC1, suffer less Tumours

Blanc et al. 2007

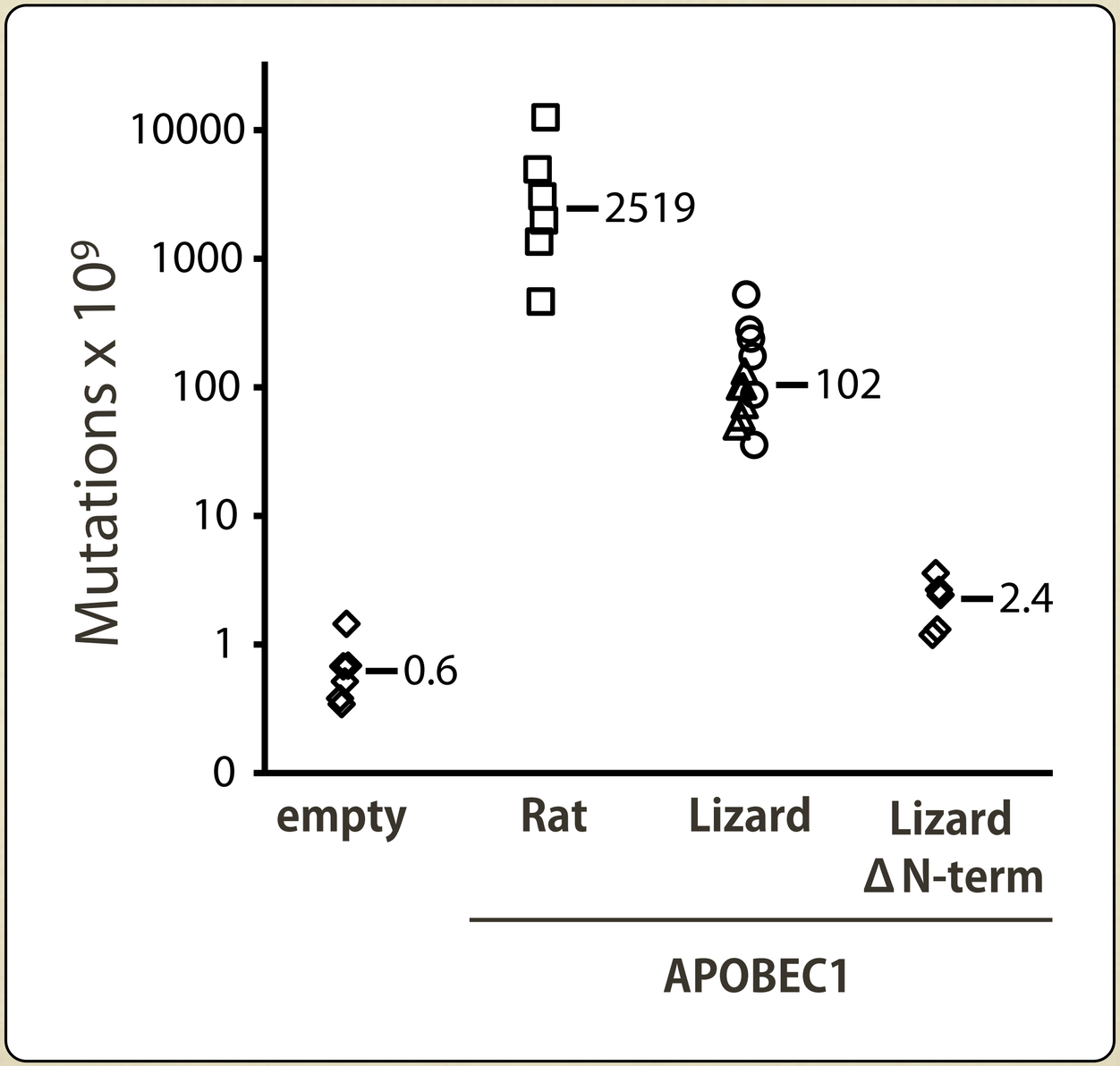
Trees, Birds, and Lizards



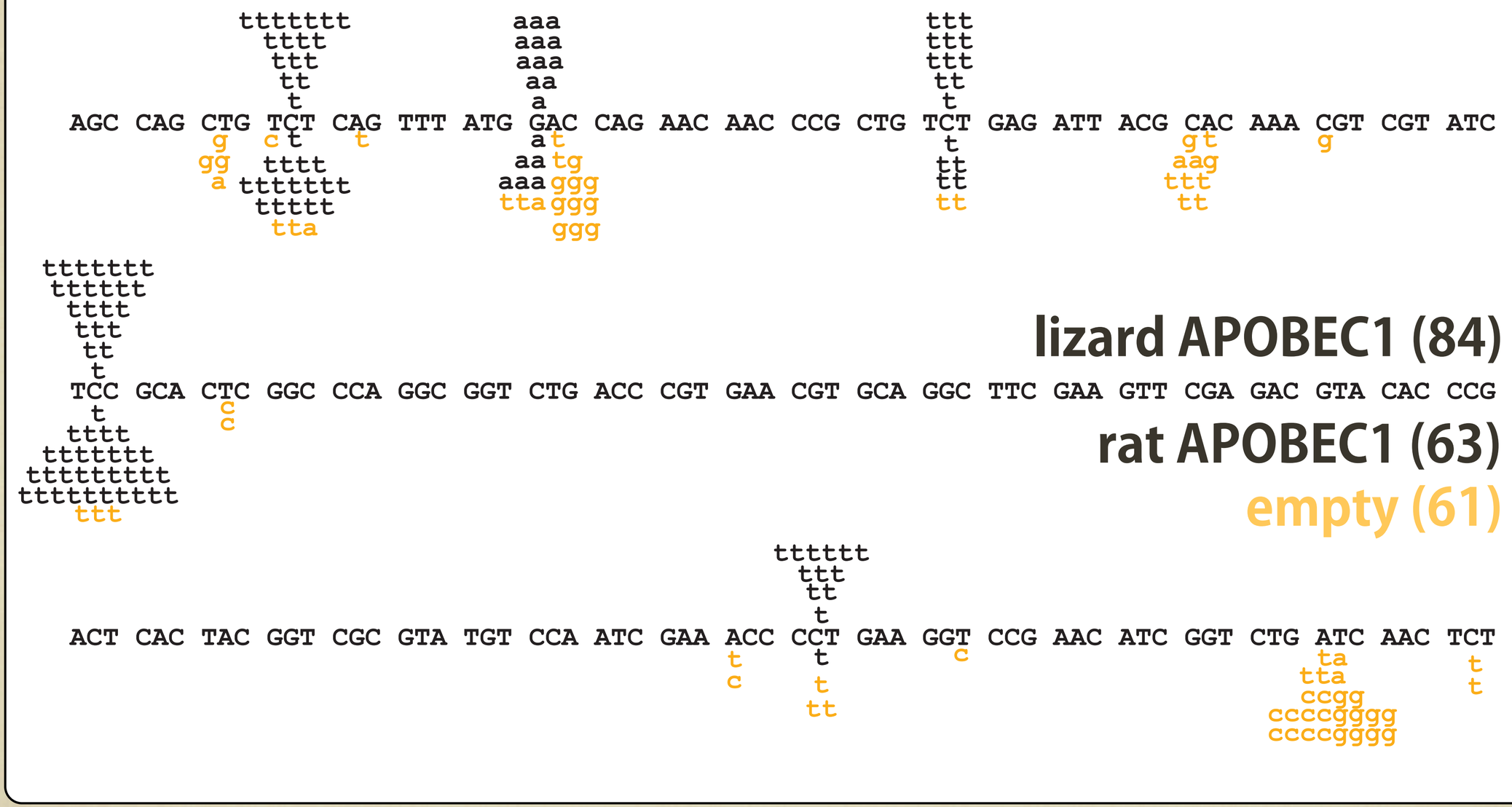
No Evidence of ApoB mRNA editing



DNA EDITING


Mutations x 10⁹
10000
1000
100
10
1
0
2519
102
2.4
0.6
empty
Rat
Lizard
Lizard
ΔN-term
APOBEC1

DNA EDITING


AGC CAG CTG TCT CAG TTT ATG GAC CAG AAC AAC CCG CTG TCT GAG ATT ACG CAC AAA CGT CGT ATC
ACT CAC TAC GGT CGC GTA TGT CCA ATC GAA ACC CCT GAA GGT CCG AAC ATC GGT CTG ATC AAC TCT
TCC GCA CTC GGC CCA GGC GGT CTG ACC CGT GAA CGT GCA GGC TTC GAA GTT CGA GAC GTA CAC CCG
lizard APOBEC1 (84)
rat APOBEC1 (63)
empty (61)

Back to Cancer

APOBEC1 transgenes develop Tumors

(hepatocellular carcinomas)

Yamanaka et al. 1995

Cancer prone (Apc^{-}) mice deficient in APOBEC1, suffer less Tumours

Blanc et al. 2007

RNA

Core Research Laboratory

ITT Istituto Toscano Tumori

Cesare Sala

Francesco Severi

Giulia Saraconi

Giorgio Mattiuz

Riccardo Pecori

Chiara Papucci

Francesca Niccheri

Silvia Pietrobono