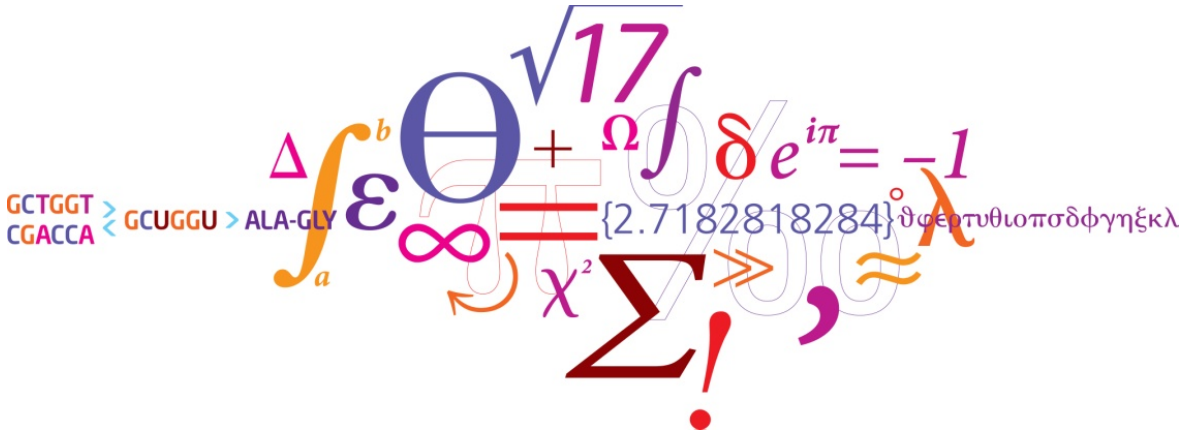
Maturation of BCRs and TCRs

Paolo Marcatili



DTU Biosys
Department of Systems Biology

Agenda

9.00 – 10.00 – BCR and TCR maturation

10.00 – 12.00 Analyse BCR and TCR sequences

12.00 - 13.00 *Lunch Break*

13.00 – 14.00 Antibody and TCR structure

14.00 – 16.00 Exercise – Prediction of antibody structure

Overview

- BCRs and TCRs have to recognize different antigens
- More than possibly encoded in the genome

BUT

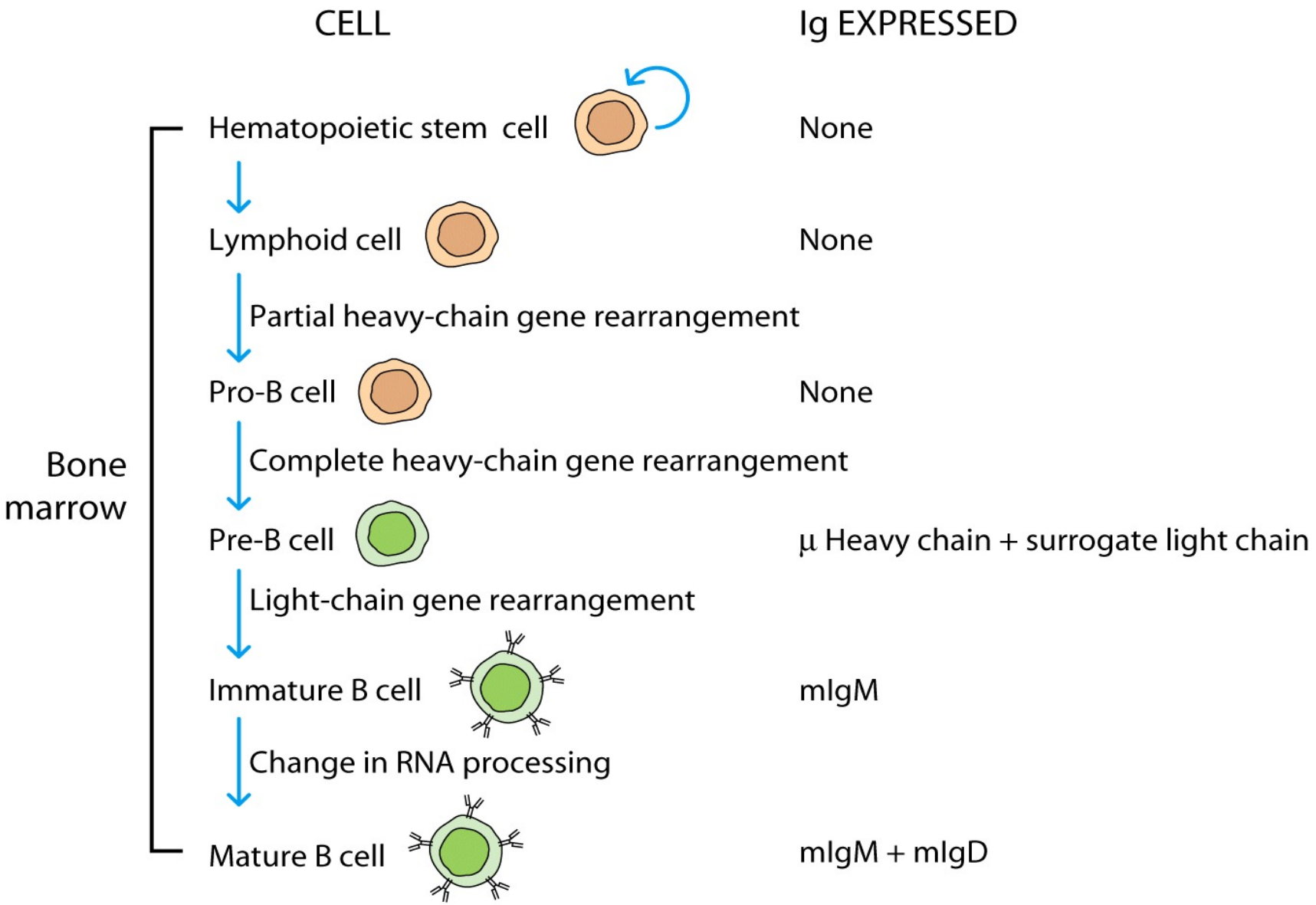
- Functional
- Properly folded
- Non cross-reactive

Solution

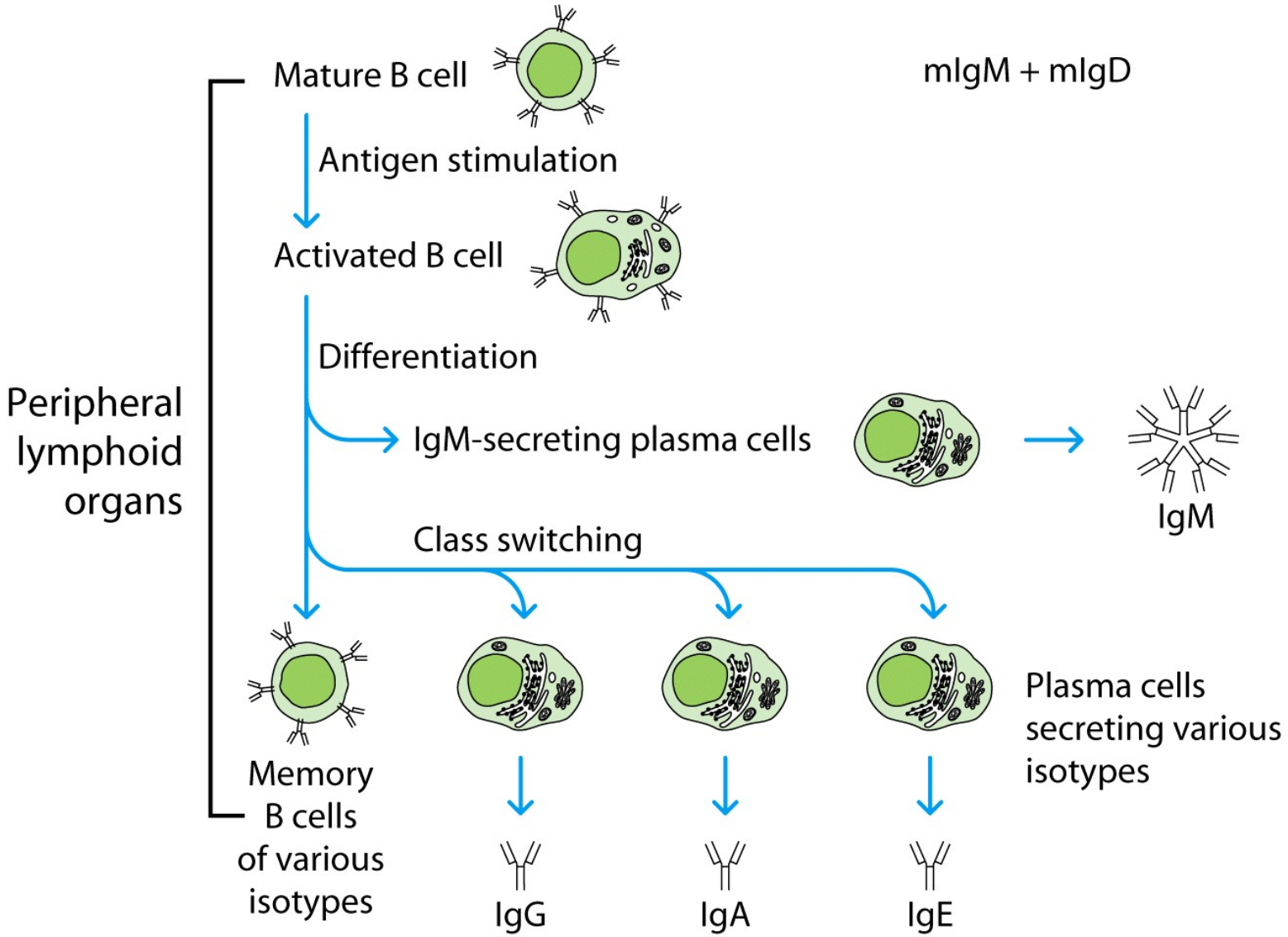
- Multiple genes that recombine
- very precise rules

- Somatic Mutations
- At specific locations

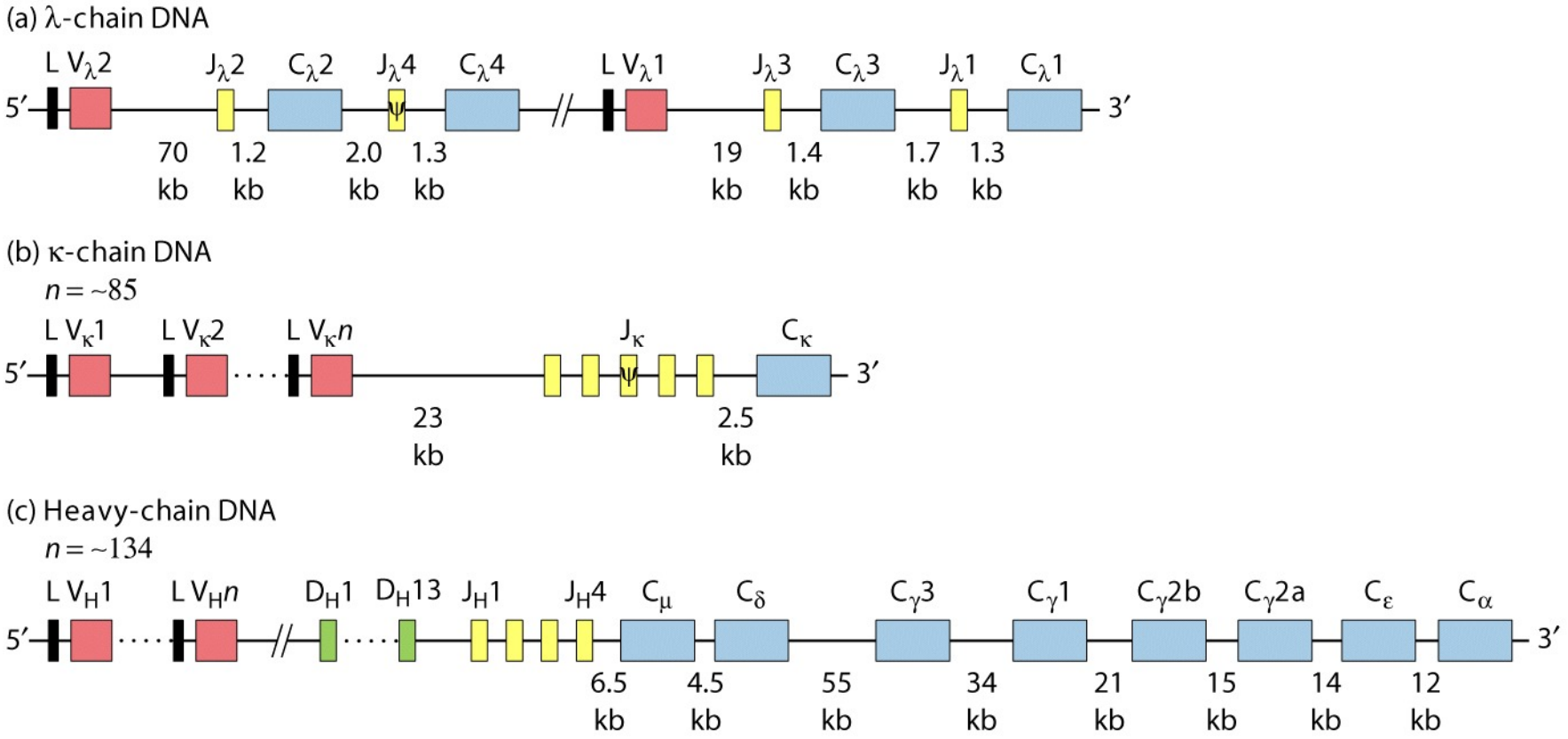
B lymphocyte development



B lymphocyte development (2)



Read Kuby pages 109-110: Multigene Organization of Ig Genes

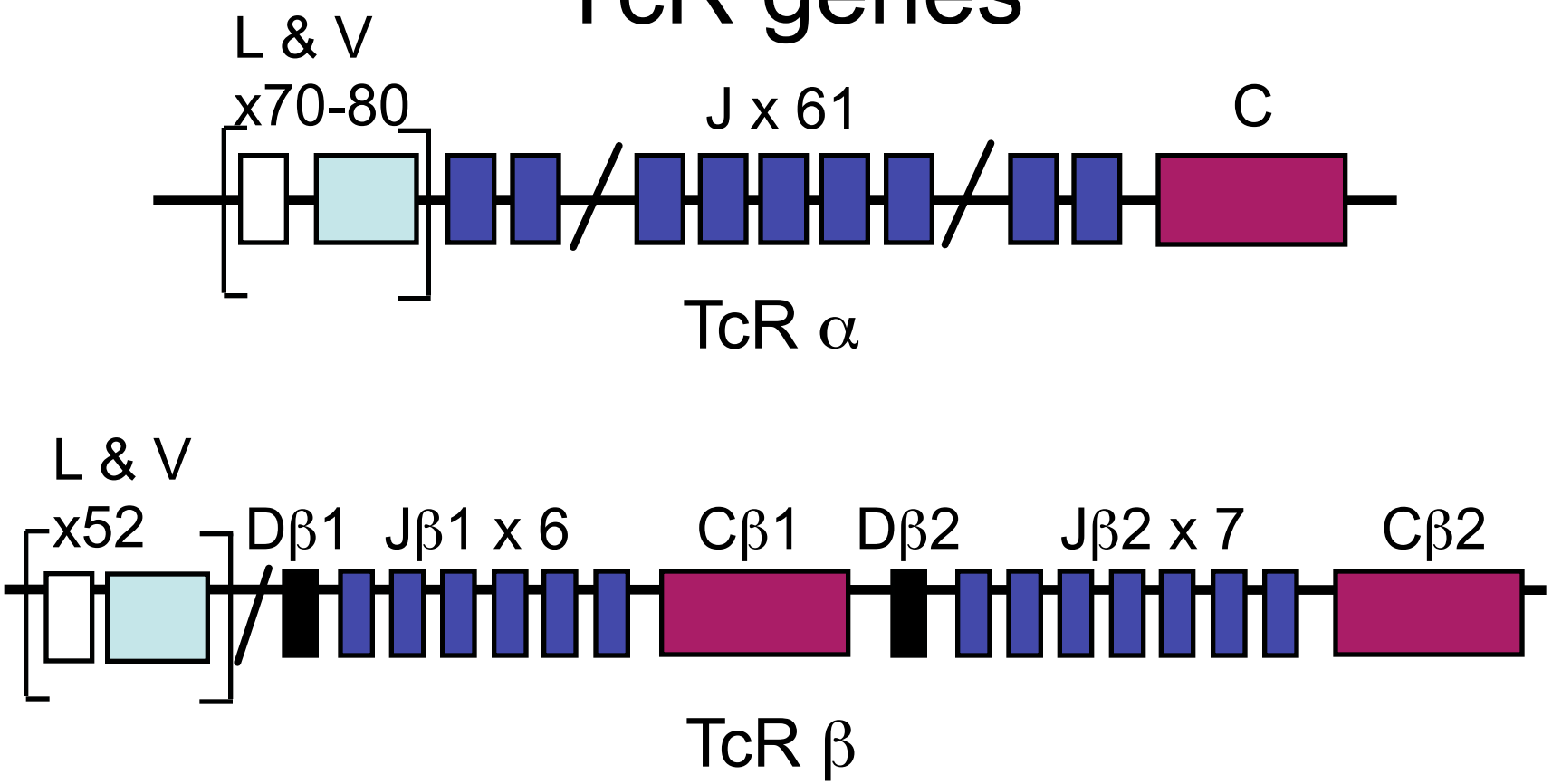


Kuby Figure 5-3

Kuby Figure 5-5

Read Kuby pages 110-112: Variable-Region Gene Rearrangements

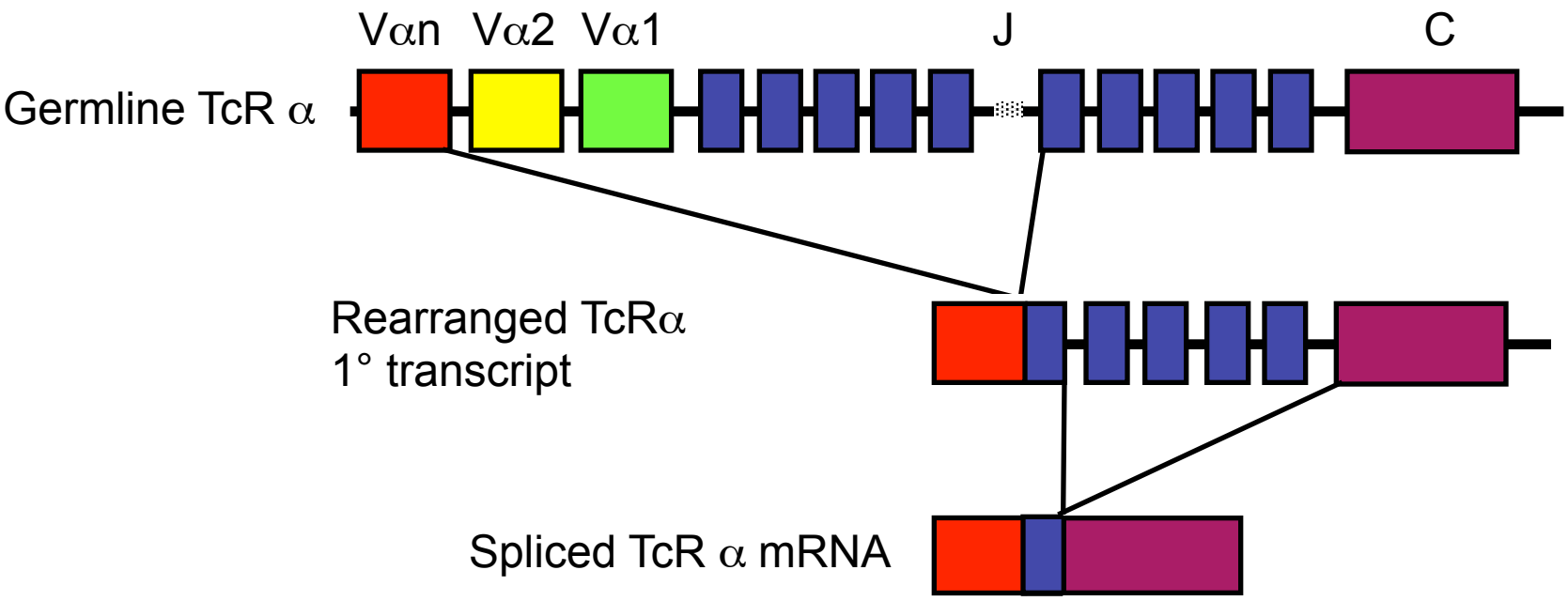
TcR genes



TcR genes segmented into V, (D), J & C elements
(VARIABLE, DIVERSITY, JOINING & CONSTANT)
Closely resemble Ig genes (α~IgL and β~IgH)

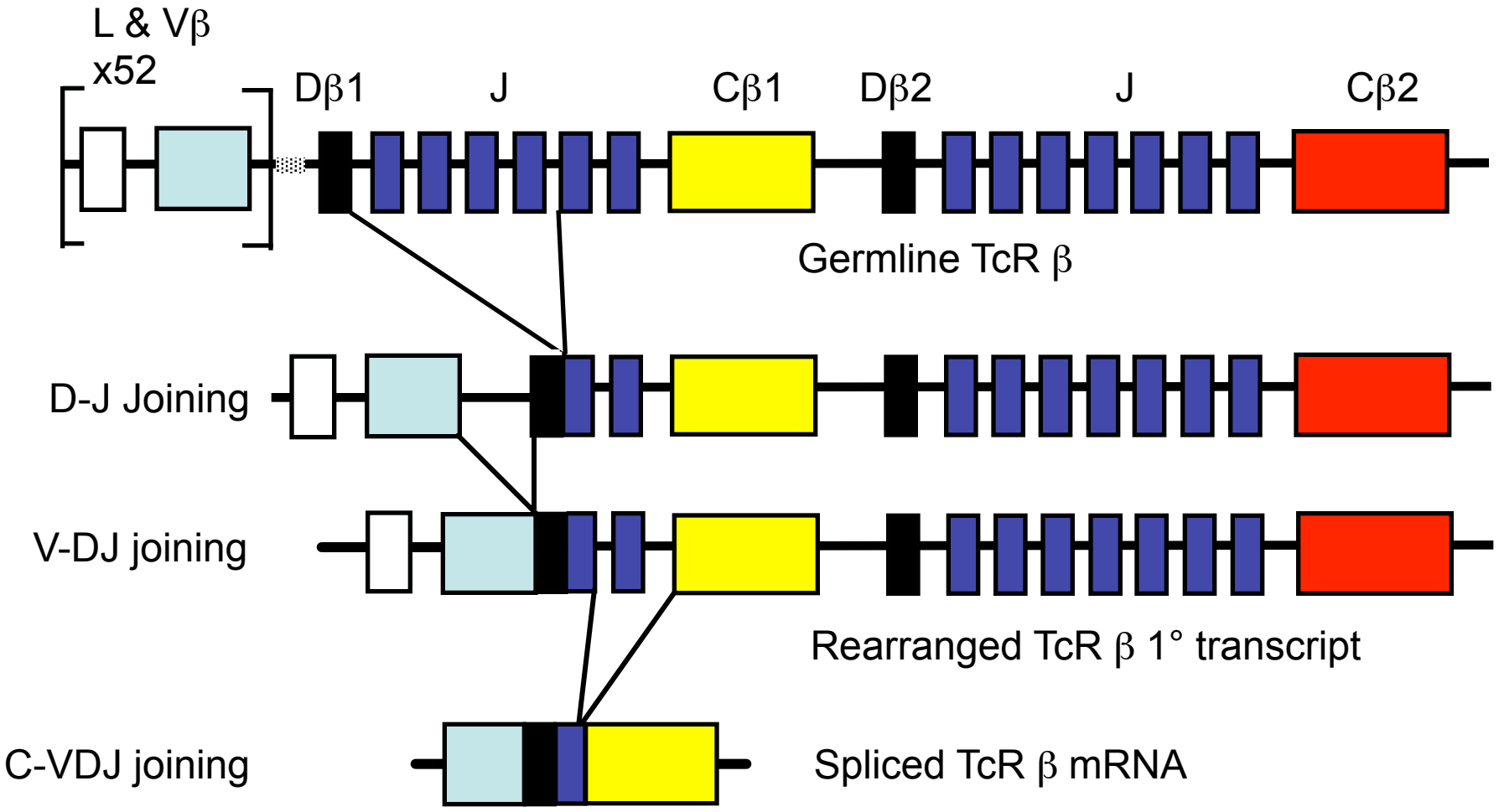
This example shows the mouse TcR locus

TcR α gene rearrangement by SOMATIC RECOMBINATION



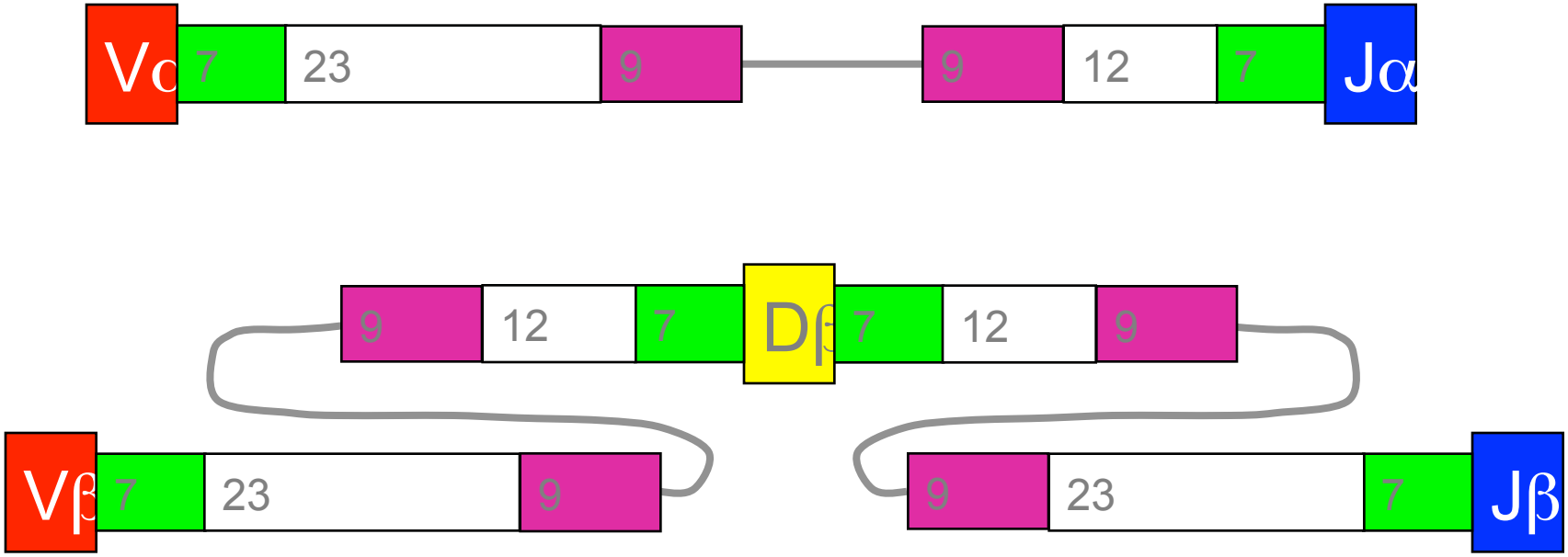
Rearrangement very similar to the IgL chains

TcR β gene rearrangement
SOMATIC RECOMBINATION

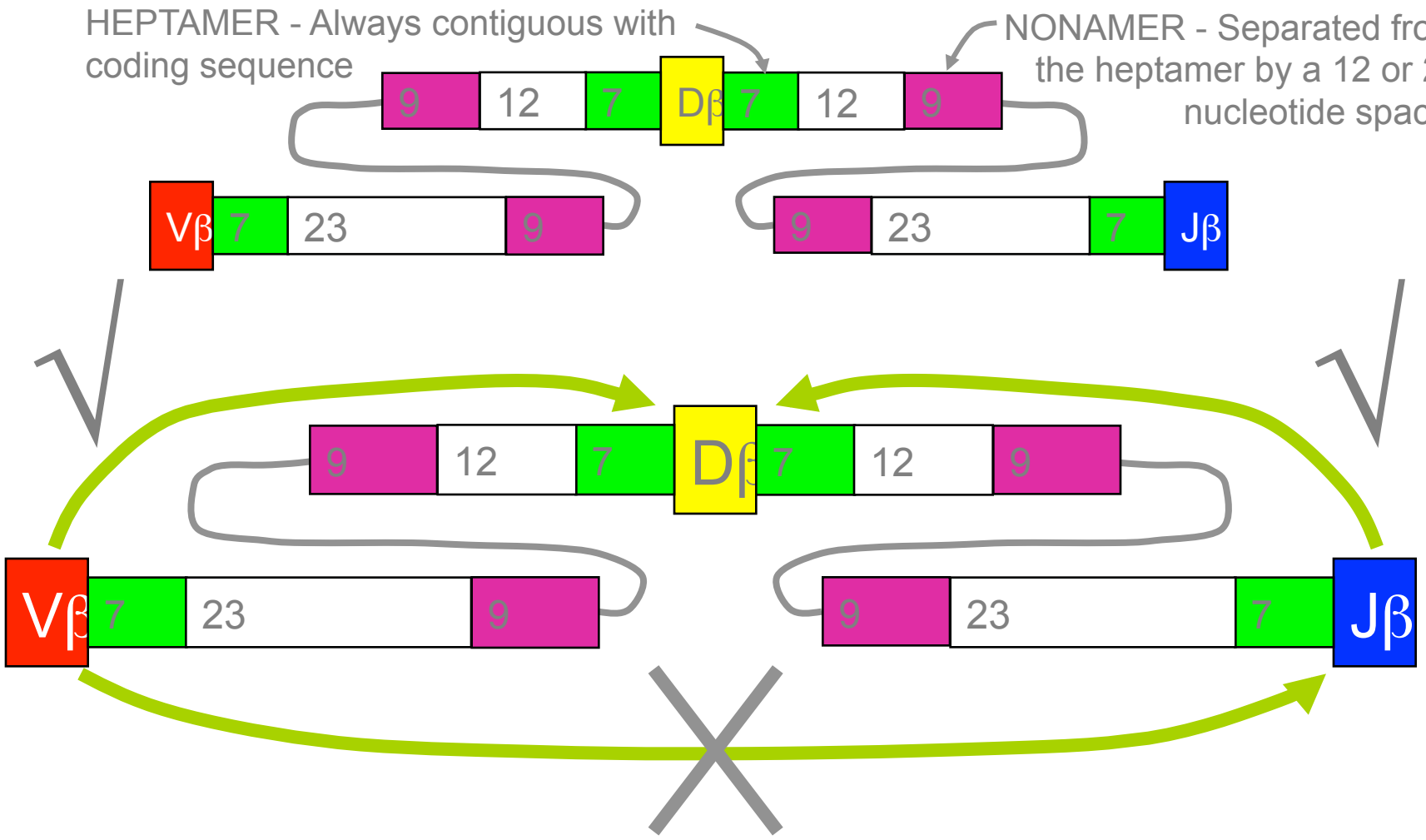


V, D, J flanking sequences

Sequencing upstream and downstream of V, D and J elements revealed conserved sequences of 7, 23, 9 and 12 nucleotides.

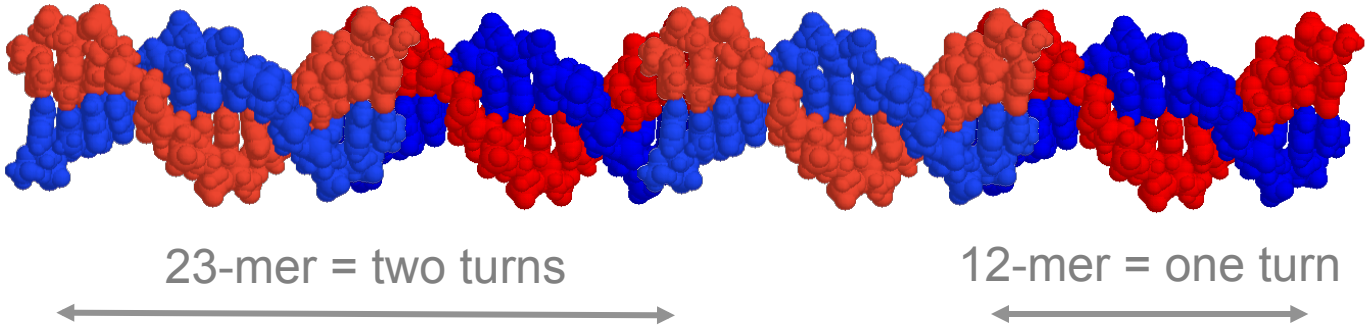


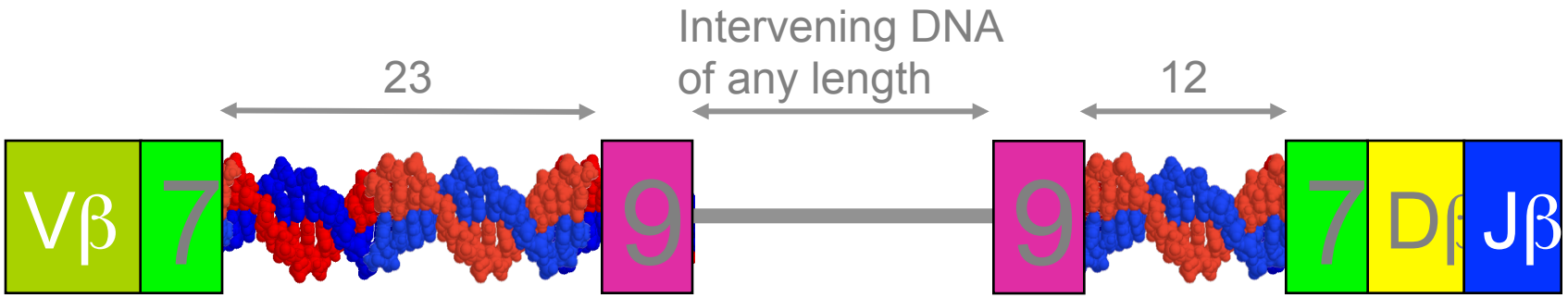
Recombination signal sequences (RSS)



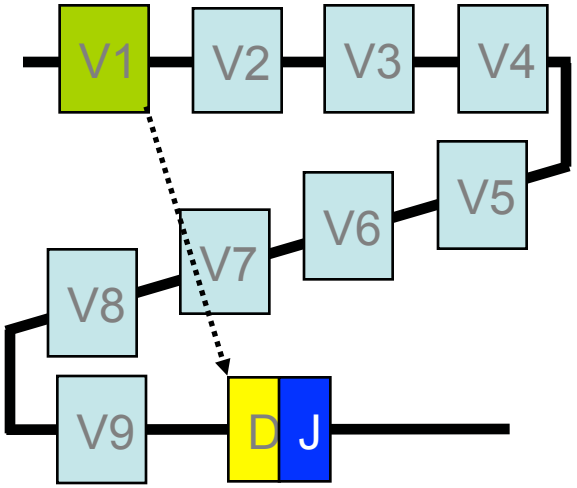
12-23 RULE – A gene segment flanked by a 23mer RSS can only be linked to a segment flanked by a 12mer RSS

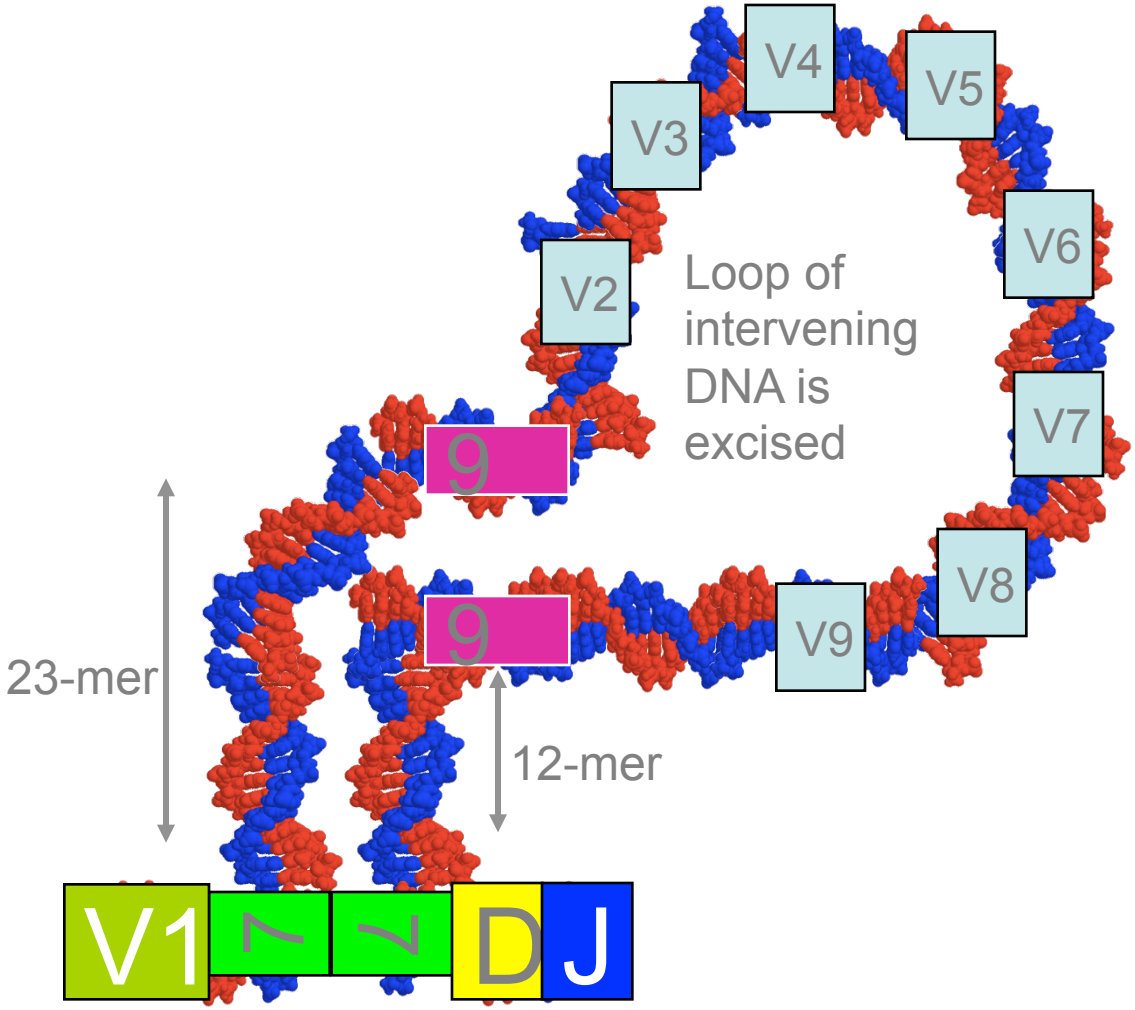
Molecular explanation of the 12-23 rule





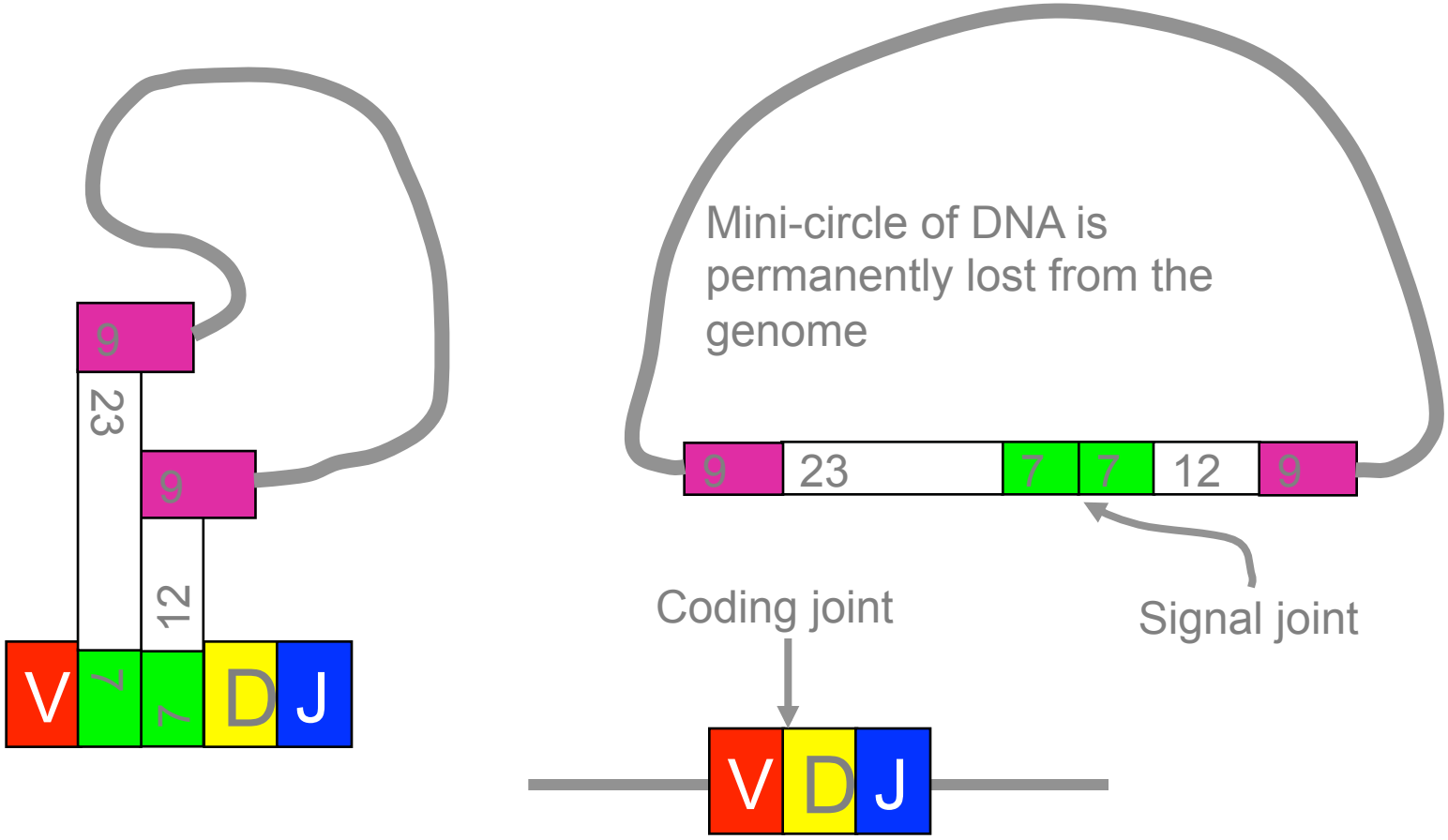
Molecular explanation of the 12-23 rule





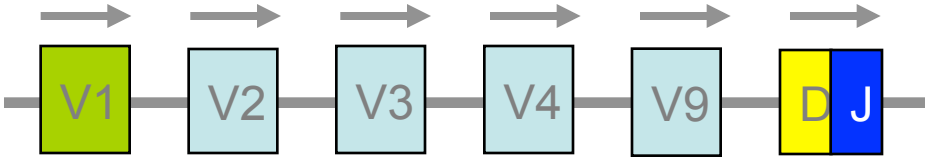
- Heptamers and nonamers align back-to-back
- The shape generated by the RSS's acts as a target for recombinases
- An appropriate shape can not be formed if two 23-mer flanked elements attempted to join (i.e. the 12-23 rule)

Junctional diversity

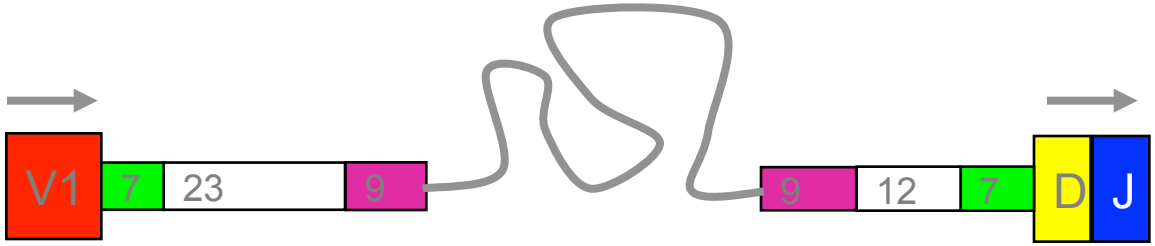


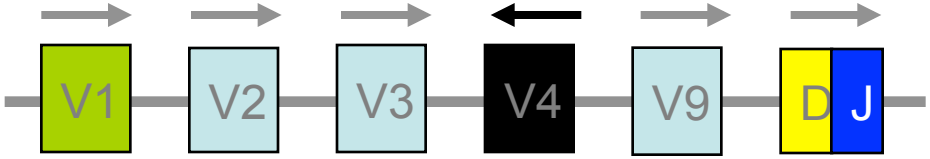
Imprecise and random events that occur when the DNA breaks and rejoins allows new nucleotides to be inserted or lost from the sequence at and around the coding joint.

Non-deletional recombination

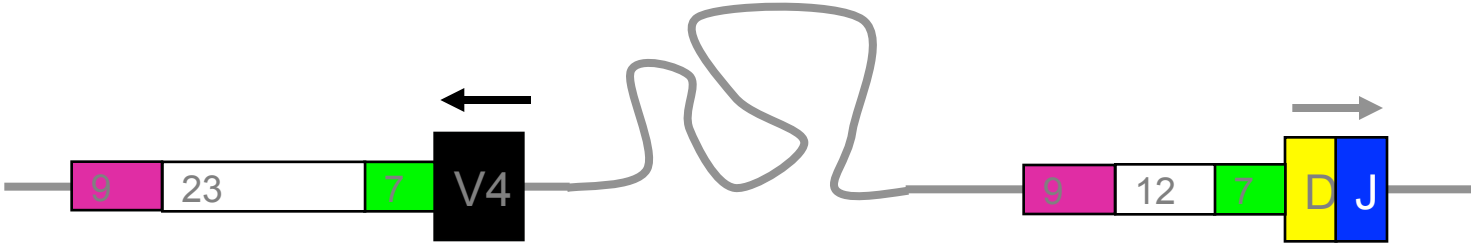


Looping out works if all V genes are in the same transcriptional orientation





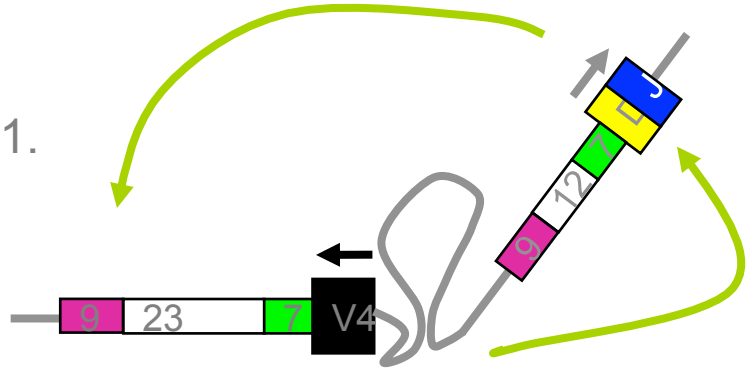
How does recombination occur when a V gene is in opposite orientation to the DJ region?



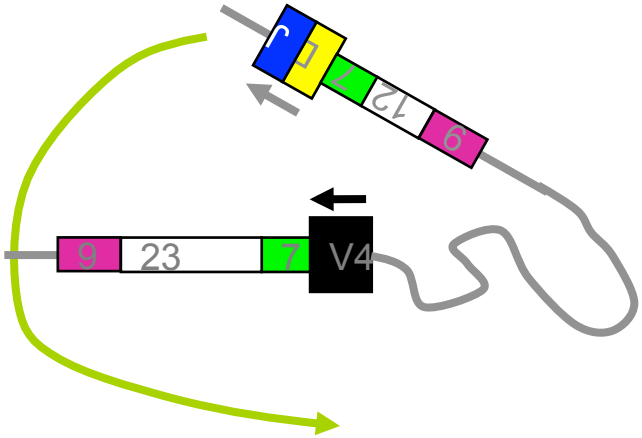
Non-deletional recombination

V4 and DJ in opposite transcriptional orientations

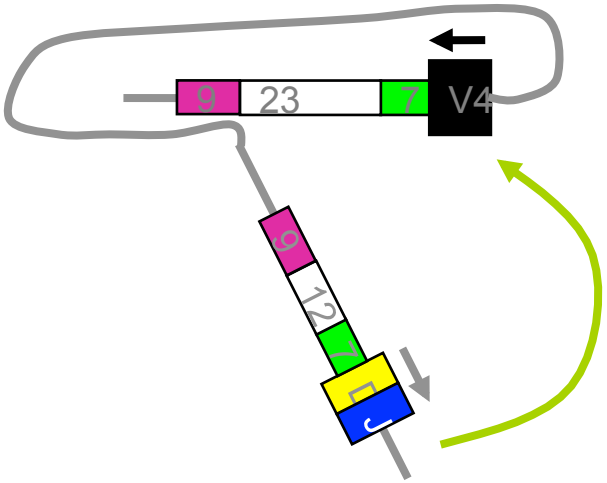
1.



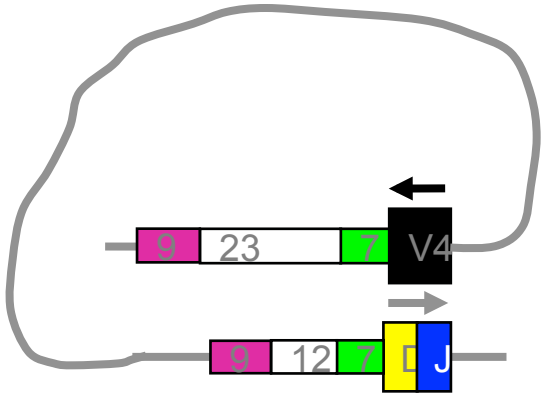
2.

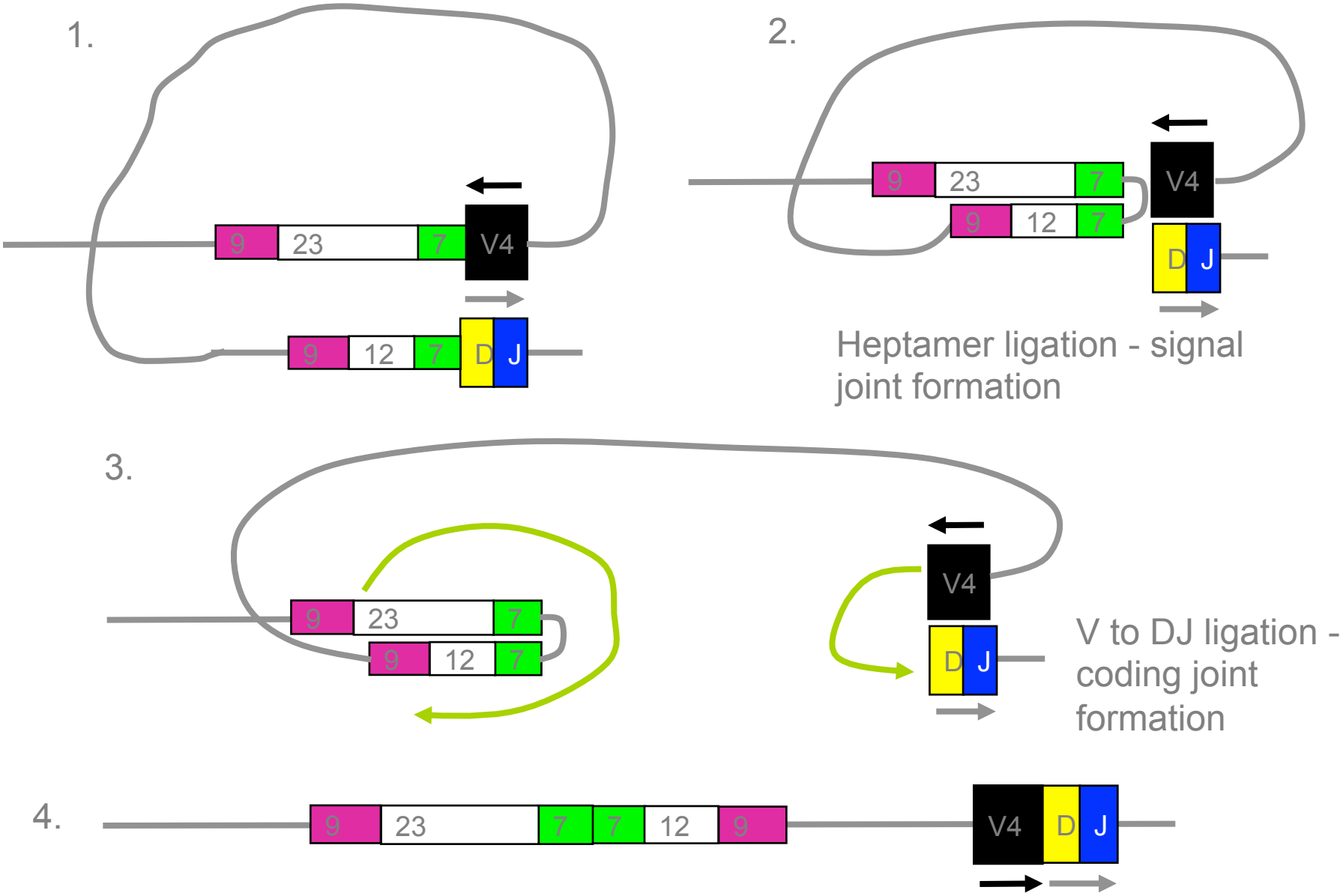


3.

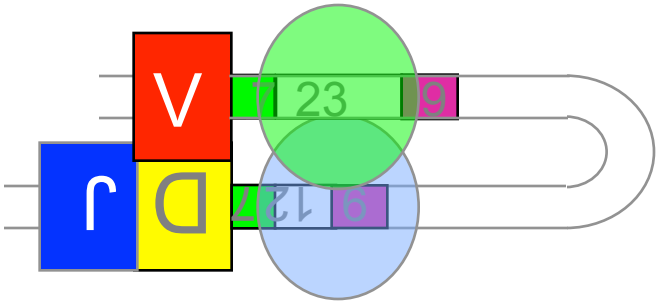


4.

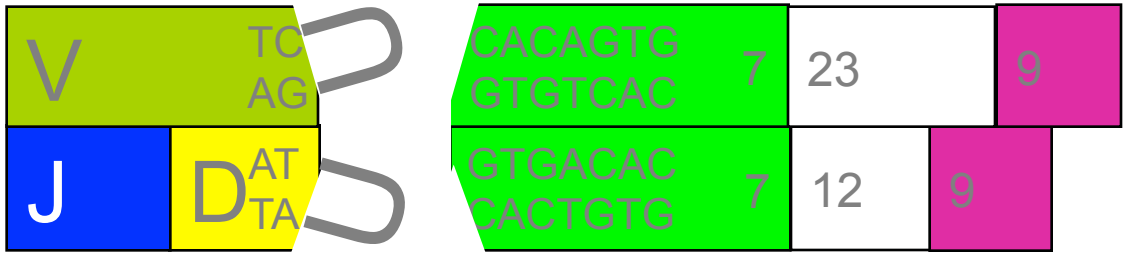



1.
9
23
7
V4
9
12
7
D
J
2.
9
23
7
V4
9
12
7
D
J
Heptamer ligation - signal joint formation
3.
9
23
7
9
12
7
V4
D
J
V to DJ ligation - coding joint formation
4.
9
23
7
7
12
9
V4
D
J
Fully recombined VDJ regions in same transcriptional orientation
No DNA is deleted

Junctional diversity: P nucleotide additions

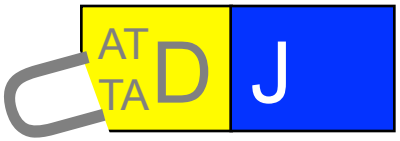


The recombinase complex makes single stranded nicks at random sites close to the ends of the V and D region DNA.

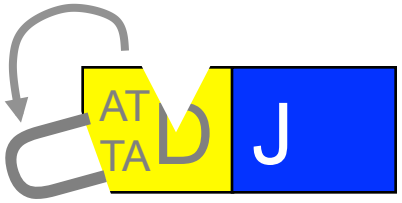


The 2nd strand is cleaved and hairpins form between the complimentary bases at ends of the V and D region.

Generation of the palindromic sequence

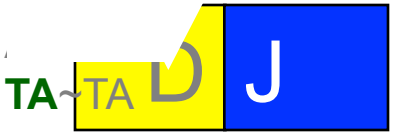


Regions to be joined are juxtaposed



Endonuclease cleaves single strand at random sites in V and D segment

The nicked strand ‘flips’ out



The nucleotides that flip out, become part of the complementary DNA strand

In terms of G to C and T to A pairing, the ‘new’ nucleotides are palindromic. The nucleotides GA and TA were not in the genomic sequence and introduce diversity of sequence at the V to D join.

Junctional Diversity – N nucleotide additions

Terminal deoxynucleotidyl transferase (TdT) adds nucleotides randomly to the P nucleotide ends of the single-stranded V and D segment DNA

Complementary bases anneal

Exonucleases nibble back free ends

DNA polymerases fill in the gaps with complementary nucleotides and DNA ligase IV joins the strands

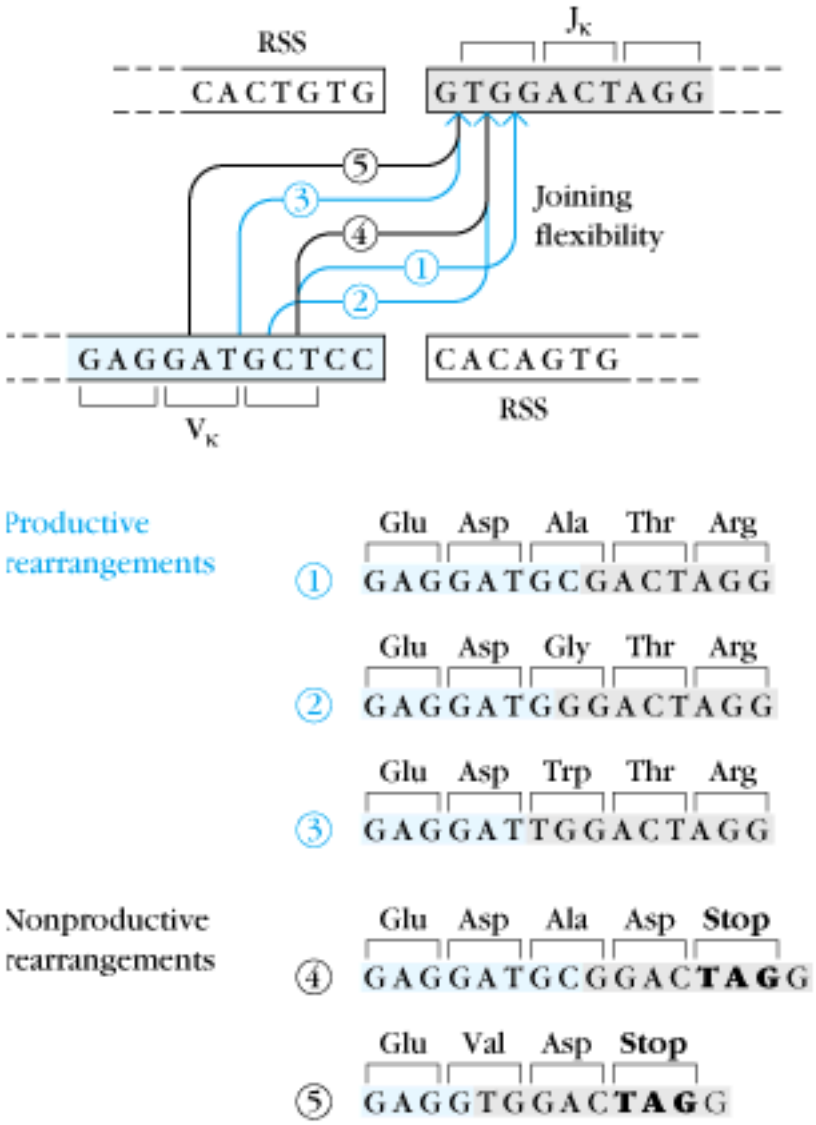
Junctional Diversity

TTTTT Germline-encoded nucleotides

TTTTT Palindromic (P) nucleotides - not in the germline

TTTTT Non-template (N) encoded nucleotides - not in the germline

Creates an essentially random sequence between the V region, D region and J region in beta chains and the V region and J region in alpha chains.



Kuby Figure 5-9

Productive and nonproductive rearrangements

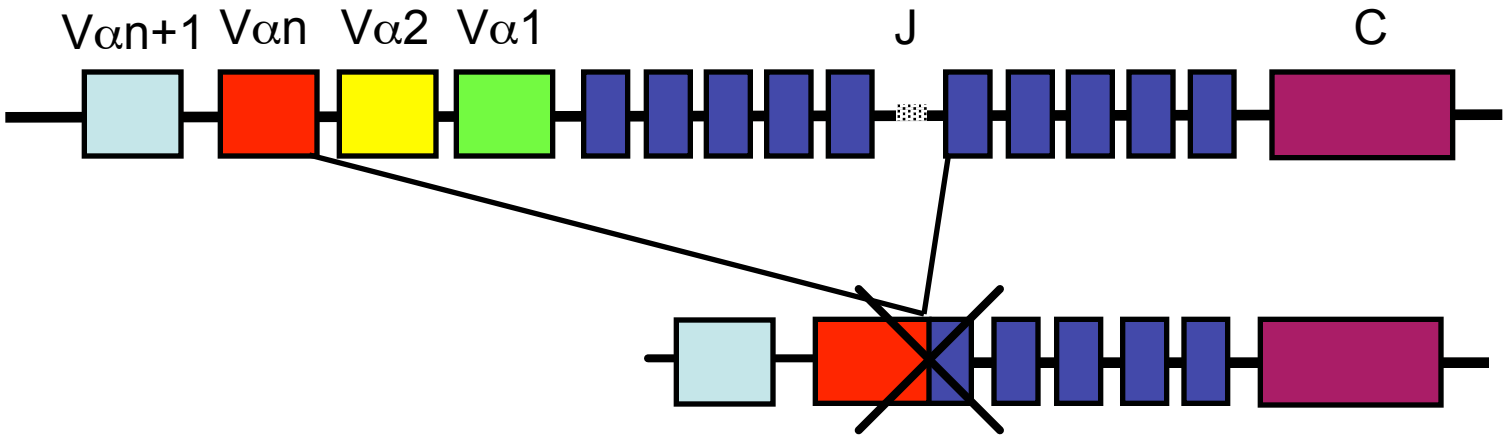
Joining of segments is not precise and may result in loss of the correct reading frame.

This may lead to introduction of stop codons --> nonproductive rearrangements.

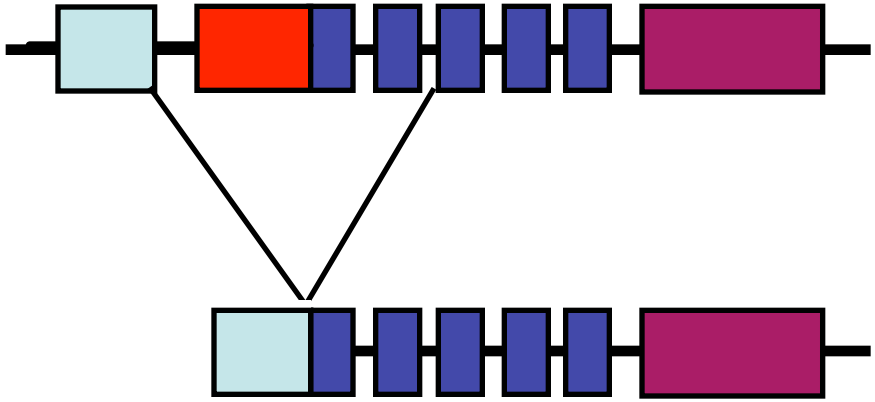
Read Kuby page 115: Ig-Gene Rearrangements May Be Productive or Nonproductive

TcR α gene rearrangement RESCUE PATHWAY

There is only a 1:3 chance of the join between the V and J region being in frame



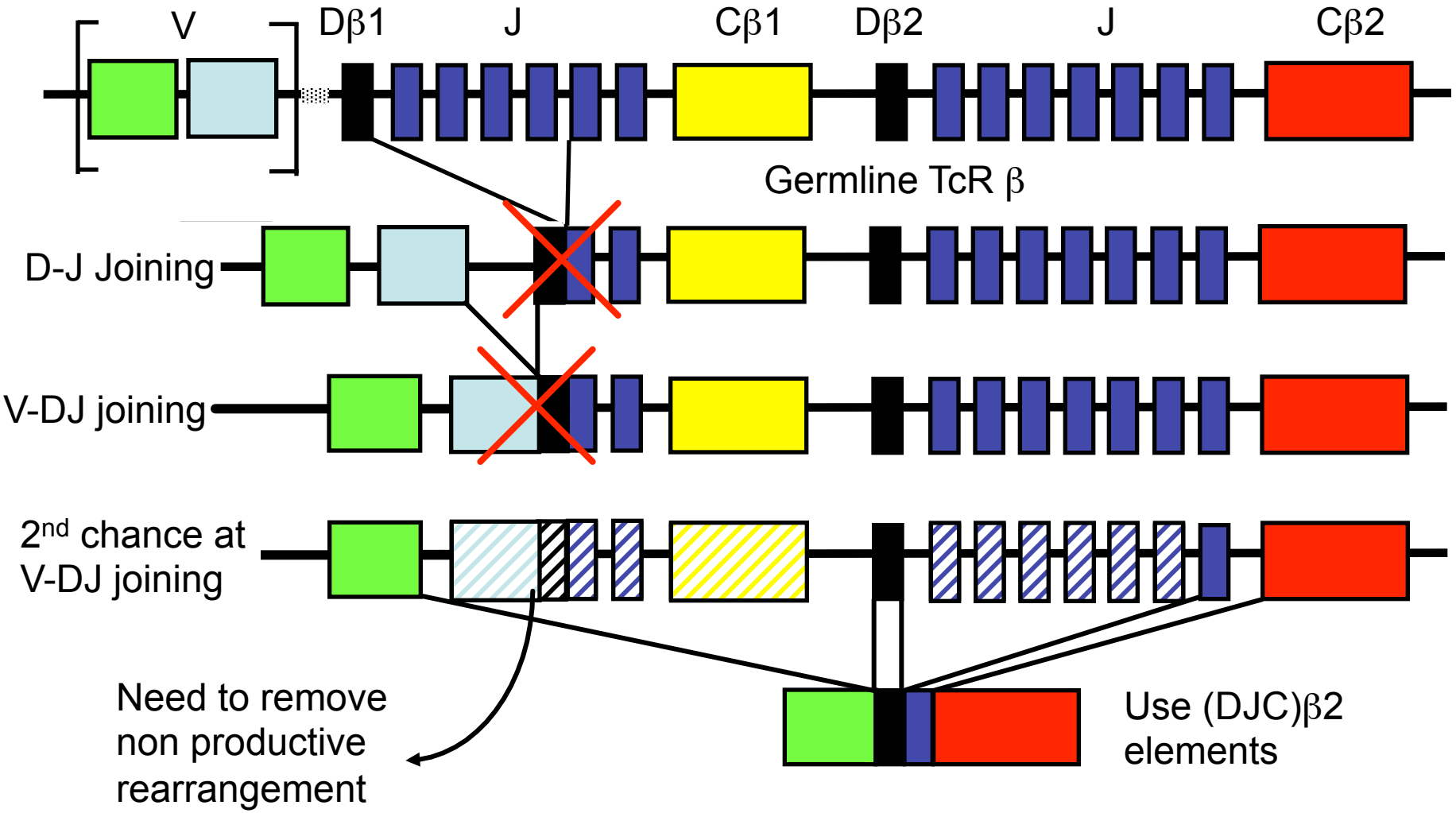
α chain tries for a second time to make a productive join using new V and J elements



Productively rearranged TcRα 1° transcript

TcR β gene rearrangement RESCUE PATHWAY

There is a 1:3 chance of productive D-J rearrangement and a 1:3 chance of productive D-J rearrangement
(i.e only a 1:9 chance of a productive β chain rearrangement)



Two alleles are available at each locus (maternal and paternal).

A B cell expresses only one heavy and light chain allele.

- ALLELIC EXCLUSION

First, one allele of the heavy chain is rearranged.

If the rearrangement is successful, the other allele will not be rearranged.

If the rearrangement is nonproductive, the other allele will be rearranged.

Once a heavy chain allele rearrangement is productive, light chain rearrangement will begin.

If rearrangement of both heavy chain alleles is nonproductive, the B cell will not mature further but will die of apoptosis within the bone marrow.

If a heavy chain allele is successfully rearranged, light chain rearrangement begins.

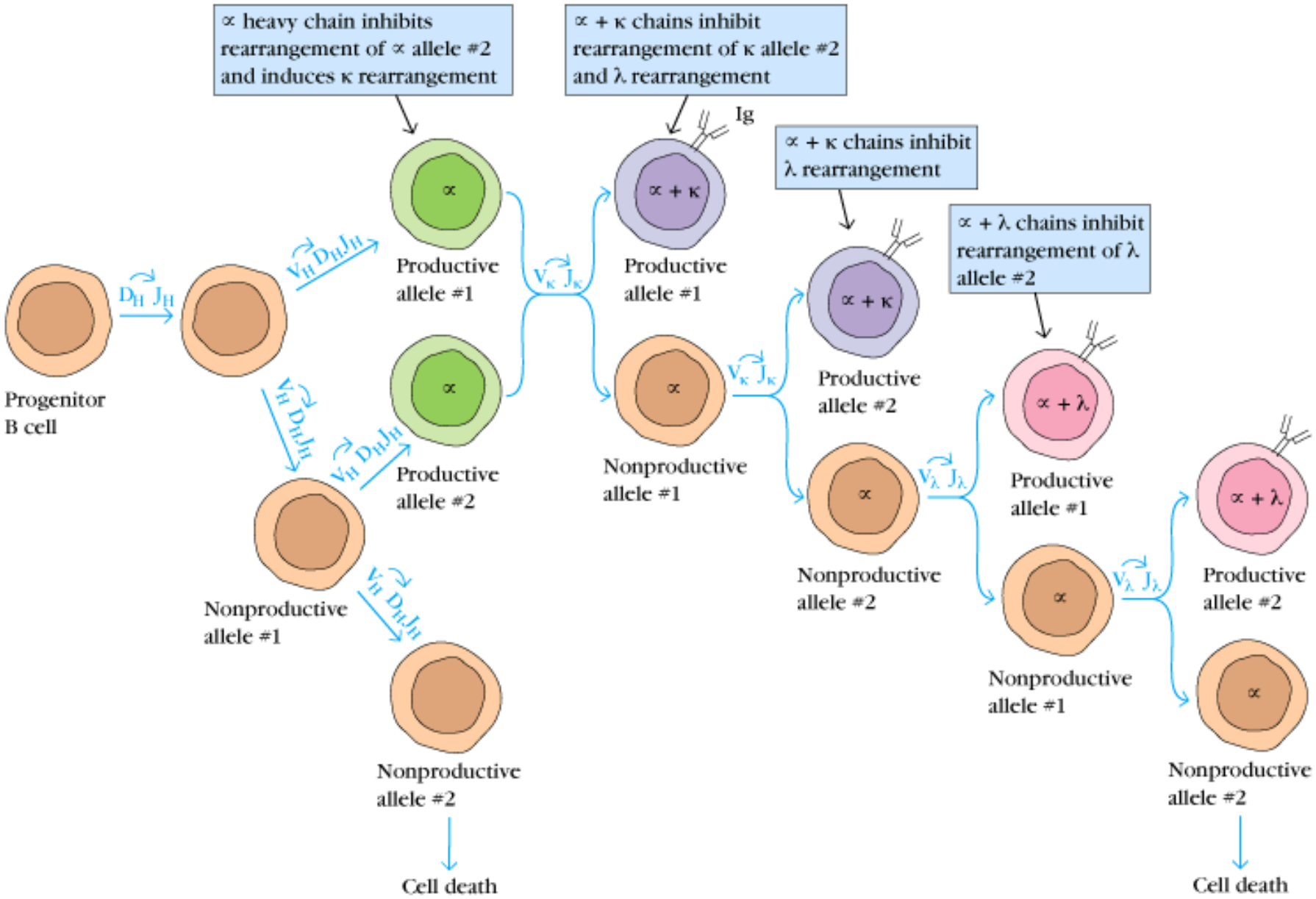
In humans, the kappa locus is rearranged first.

Rearrangement occurs at one allele at a time and continues until a productive rearrangement occurs.

If both kappa alleles rearrange nonproductively, rearrangement will begin at the lambda locus.

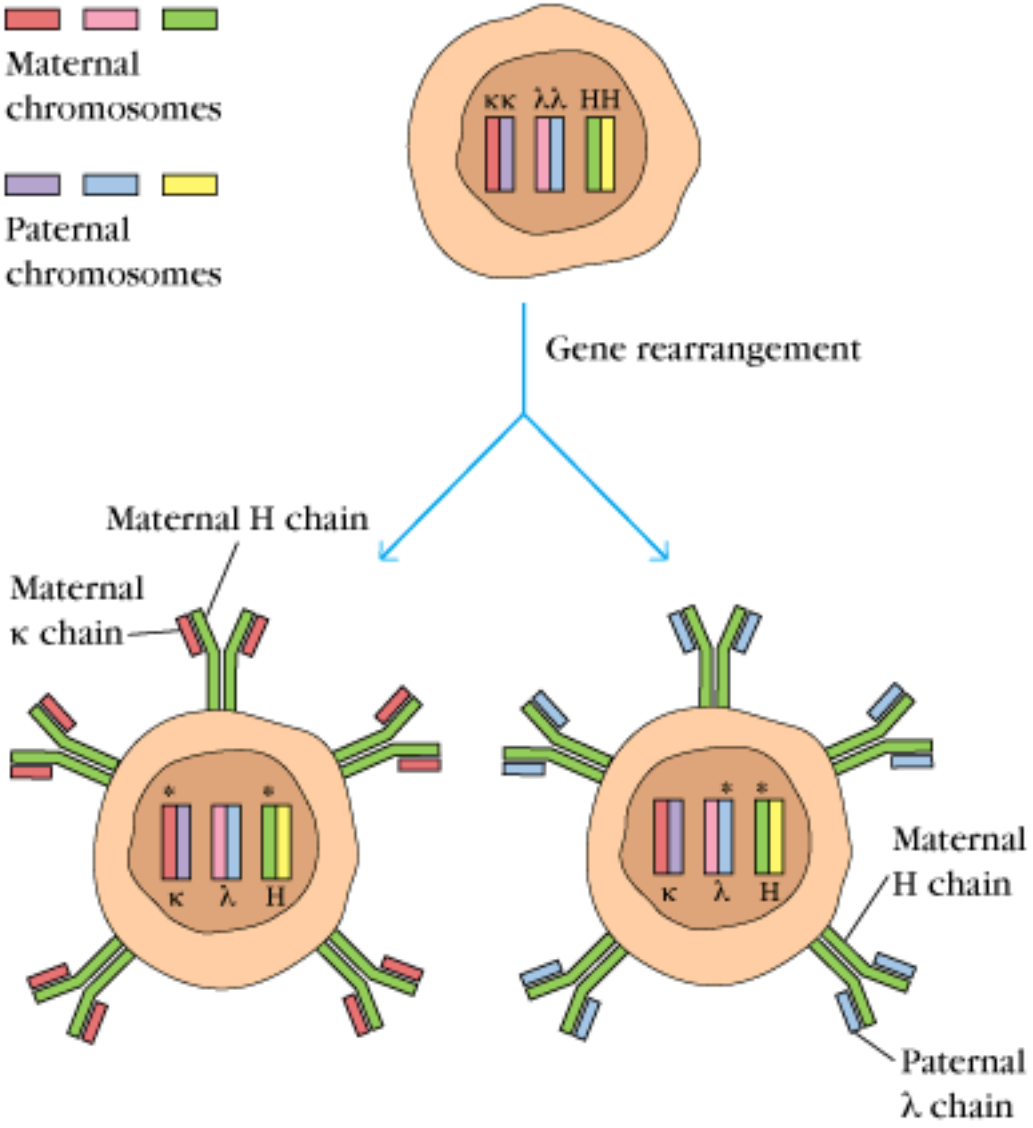
If all 4 alleles (both kappa alleles and both lambda alleles) rearrangements are nonproductive, the B cell will not mature but will instead die of apoptosis within the bone marrow.

If <u>either</u> both heavy chain alleles, <u>or</u> all four light chain alleles, rearrange nonproductively, the B cell will not mature.



Kuby Figure 5-11

Allelic exclusion



Kuby Figure 5-10

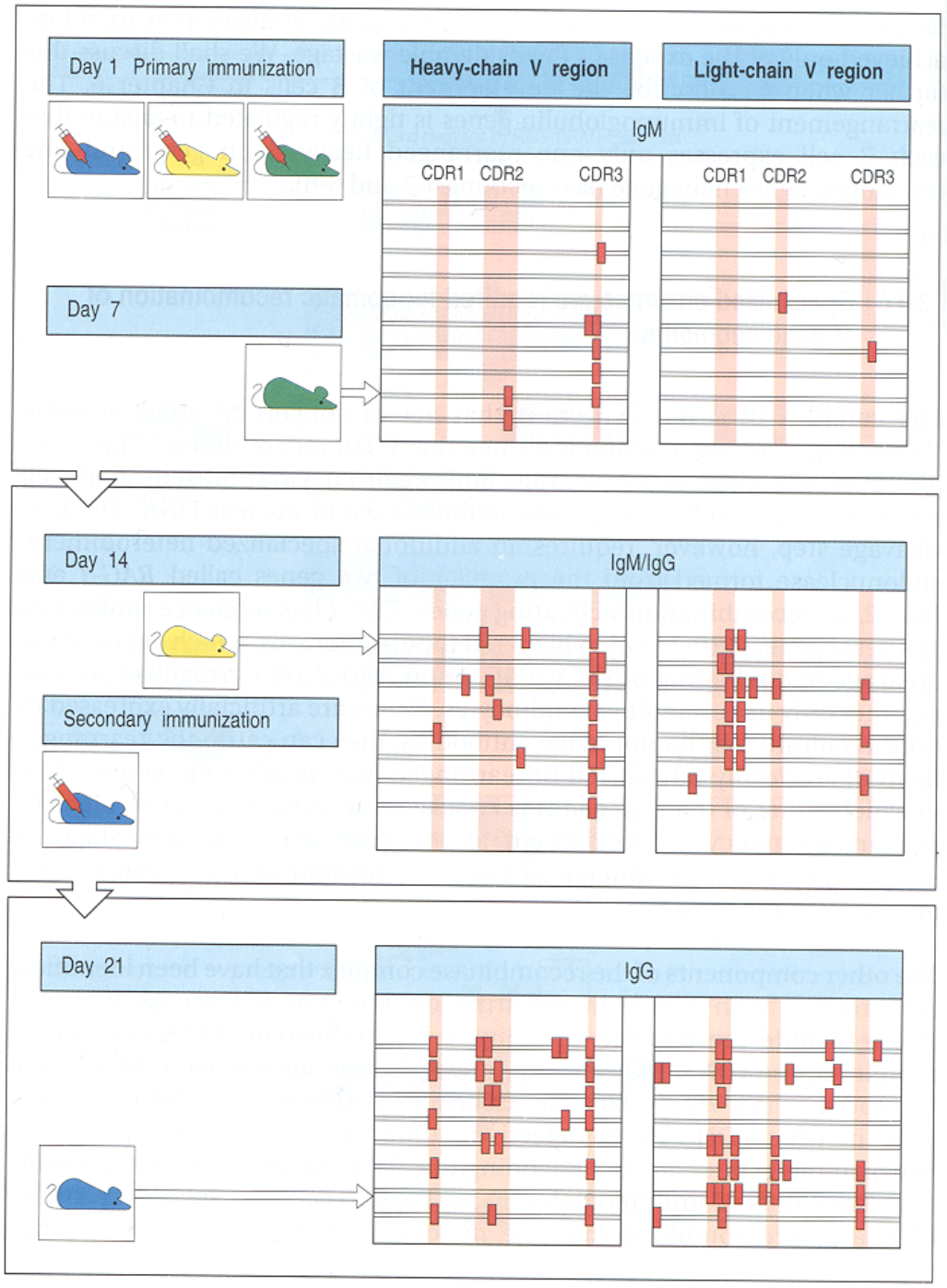
Read Kuby pages 115-117: Allelic Exclusion Ensures a Single Antigenic Specificity

7 means of generating antibody diversity

- Multiple germ-line gene segments
- Combinatorial V-(D)-J joining
- Junctional flexibility
- P-region nucleotide addition (P-addition)
- N-region nucleotide addition (N-addition)
- Somatic hypermutation
- Combinatorial association of light and heavy chains

Although the exact contribution of each of these avenues of diversification to total antibody diversity is not known, they each contribute significantly to the immense number of distinct antibodies that the mammalian immune system is capable of generating.

Accumulation of V-region point mutations during the antibody response.



Is antibody mutation induced?

- Natural mutation rate in the absence of repair is high
  ~10^{-5}/bp/generation
- With repair, spontaneous mutation rate is ~10^{-9} or less.
- Repair pathways

-DNA polymerase 3'-->5' exonucleolytic proofreading
improves fidelity ~100X

-mismatch repair system
improves fidelity ~100X

- Initial estimates found values of 10^{-3}-10^{-5}/bp/generation
  in clonally related B cells carrying mutations.
- Suggested that repair is either turned off or mutation is
  induced.

Characteristics of Somatic Mutation

1. Occurs at high rates: 10^{-4} -10^{-3} /bp/generation.
2. Occurs by untemplated single base substitutions.
3. Restricted to a brief period of B cell differentiation.
4. Restricted to the rearranged V region and its immediate flanking sequences.
5. Occurs in germinal centers with T cell help.
6. Occurs throughout the V region but more frequently in RGYW (A/G **G** C/T A/T) motifs.
7. Mutations in kappa light chain transgenes require intronic and 3' enhancers but not in the V region promoter or V coding region.

BioEssays 20:227–234, 1998

Mutation models involving error-prone DNA polymerases

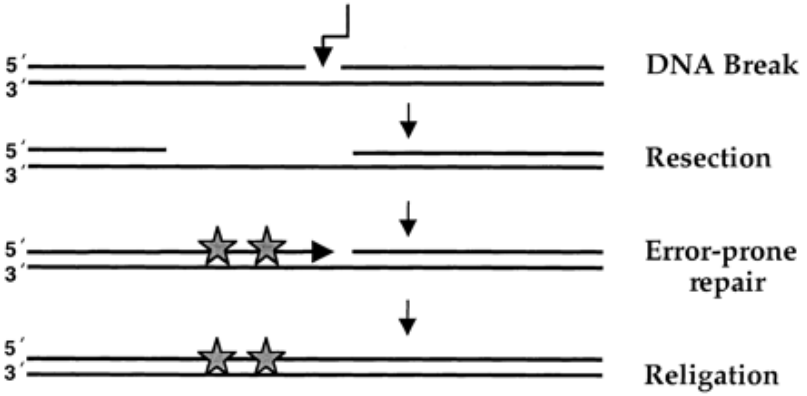
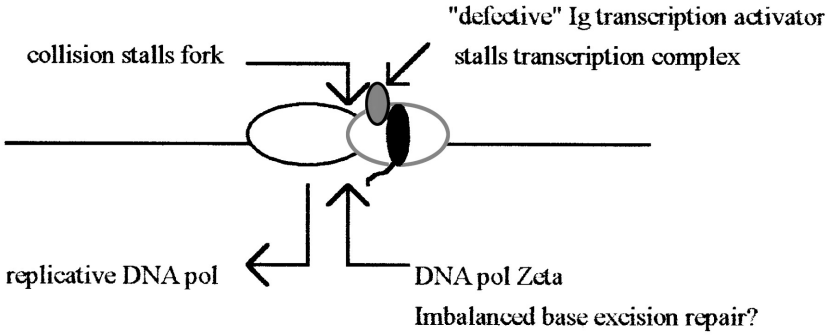


Table 1 | **Fidelity of eukaryotic polymerases on undamaged DNA**

Pol (catalytic subunit)	Gene	Mut/bp‡	Functions	Reference
α	*POLA*	2×10^{-4}	Primase in replication	75
β	*POLB*	7×10^{-4}	Base excision repair	75
γ	*POLG*	$<1 \times 10^{-6}$	Mitochondrial replication	75
δ	*POLD1*	1×10^{-5}	Replication and repair	75
ε	*POLE1*	$<1 \times 10^{-6}$	Replication and repair	75
ζ*	*REV3L*	5×10^{-4}	Lesion bypass	45
η*	*POLH*	3×10^{-2}	Lesion bypass	59
ι*	*POLI*	3×10^{-1}	Lesion bypass	65
κ	*POLK*	6×10^{-3}	Lesion bypass	39
θ	*POLQ*	?	Crosslink repair?	76
λ	*POLL*	?	DNA repair in meiosis?	41
μ*	*POLM*	?	Lymphoid-specific function?	68
Rev1	*REV1L*	?	Lesion bypass	77
TRF4		?	Sister chromatid cohesion	42

*These enzymes have been proposed to be involved in somatic hypermutation (see text).
‡Frequency is averaged for the 12 possible mutagenic events, but the range can vary considerably depending on the specific substitution. (Mut/bp, mutations per base pair; Pol, polymerase)



Take Home 1

- Genetic recombination to increase variability
- Somatic mutations in B-cells

- Failsafe mechanisms:

  12/23 rule
  V(D)J trial and error
  allelic switch and exclusion
  kappa to lambda switch
  Apoptosys

Junction: gene encoded + P + N nucleotides

Idea

- Antigen-fit mutations are favoured
- They leave a "fingerprint" of the antigen
- Positive (missense) and negative (synonymous) pressure
- Binding/important residues -> engineering

We need a way to properly align Igs and TCRs to study this

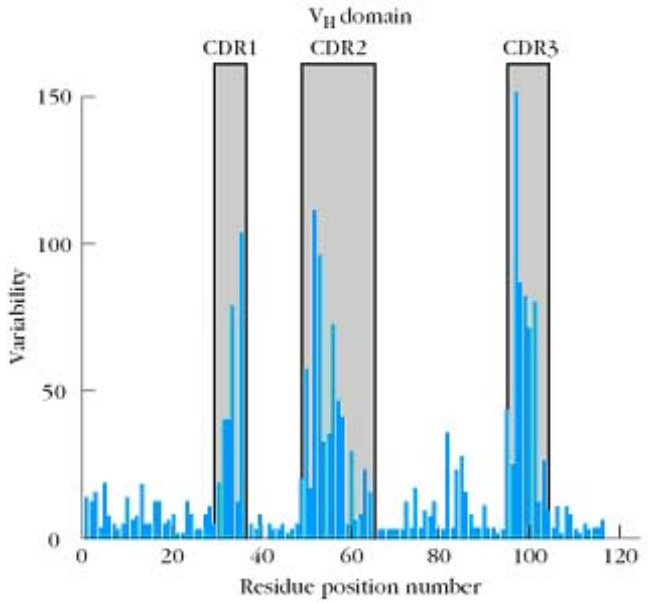
Sequence classification

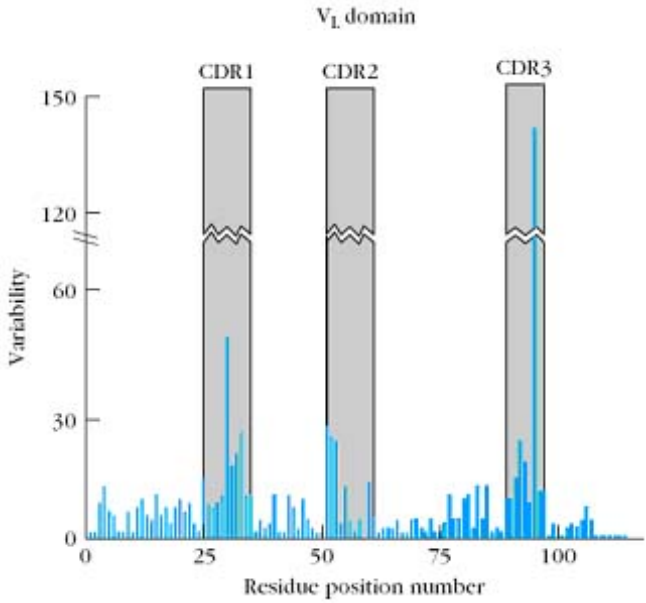
Kabat, 1972, from the analysis of antibody variability

CDR: complementarity-determining regions
FR: Framework regions

V-J and VDJ joining regions are in the CDR3

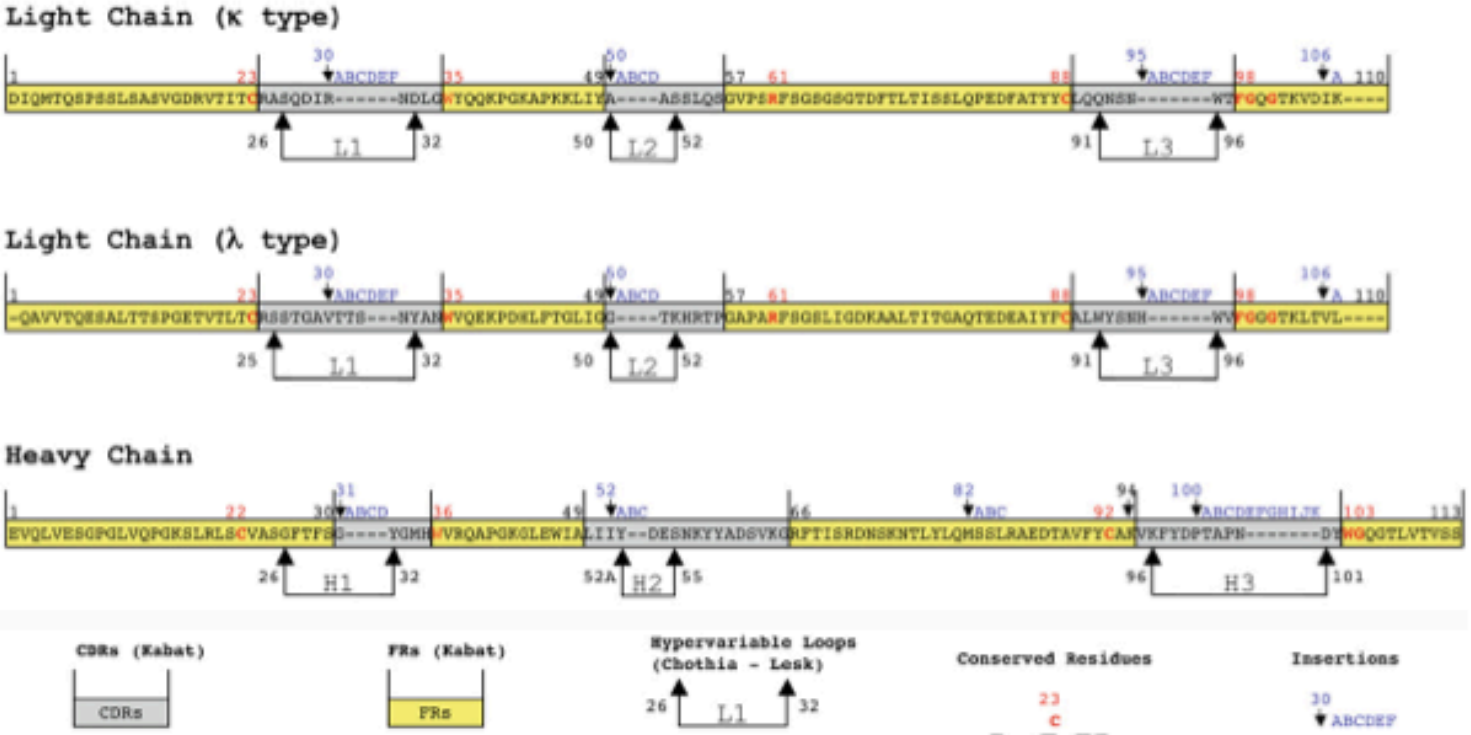
CDR1-2 variability is encoded + somatic mutations





Sequence numbering

Kabat
Kabat+Chothia
IMGT



Modeling of Antibody Structures, Fig. 2 Kabat-Chothia numbering of VK, VL, and VH. The numbers above the sequences represent the KC numbering of specific residues, the remaining residues are numbered consecutively. Letters in *blue* correspond to insertions. Kabat definition of FRs and CDRs are depicted in *yellow* and *gray*, respectively; Chothia and Lesk definition of hypervariable loops is indicated by *arrows*. Conserved residues are reported in *red*

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Sequence numbering

Important residues to identify CDRs:

CDR1: after Cysteine
CDR2: ~ 15 residues after Tryptophan
CDR3: after 2nd Cysteine

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