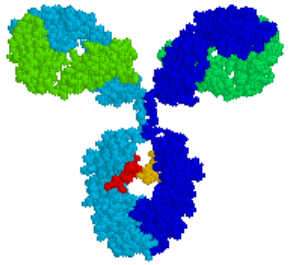


Humoral immunity

Ig Biosynthesis

Ig genes

And

generation of diversity

Ig Diversity

- We can have Abs with same C region with many V regions
  - Different IgG molecules with different specificities
- We and also have Abs with different C regions and same V region
  - IgG, IgM, IgD can have specificity for same antigen
- How does it happen?
- Can be understood by studying genetics of Ig

History

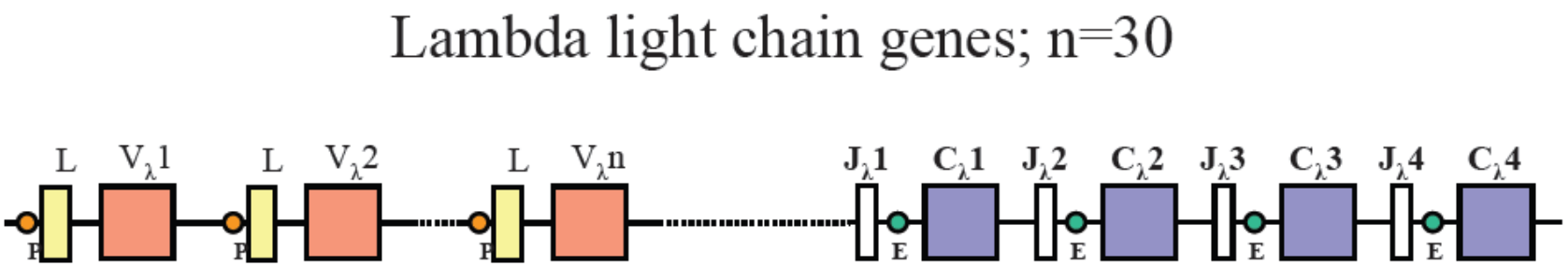
- What is happening?
- May be proteins are fusing
- May be mRNA is fusing
- Is some thing happening at DNA level
- By comparing B cells genome with other cells genome…. We know now that at DNA level Igs are encoded by 3 gene families

History

- Igs are encoded by 3 gene families on separate chromosomes
  - λ light chain
  - κ light chain
  - Heavy chain family

Light chain gene families

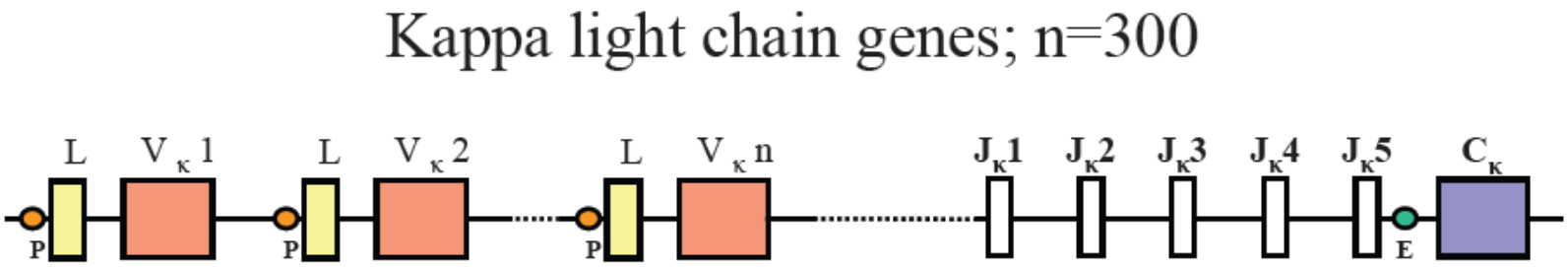
- Germ line organization of genes



- 4 C regions
- Several V regions (30)
- Several J segments
- Several L segments

Light chain gene families

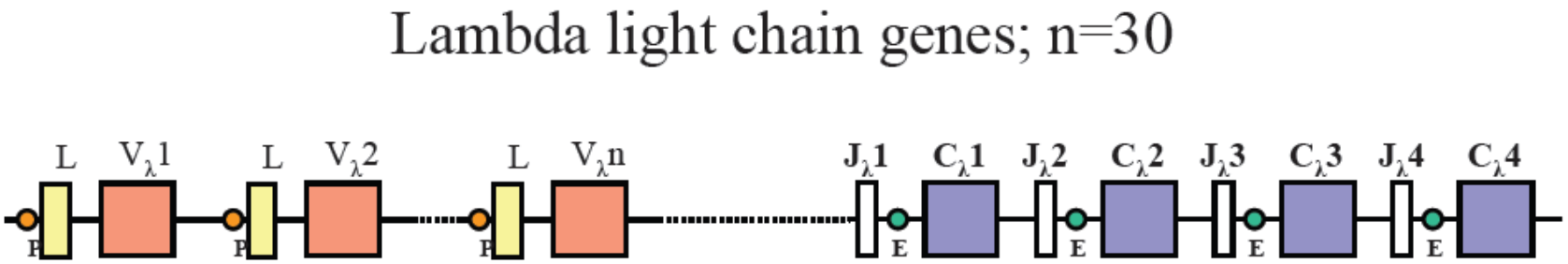
- Germ line organization of genes



- 1 C regions
- Several V regions (300)
- Several J segments
- Several L segments

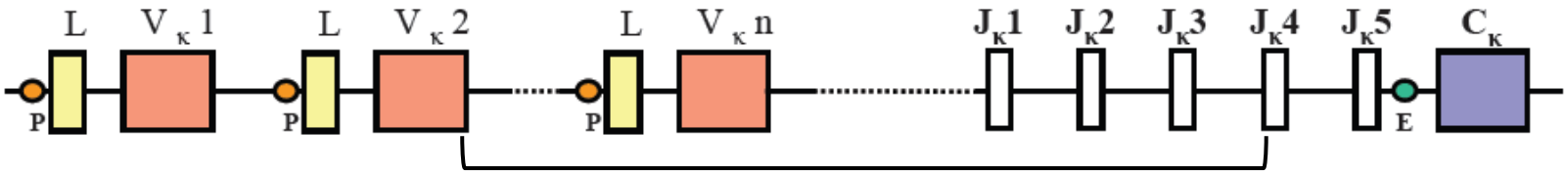
Light chain gene families

- Gene rearrangements in B cell

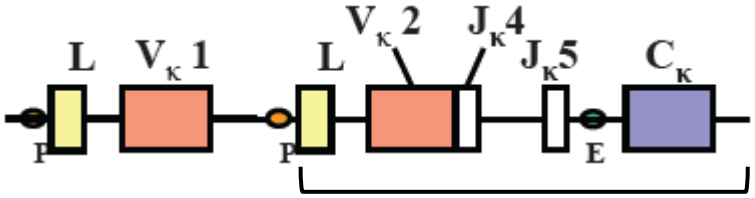


- DNA rearrangement occurs via recombination events

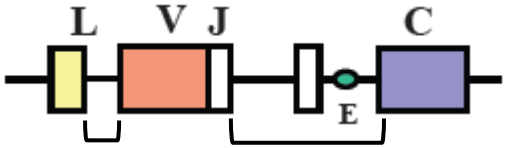
Light chain gene families



DNA rearrangement by recombination

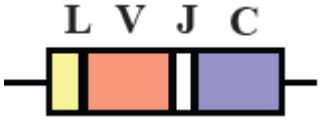


Transcription



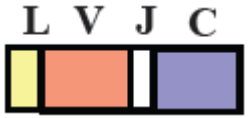
Primary transcript

RNA processing

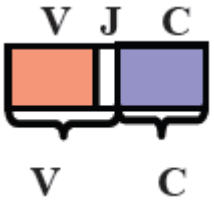


mRNA

Translation



Transport to ER



Heavy chain gene families

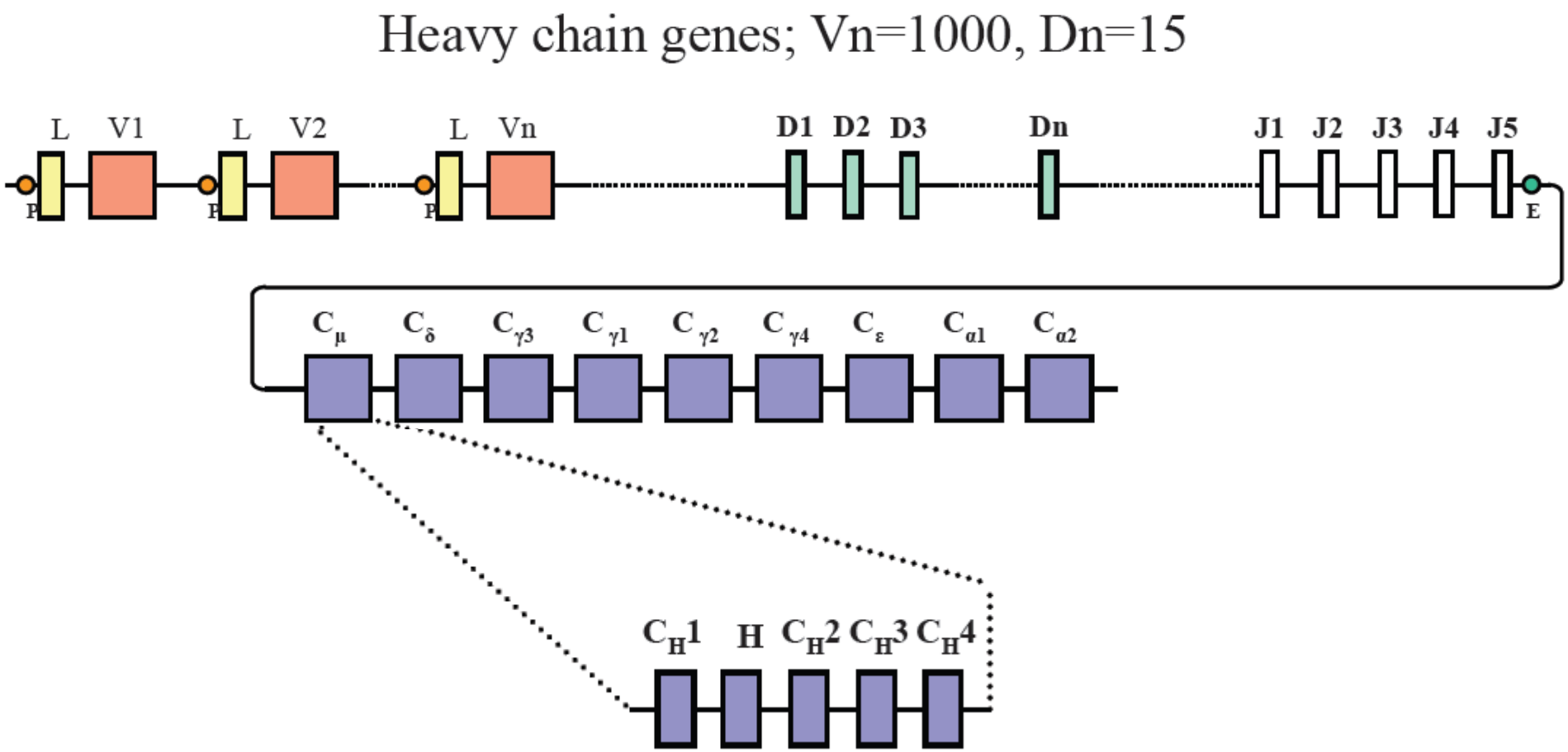
- Germ line organization of heavy chain genes on different chromosome
  - Variable regions (1000)
  - One constant region gene for every class and subclass of Ig
  - arranged in order μ, δ, γ, ε, α
  - Every constant region has introns ans exons
  - J gene segments
  - And D segments (15)

Sub classes of Ig

Ig Class	Heavy chain	subclasses	Light Chain	Molecular formula
IgG	γ	$\gamma 1$ $\gamma 2$ $\gamma 3$ $\gamma 4$	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgA	α	$\alpha 1$ $\alpha 2$	κ or λ	$(\alpha_2\kappa_2)_{1,2,3,\text{or }4}$ $(\alpha_2\lambda_2)_{1,2,3,\text{or }4}$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_{1\text{ or }5}$ $(\mu_2\lambda_2)_{1\text{ or }5}$
IgE	ε	None	κ or λ	$\varepsilon_2\kappa_2$ $\varepsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

Heavy chain gene families

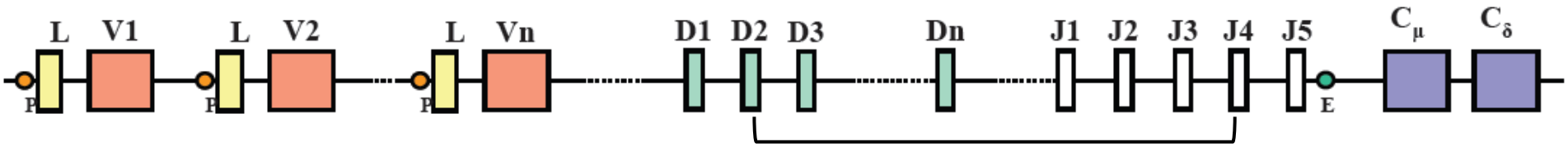
- Germ line organization of genes



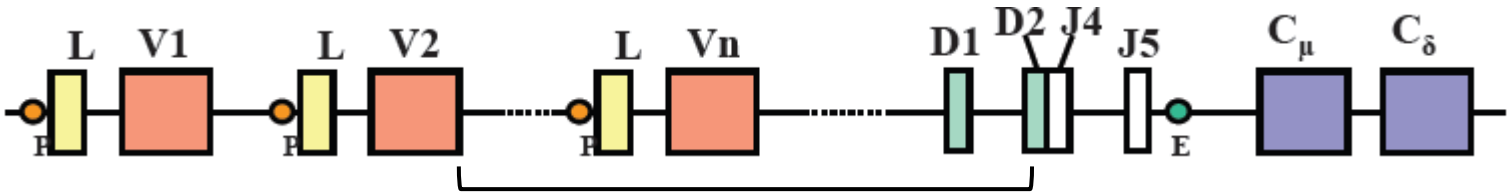
Heavy chain gene families

- Gene rearrangements in B cell
- DNA re arrangement occurs twice in case of heavy chain genes
- Primary transcript can be processed in two ways
- RAG1 and RAG2 enzymes mediate recombination events

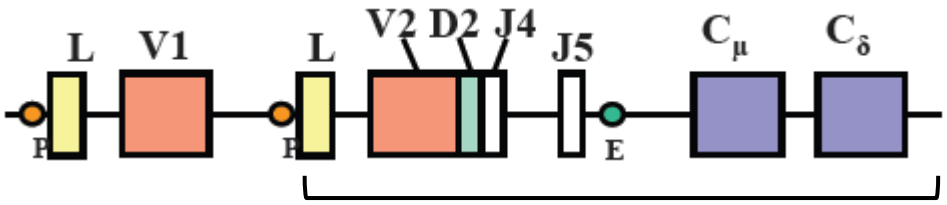
Heavy chain gene families



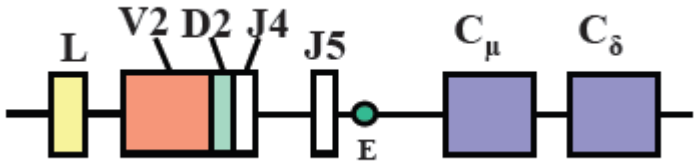
D J arrangement



V DJ arrangement

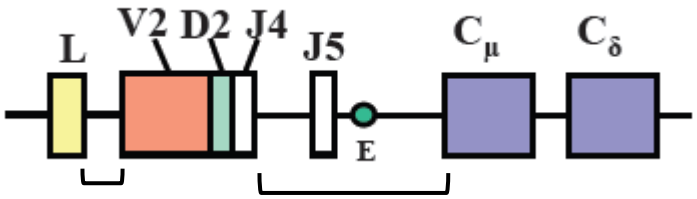


Transcription

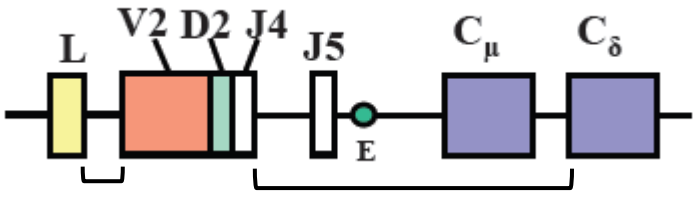


Primary transcript

Heavy chain gene families



Primary transcript



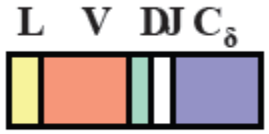
RNA processing

mRNA for C_μ

mRNA for C_δ

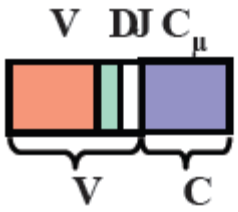
Translation

C_μ heavy chain

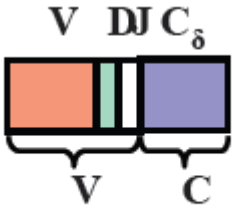


C_δ heavy chain

Transport to ER



C_μ heavy chain

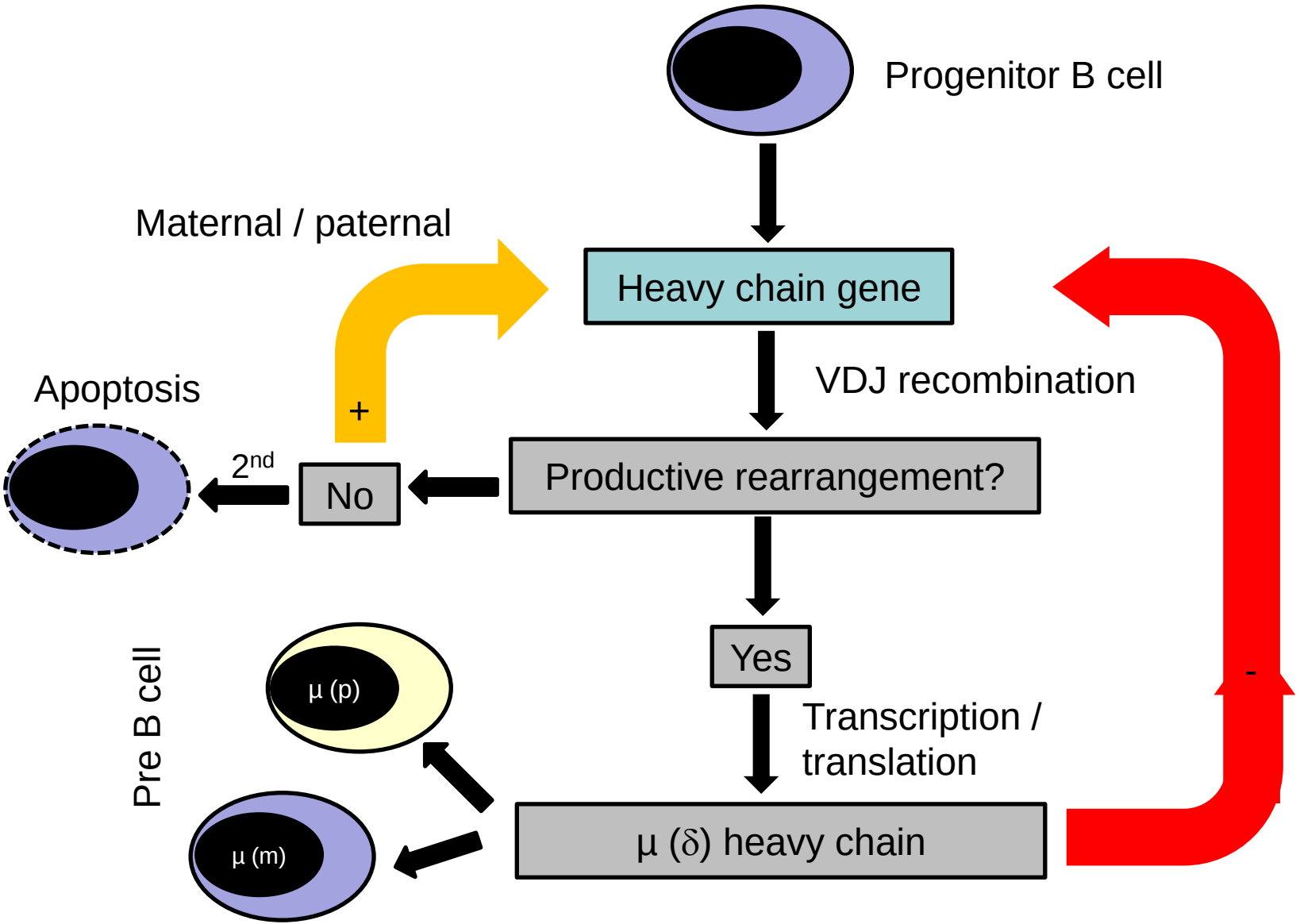


C_δ heavy chain

Heavy chain gene families

- Resulted heavy chain joins with light chain (λ or κ)
- Even though there is chance to express two heavy chains but the variable region is the same
- Maternal / paternal gene are simultaneously switched off

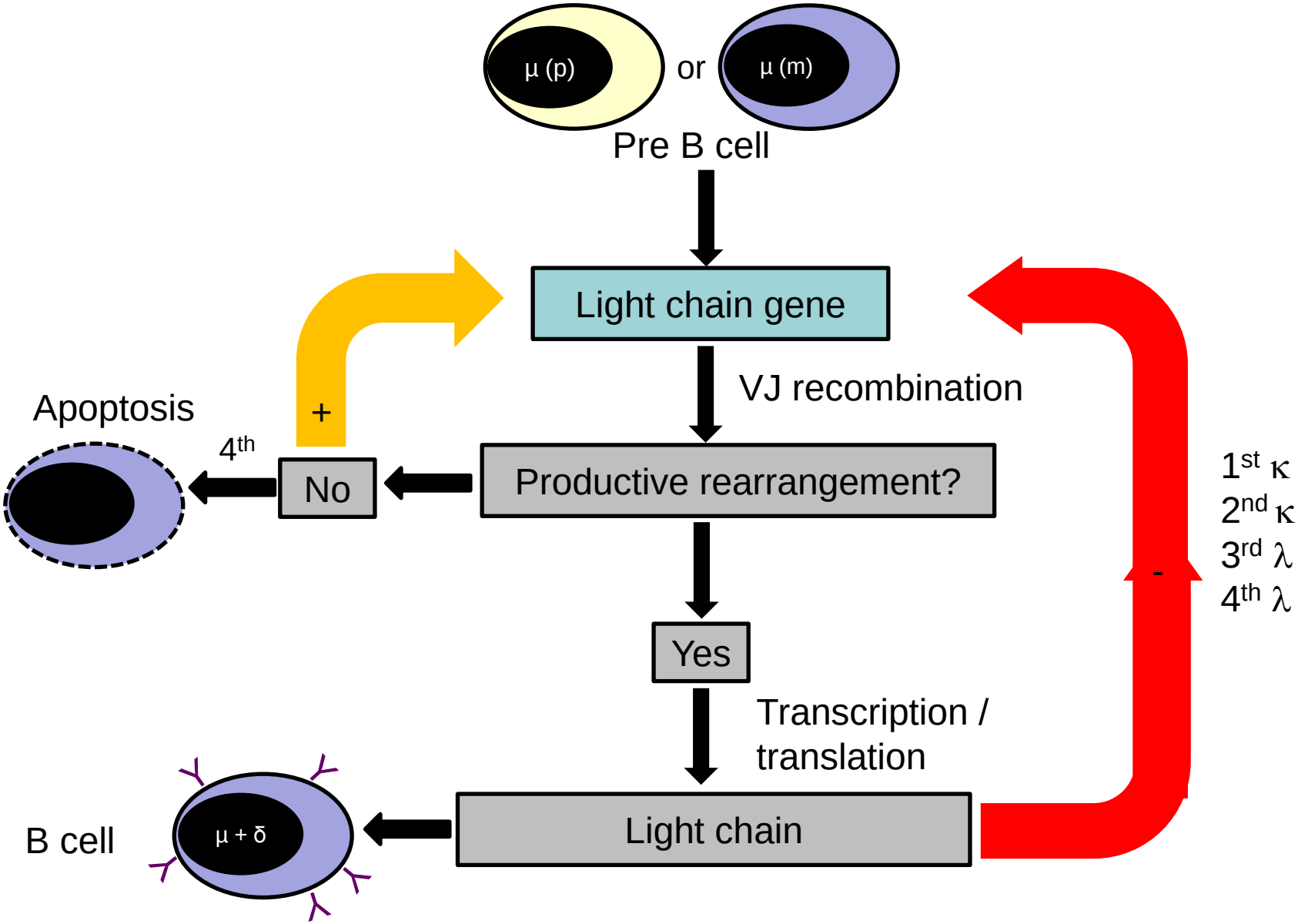
Order of Ig gene expression



Origin of Ab diversity

- B cell after rearranging heavy chain is called pre B cell
- Pre B cell express only heavy chain

Order of Ig gene expression



Ig gene expression

- B cell gets 2 chances to rearrange heavy chains
- 4 chances to rearrange light chains
- Order of gene expression explains
  - Why B cell only produces one kind of Ig
  - Why one B cell makes Abs of one specificity
  - Why allelic exclusion ?
    - Loss of specificity
    - No immunological memory

Ig Diversity

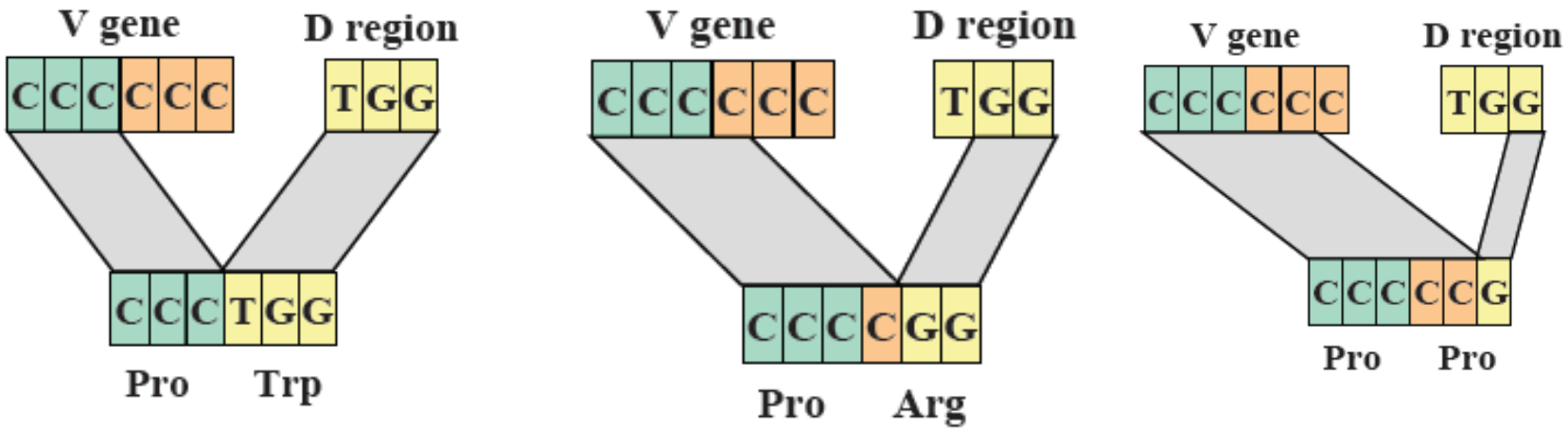
- Diversity
  - Diversity is the total of all the Antibody specificities that an organism is capable of expressing
- Diversity is mainly in the hyper variable region (HVR)
- Humans are capable or producing a minimum of 70 million different types of antibody specificities

Ig Diversity

- Diversity
- History
  - Two theories tried to explain the diversity
  - Germ line theory
    - Says we have all the genes responsible for the diversity (amount of DNA)
  - Somatic mutation theory
    - Says only have one gene for Ab and mutations generate the diversity

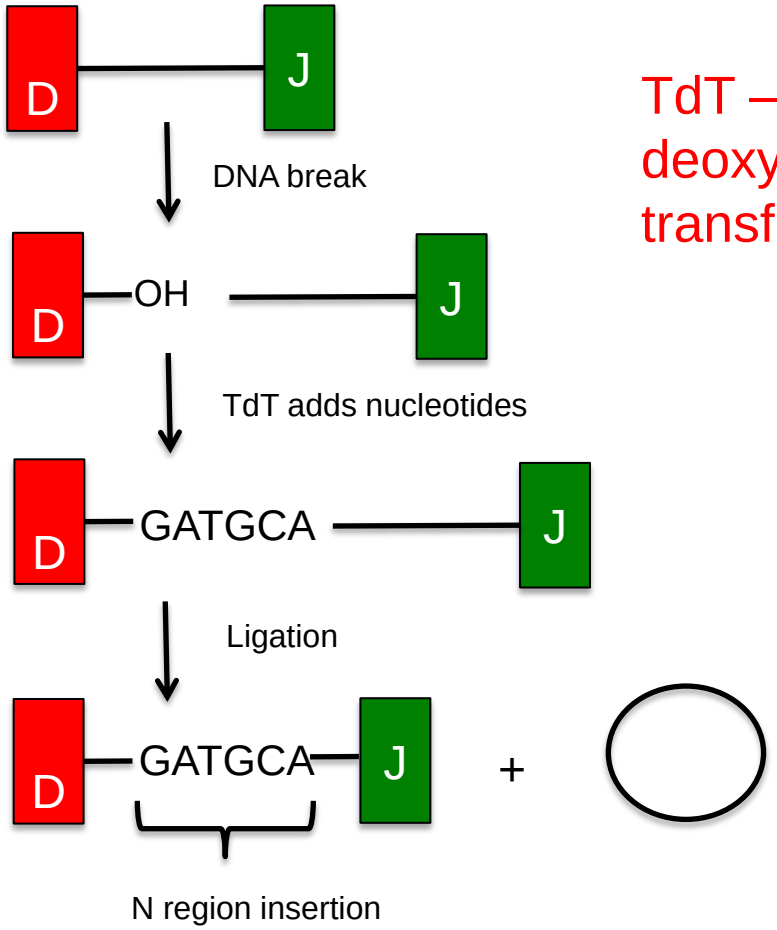
Origin of Diversity

- Multiple V regions
- V-J and V-D-J joining
- Junctional diversity
  - Slight inaccuracy in recombination process also leads to generation of diversity



Origin of Diversity

- N region insertion
  - Amino acid sequences not encoded in germ line



TdT – Terminal deoxynucleotidyl transferase

Origin of Diversity

- Somatic mutations
- Combinatorial association

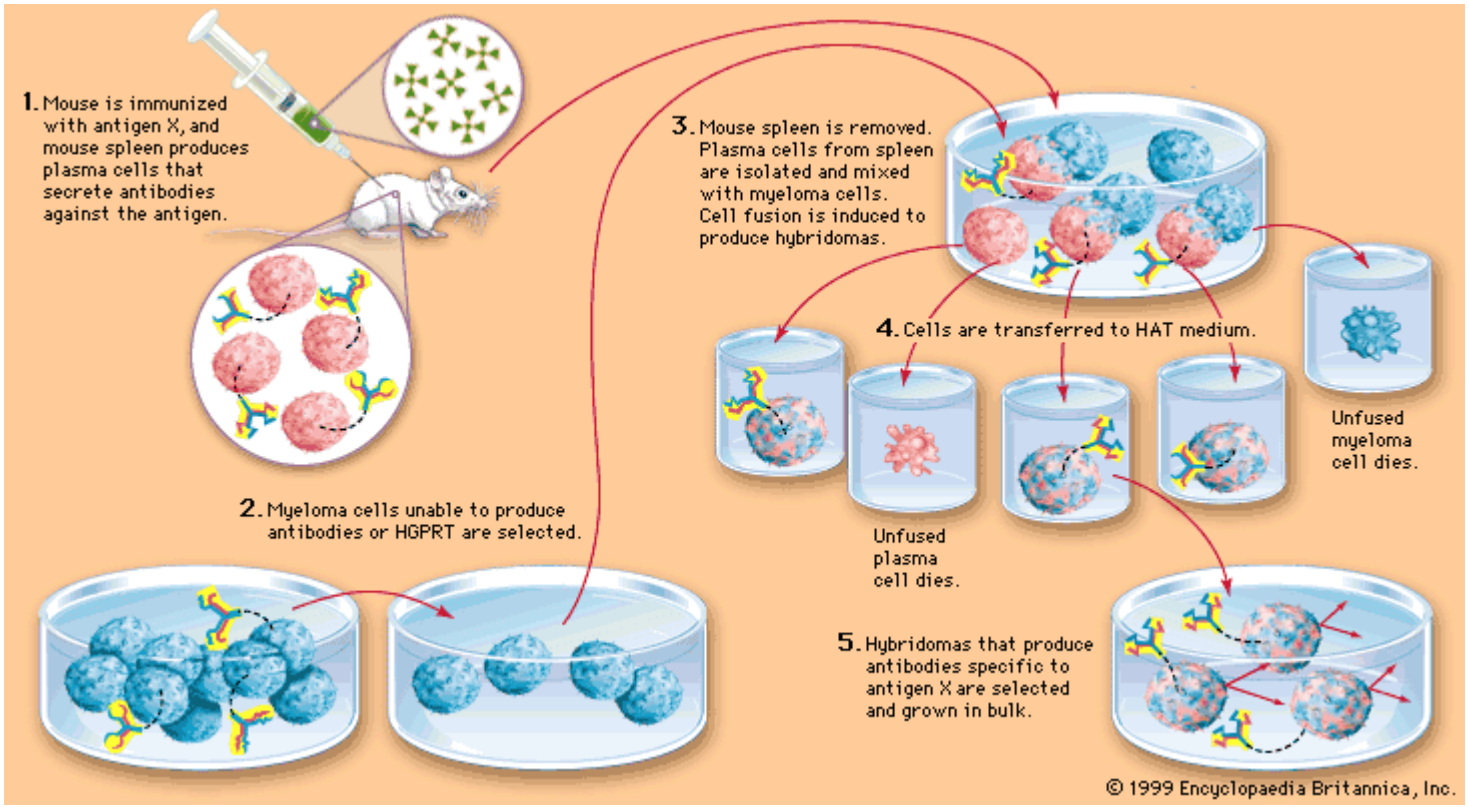
	B cell receptor (Immunoglobulin)	
	Heavy	Light
V gene segments	1000	300
D gene segments	15	-
J gene segments	4	4
N region insertion	++	-
Junctional diversity	+++	+
Somatic mutation	+	+
	V x D x J 1000 x 15 x 4	V x J 300 x 4
Total	6×10^4	1.2×10^3
Combinatorial association	7.2×10^7	

Origin of Diversity

- All these rearrangements happening right now in our body
- Without exposure to Ag (independent of Ag)
- B cell prepares Ab without seeing Ag
- When Ag enters it will select one of the B cell clone which is producing specific Ab for it
- This is CLONAL SELECTION
- Once B cell encounter Ag, B cell start to secrete Abs and class switching also occurs
- Reason why IgM and IgD are cell surface bound

Monoclonal Anti bodies (mAbs)

- Monoclonal antibodies are monospecific antibodies – applications
- Hybridoma technology

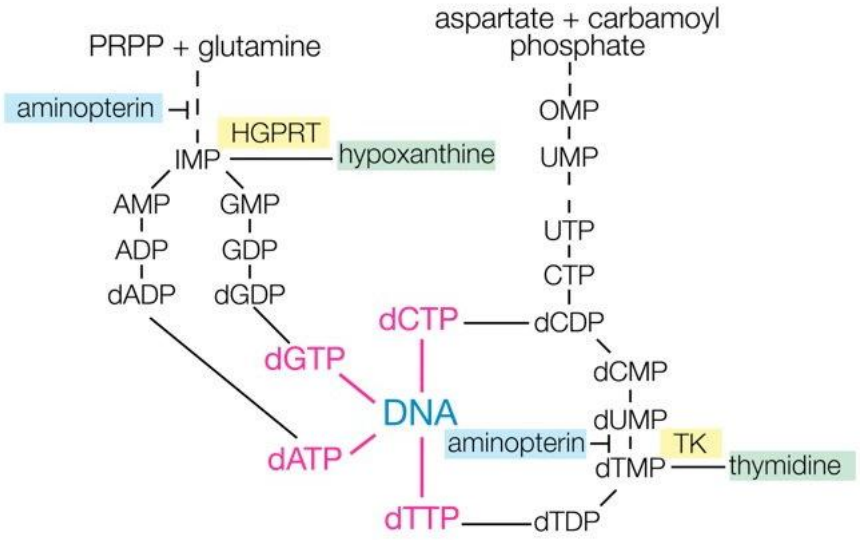


Monoclonal Anti bodies (mAbs)

- Myeloma cells don’t have HGPRT (hypoxanthine-guanine phosphoribosyltransferase)
  - Enzyme required for synthesis of nucleotides
- B cells can not grow indefinitely
- After fusion… cells will be selected in HAT medium (hypoxanthine-aminopterin thymidine medium)
- Only hybridomas will be selected and survive
- This hybridoma will produce monospecific Abs.

Monoclonal Anti bodies (mAbs)

Pathways involved in nucleotide synthesis in mammalian cells



Monoclonal Anti bodies (mAbs)

1. HAT contains a drug Aminopterin which blocks one pathway for nucleotide synthesis making the cells to depend upon other pathway that needs HGPRT enzyme, which is absent in Myeloma cells.
2. Therefore, Myeloma cells which do not fuse with the B cells will die, since they are HGPRT-. B cells which do not fuse with the Myeloma cells will die, because they lack tumorgenic property of immortal growth.
3. Therefore HAT medium allows selection of Hybridoma cells which inherit HGPRT gene from B cells and tumorgenic property from Myeloma cells.

Monoclonal Anti bodies (mAbs)