

Název:

Genová kontrola tvorby protilátek Genetics of the immunoglobulins

Školitel:

Ing. Soňa Křížková, Ph.D
Mgr. Markéta Vaculovičová, Ph.D.

Datum:

20. 6. 2014

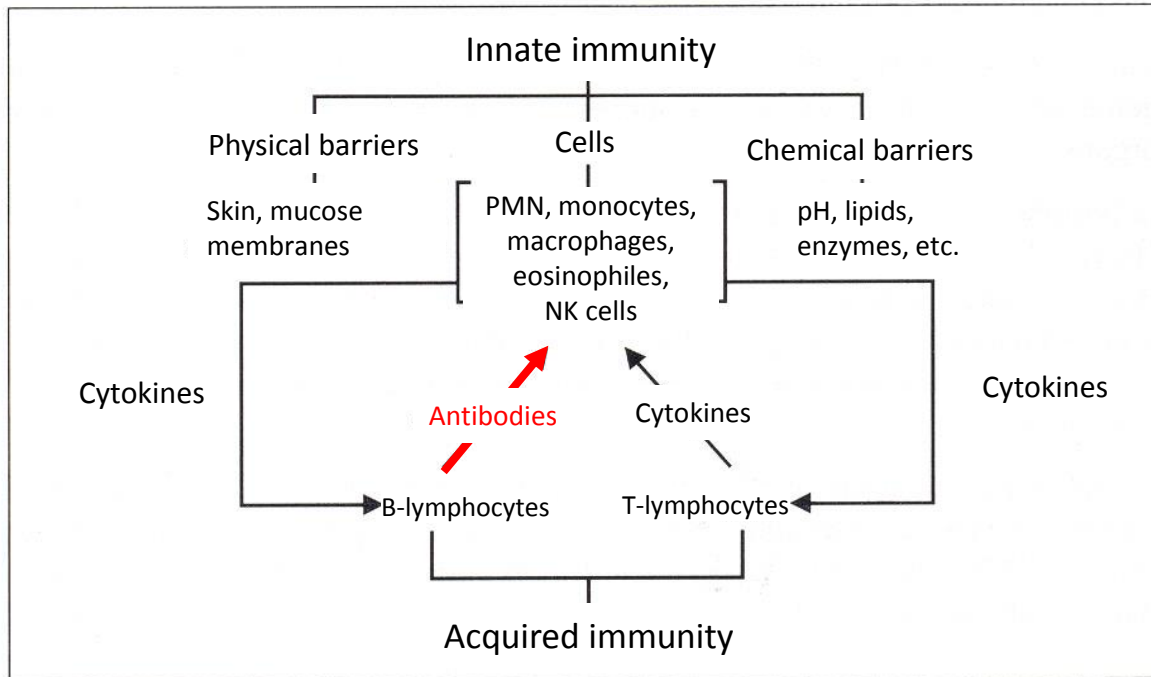


Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

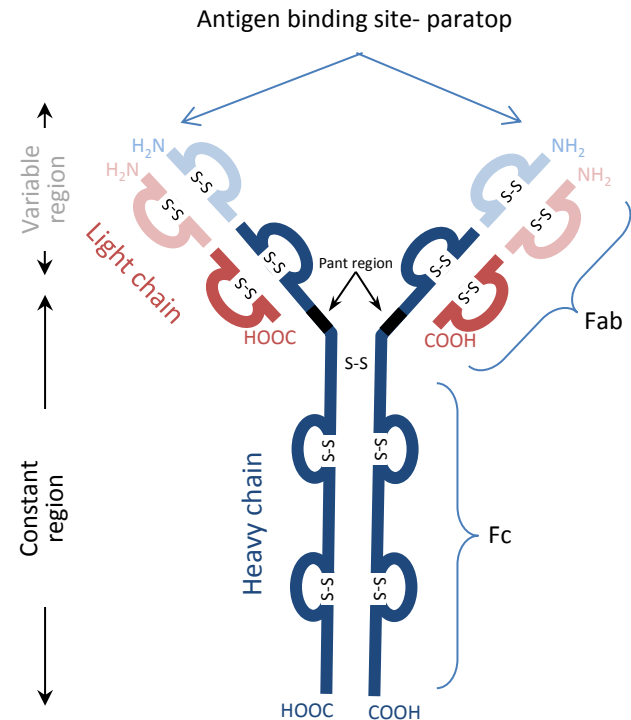
- Antibodies – structure
- How the antibodies are created?
 - V(D)J recombination
 - Affinite maturation
 - Somatic hypermutation
 - Clonal selection

Immune system:



Structure of the immunoglobulins

- 2 x **Light chain(L)**
 - 1 x Variable domain (VL)
 - 1 x Constant domain (CL)
 - 2 types: κ , λ . differences in constant region
- 2 x **Heavy chain (H)**
 - 1 x Variable domain (VH)
 - 3 x Constant domain (CH 1-3) (IgA, IgD, IgG)
 - 4 x Constant domain (CH 1-4) (IgE, IgM)
 - 5 types: α , δ , ϵ , γ , μ
 - Binding of polysaccharides
- Disulfidic bridges
 - Covalent connection of the chains
 - 1 disulfide between L and H chains
 - Between H and H chains – varies according to Ig subtype
- Antigen binding site
 - Hypervariable regions
 - Formed by variable domains of L and H chains
- **Pant region**
 - Cleavage by papain (plant protease)
 - 2 x Fab fragment – antigen binding
 - 1 x Fc fragment – binding to surface receptor of the leucocytes
 - Cleavage by pepsin (animal protease)
 - 1 x Fc fragment
 - 1 x bivalent Fab fragment



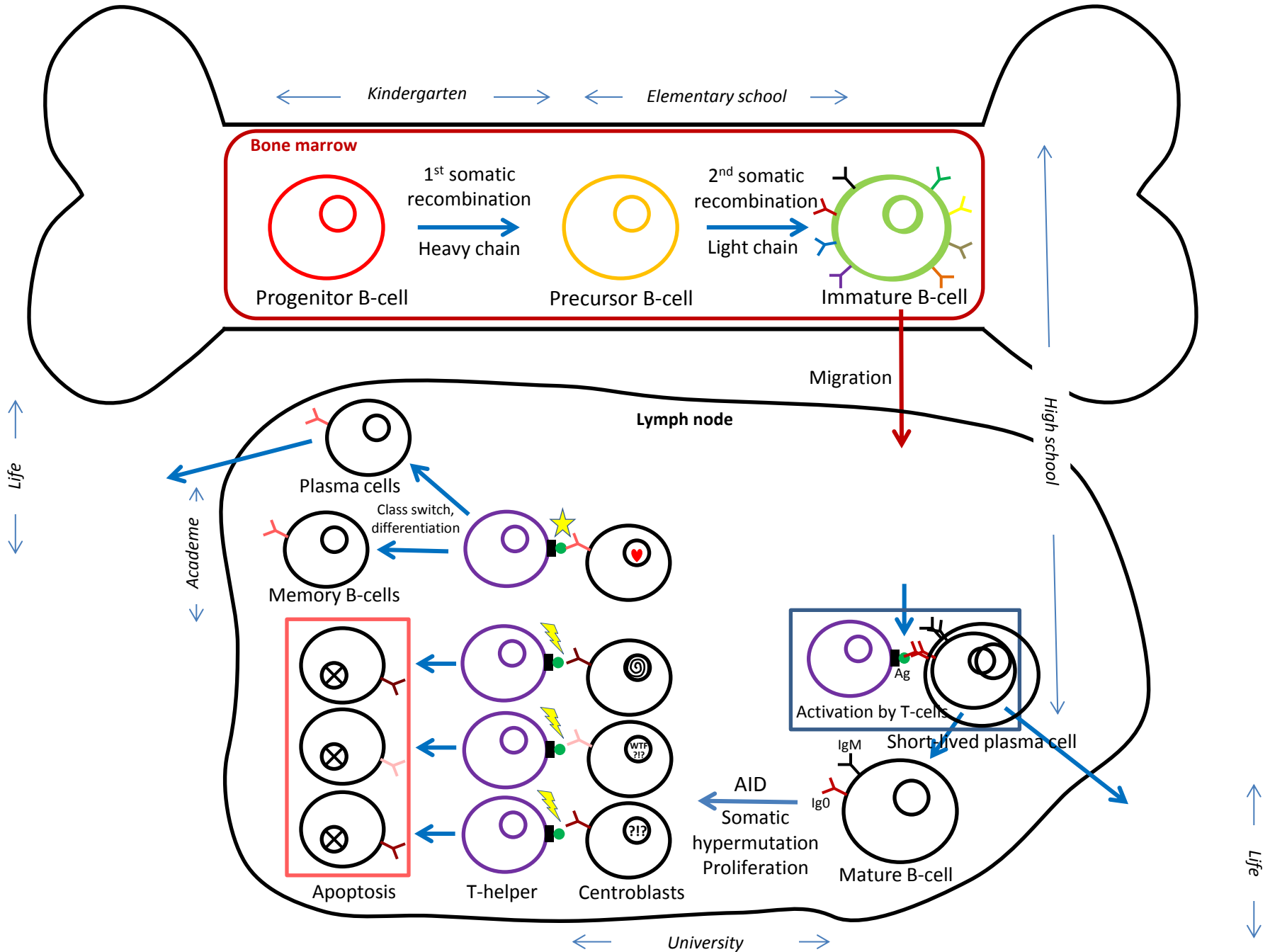
Immunoglobulins

- 1 B-lymphocyte – 1 clone of single antigen-specific antibodies (identical in primary sequence)
- 1 gene – 1 protein (except alternative splicing)
- > 1000000 antibodies clones in human organism
- ~ 22000 protein-coding genes in human genome
- e. g. 45-times less...
 - How is that possible?
 - How the immunoglobulins variability is ensured?
 - How the specific binding site is created after meeting with antigen?
 - Is the genetic information identical in all cells of an individual?
 - Can the genetic information of be adaptively changed during the life of an eucaryotic cell?
 - Is DNA replication always so precise?
 - Does random have its place in biological systems?
 - Can the mutagenesis be a component of molecular-genetic mechanisms?
 - Can uracil be found in DNA?

How the antibodies are created?

- Adaptive immunity
- Occurs in bone marrow and lymph nodes
- Produced by **B-cells**
- 1st somatic recombination – heavy chain.
 - Progenitor B-cells → precursor B-cells
- 2nd somatic recombination – light chain
 - Precursor B-cells → immature B-cells
- Immature B-lymphocytes are activated by a T-cell
 - and either released as short-lived plasma cells
 - or undergo somatic hypermutation
- Somatic hypermutation
 - increases the affinity of immunoglobulins to an antigen
- Differentiation and class switching
 - formation of memory B-cells
 - and long-lived plasma cells





V(D)J recombination (somatic recombination)

- Occurs in bone marrow
- Random combination of V(variability), D(diversity) and J (joining) genes
- 3 gene families for immunoglobulin chains
- **H** family - locus **14q** – genes for heavy chains
 - V(variability) – 40 genes
 - D(diversity) – 27 genes + ½ CDR3
 - J (joining) – 6 genes + ½ CDR3
 - RSS – recombination signal sequences recognized by proteins Rag 1 a Rag 2
- **κ** family - locus **2q** – genes for light chains
 - V(variability) – 29 genes
 - J (joining) – 5 genes
- **λ** family - locus **22q** – genes for light chains
 - V(variability) – 30 genes
 - J(joining) – 4 genes
- 1st somatic recombination – heavy chain.
 - Progenitor B-cells → precursor B-cells
- 2nd somatic recombination – light chain
 - Precursor B-cells → immature B-cells

Proteins involved in V(D)J recombination:

Rag1

Rag2

DNA-dependent protein kinase (DNA-PK)

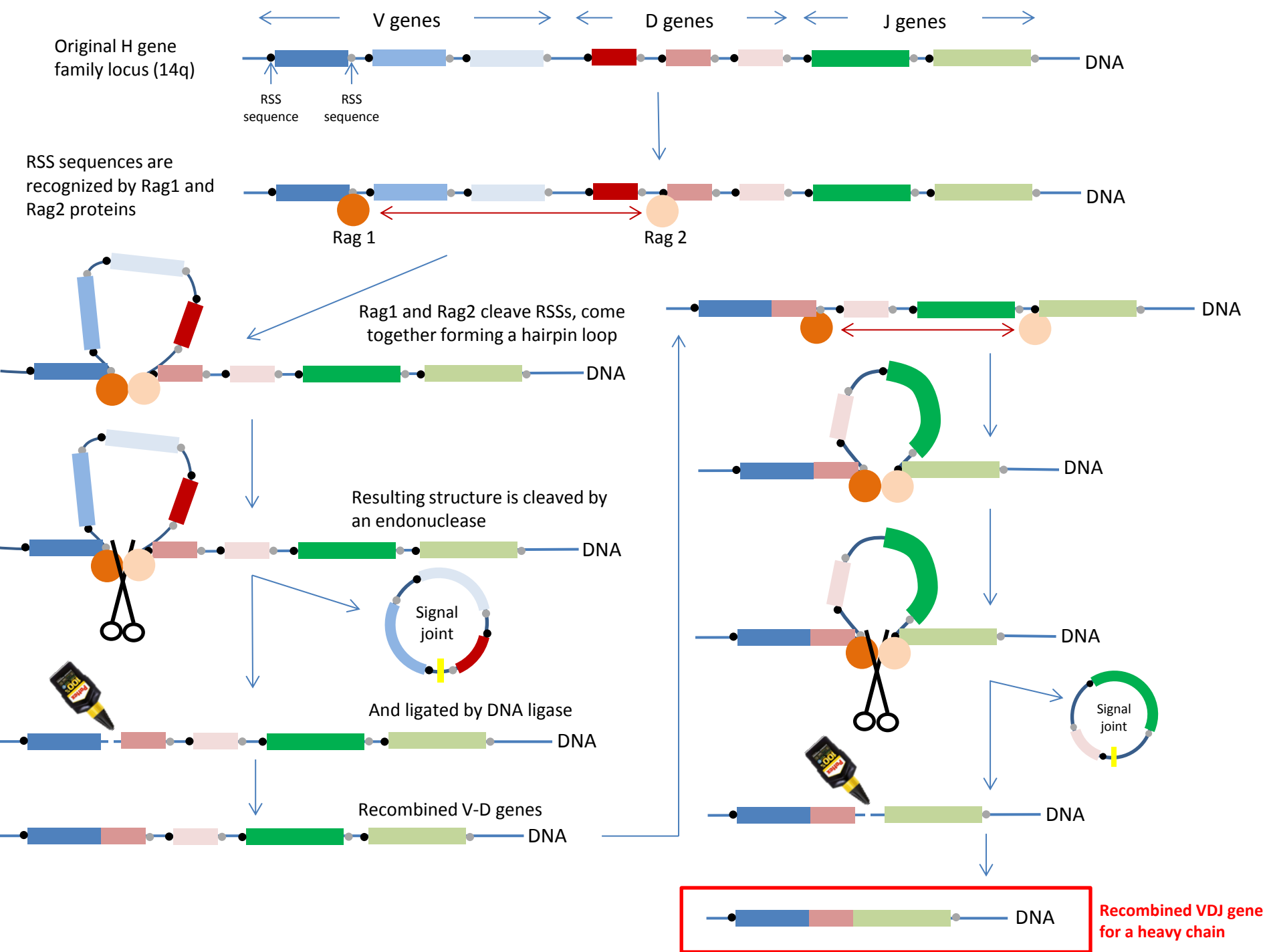
Ku

Artemis

DNA ligase XRCC4 dimer

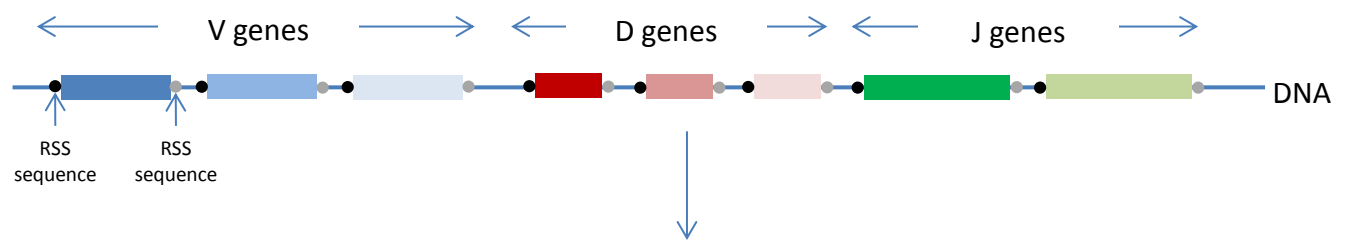
Terminal deoxynucleotidyl transferase (TdT)

Together: Artemis complex



- <http://www.youtube.com/watch?v=IbmDKX-cSMQ>

Example H gene family locus (14q)



Example locus:

$3 \times 3 \times 2 = 18$ possible combinations

In human genome (allelic exclusion):

Heavy chain:

$40 \times 27 \times 6 = 6\,580$

D can be read in 3 frames:

$6\,580 \times 3 = 19\,440$

κ light chain:

$29 \times 5 = 145$

λ light chain:

$30 \times 4 = 120$

If H and L chains pair randomly:

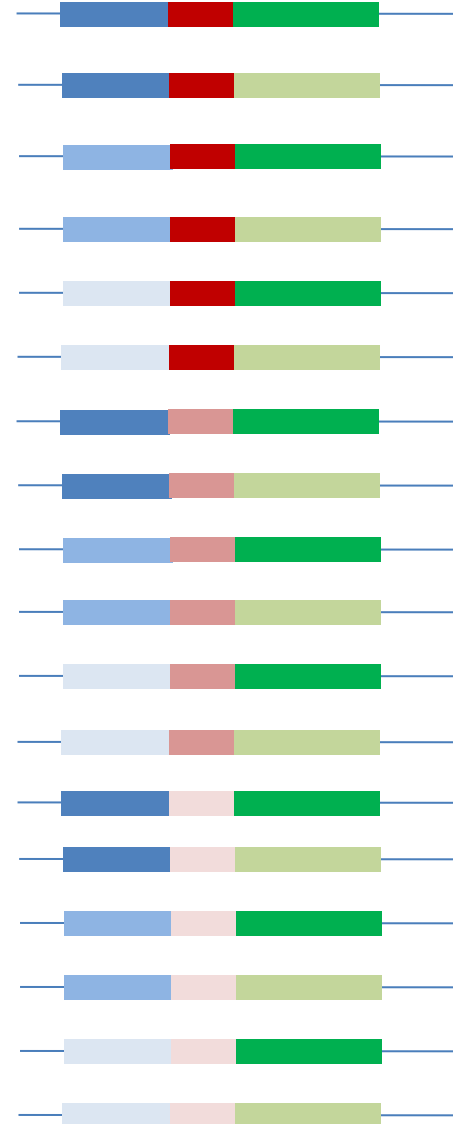
$19\,440 \times (145 + 120) = 5\,151\,600$

... but some H and L

combinations are unstable and

the recombinations create

new extra codons

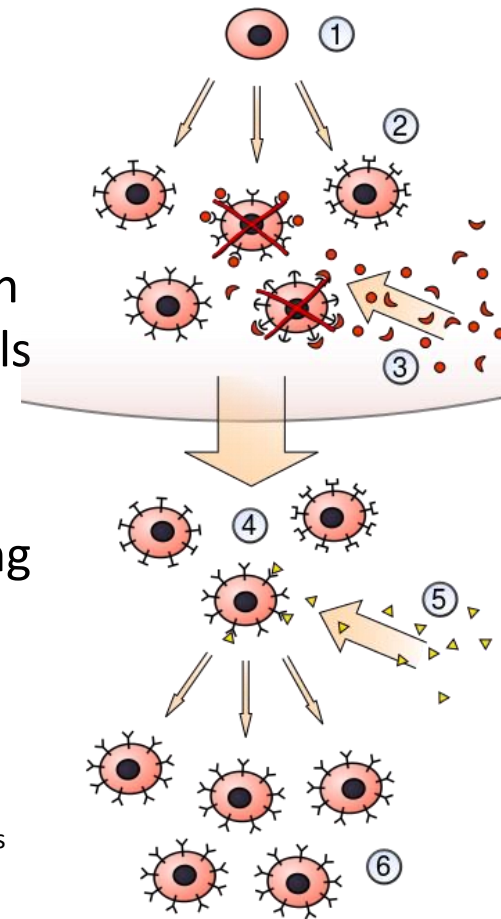


Affinity maturation

- Selection of B cells that express immunoglobulin receptors possessing an enhanced ability to recognize and bind a specific foreign antigen
- Clonal selection
- Somatic hypermutation

Clonal selection

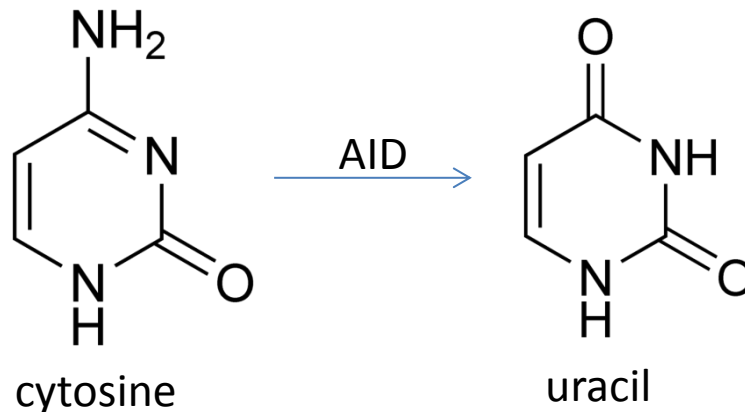
- Selection of B cells that express immunoglobulin receptors possessing an enhanced ability to recognize and bind a specific foreign antigen
- Each lymphocyte has a unique antibody on its surface.
- If the immature cells have antigen receptors that match any of the bodies own tissues, then those particular cells are destroyed.
- When the lymphocyte and antigen connect, a chemical change is triggered. The lymphocyte is activated, causing it to rapidly multiply and create many clones of itself.

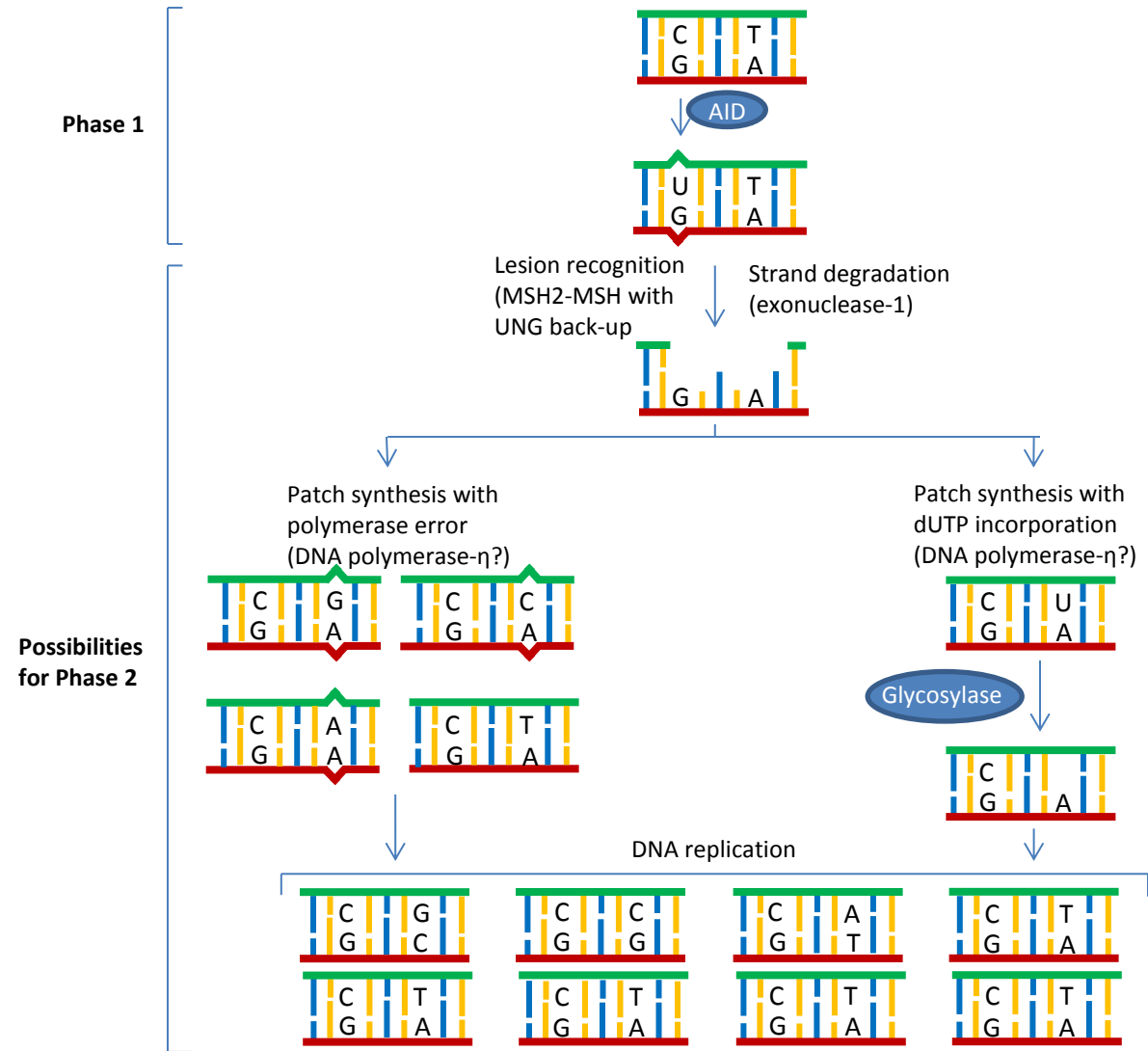


Clonal selection of lymphocytes: 1) A hematopoietic stem cell undergoes differentiation and genetic rearrangement to produce 2) immature lymphocytes with many different antigen receptors. Those that bind to 3) antigens from the body's own tissues are destroyed, while the rest mature into 4) inactive lymphocytes. Most of these will never encounter a matching 5) foreign antigen, but those that do are activated and produce 6) many clones of themselves.

Somatic hypermutation

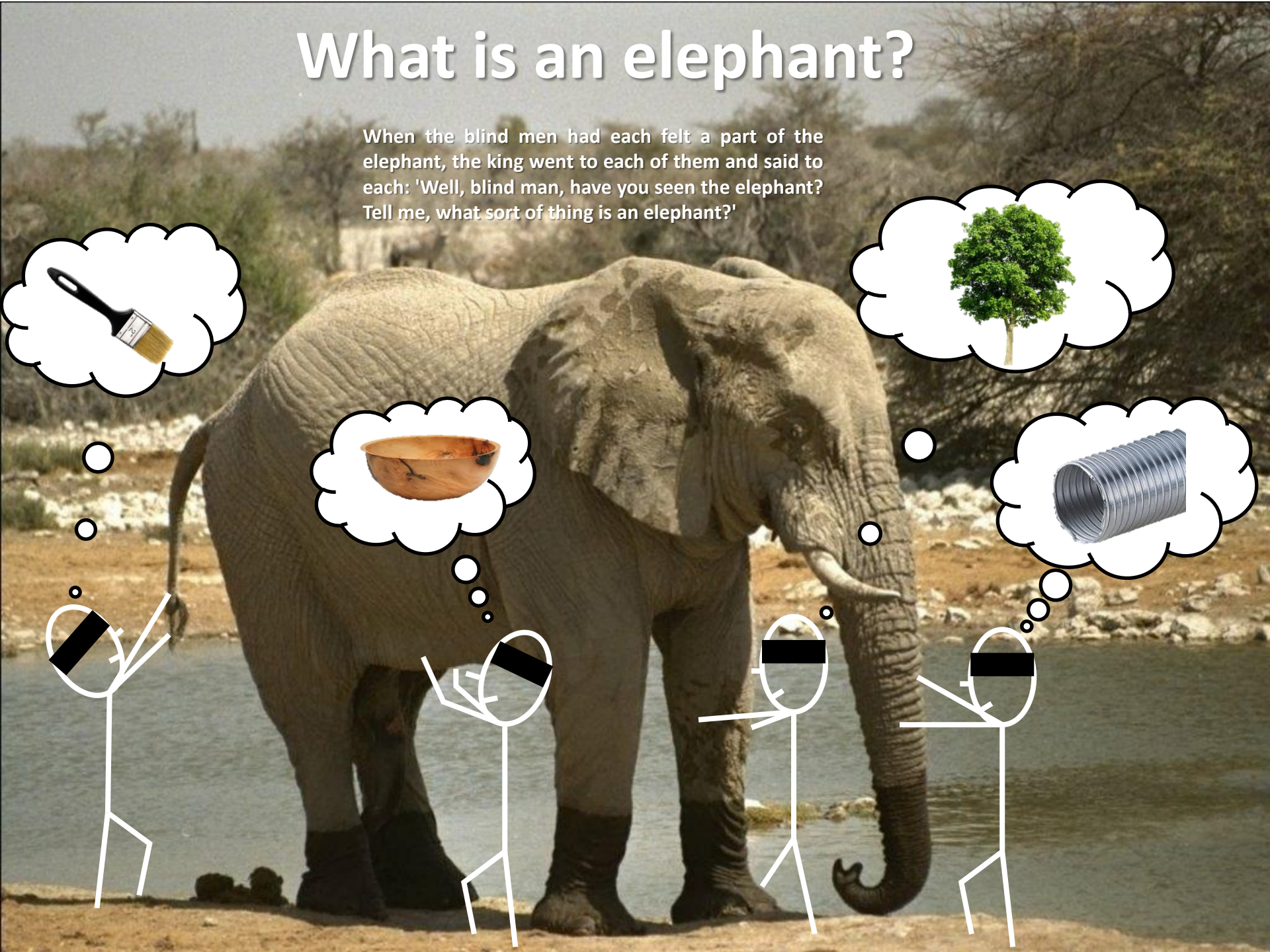
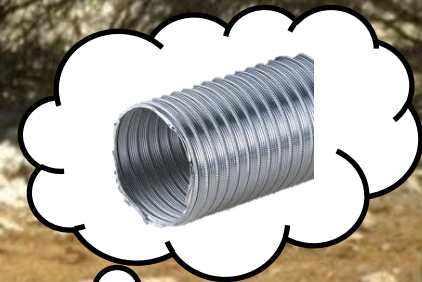
- Creates B cells that express immunoglobulins with mutations in variable regions, that may increase or decrease their specificity to the antigen
- In lymphatic nodes
- AID - (Activation-Induced (Cytidine) Deaminase)
 - introduces point mutations into variable regions of Ig genes
 - Cytosine:Guanine pair is directly mutated to a uracil:guanine mismatch
 - Those mutation are repaired by high-fidelity DNA mismatch repair enzymes
 - The uracil bases are removed by the repair enzyme, uracil-DNA glycosylase
 - Error-prone DNA polymerases are then recruited to fill in the gap and create mutation
 - extremely high rate of somatic mutation, at least 10^5 - 10^6 fold greater than the normal rate of mutation across the genome



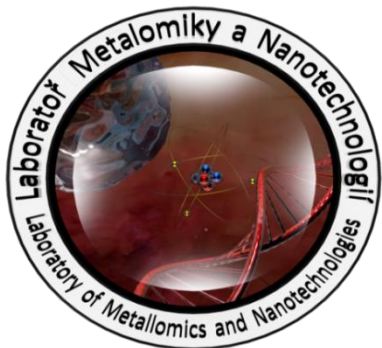
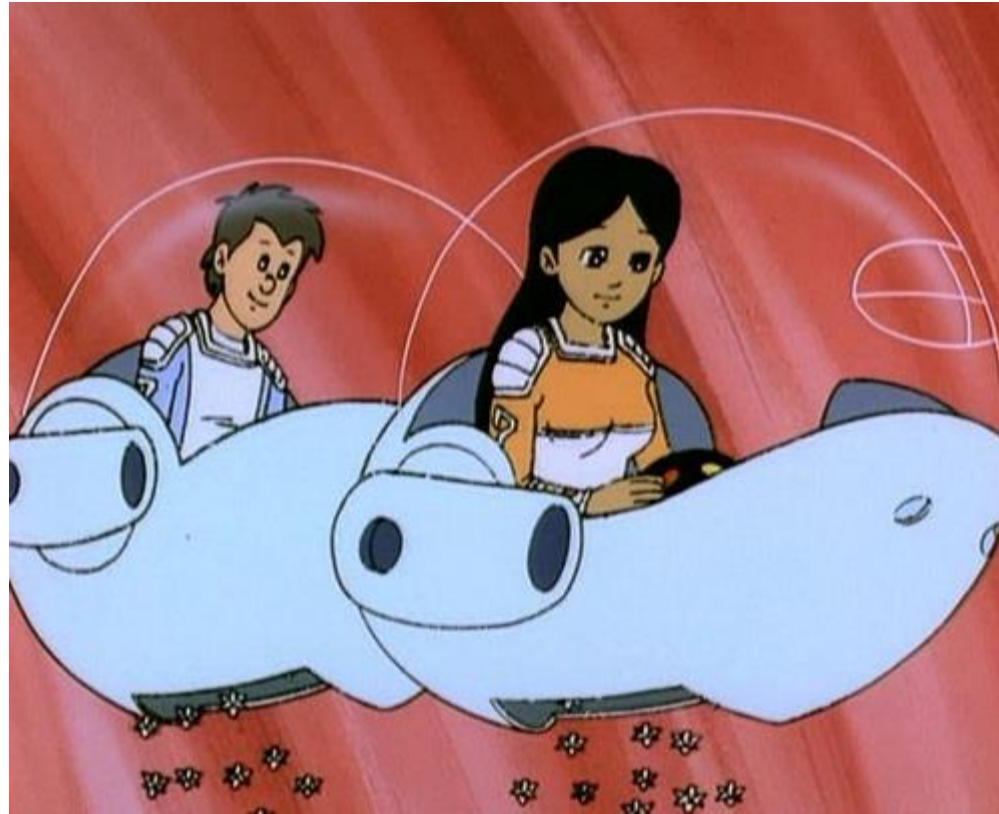


What is an elephant?

When the blind men had each felt a part of the elephant, the king went to each of them and said to each: 'Well, blind man, have you seen the elephant? Tell me, what sort of thing is an elephant?'



Děkuji za pozornost



evropský
sociální
fond v ČR



EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Mendel
University
in Brno

