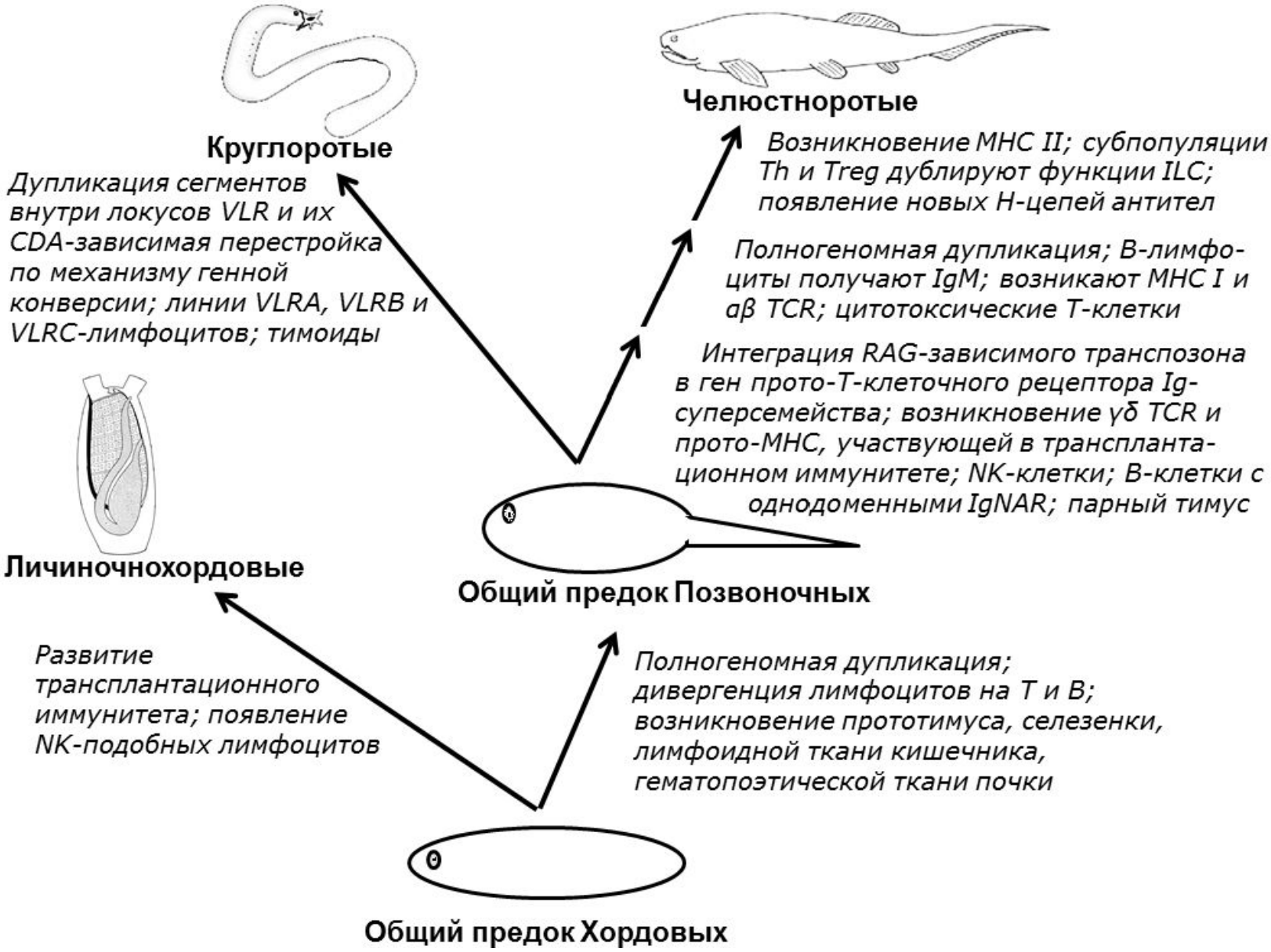
Эволюционная иммунология
Лекция 10 «Разнообразие в иммунной системе позвоночных»

Шилов Е.С.

16 апреля 2018

Основные этапы возникновения адаптивного иммунитета

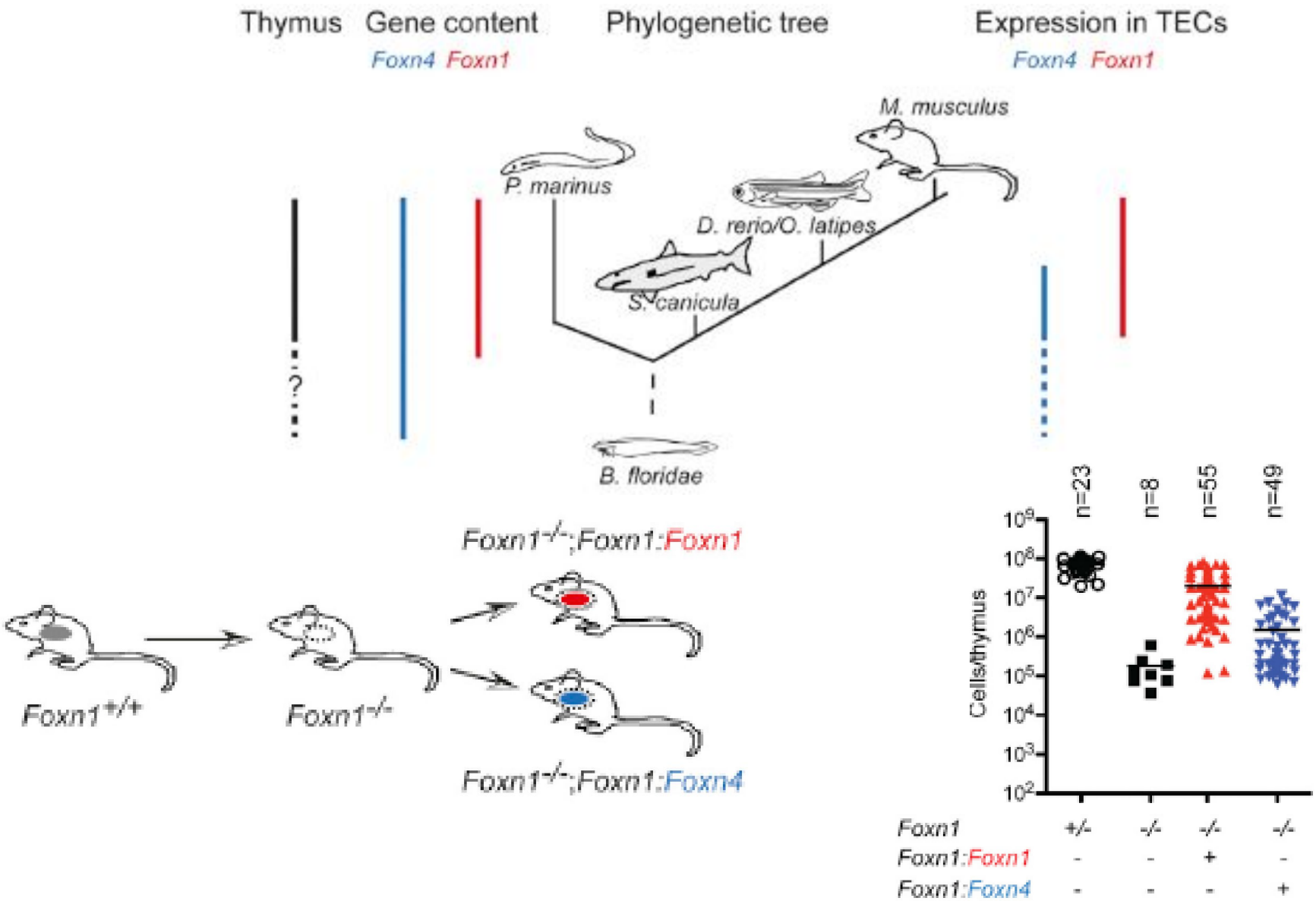


РАЗНОВИДНОСТИ ЛИМФОИДНЫХ ОРГАНОВ ПОЗВОНОЧНЫХ

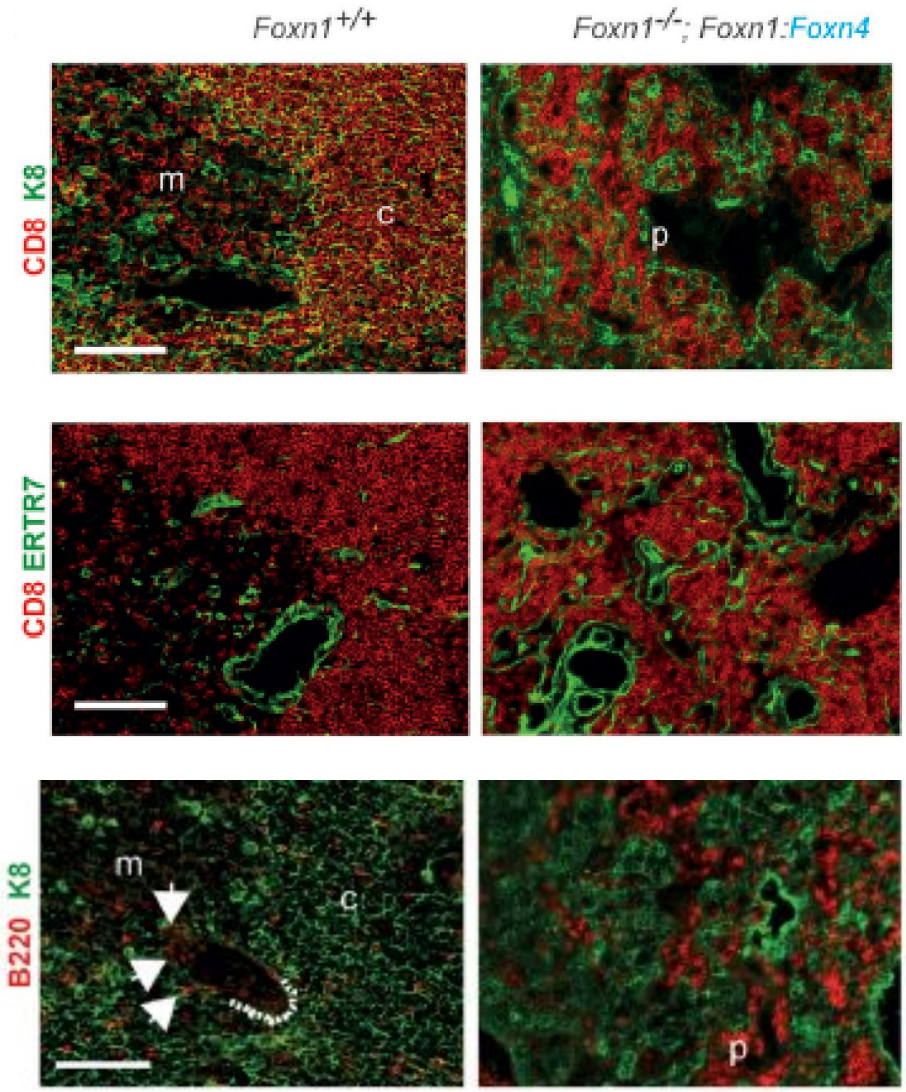
Тип органов	Название органов	Функция
Первичные	Костный мозг, тимус, Фабрициева сумка (у птиц)	Развитие клеток
Вторичные: Инкапсулированные	Лимфоузлы, селезенка, пейеровы бляшки	Индукция (частично реализация) ответа
Неинкапсулированные	Миндалины, лимфоидные фолликулы, криптопатчи	
Диффузная лимфоидная ткань	Слизистых оболочек, кожи, нелимфоидных органов	Реализация иммунного ответа

Слайд взят из материалов лекции А.А.Ярилина

Дифференцировка лимфоцитов в тимусе может определяться одним единственным мастер-геном

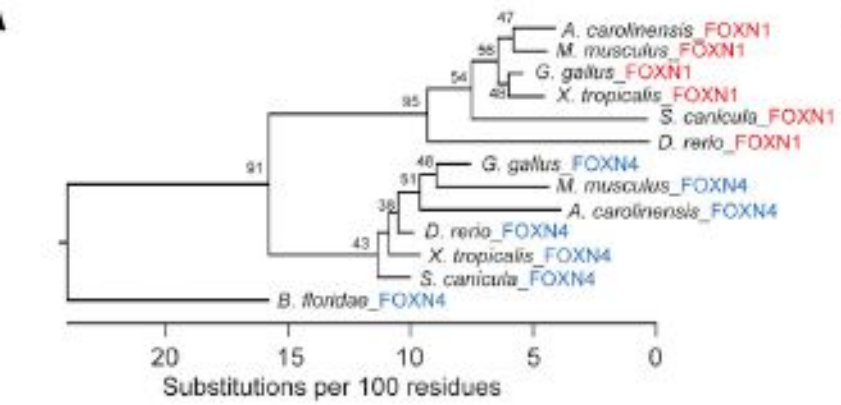


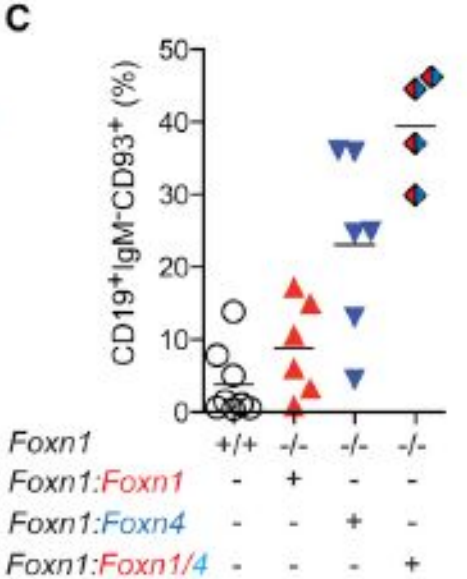
Замена гена *Foxn1* на его паралог *Foxn4* приглашает В-клетки в тимус

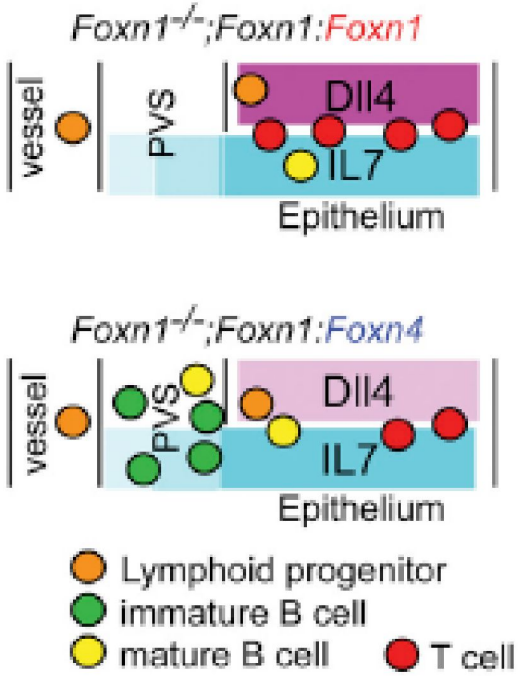


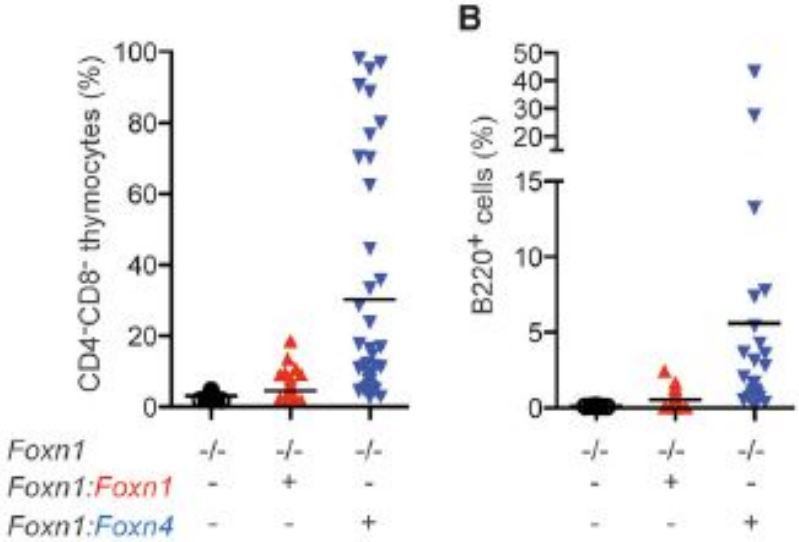
(Swann, et al 2014)

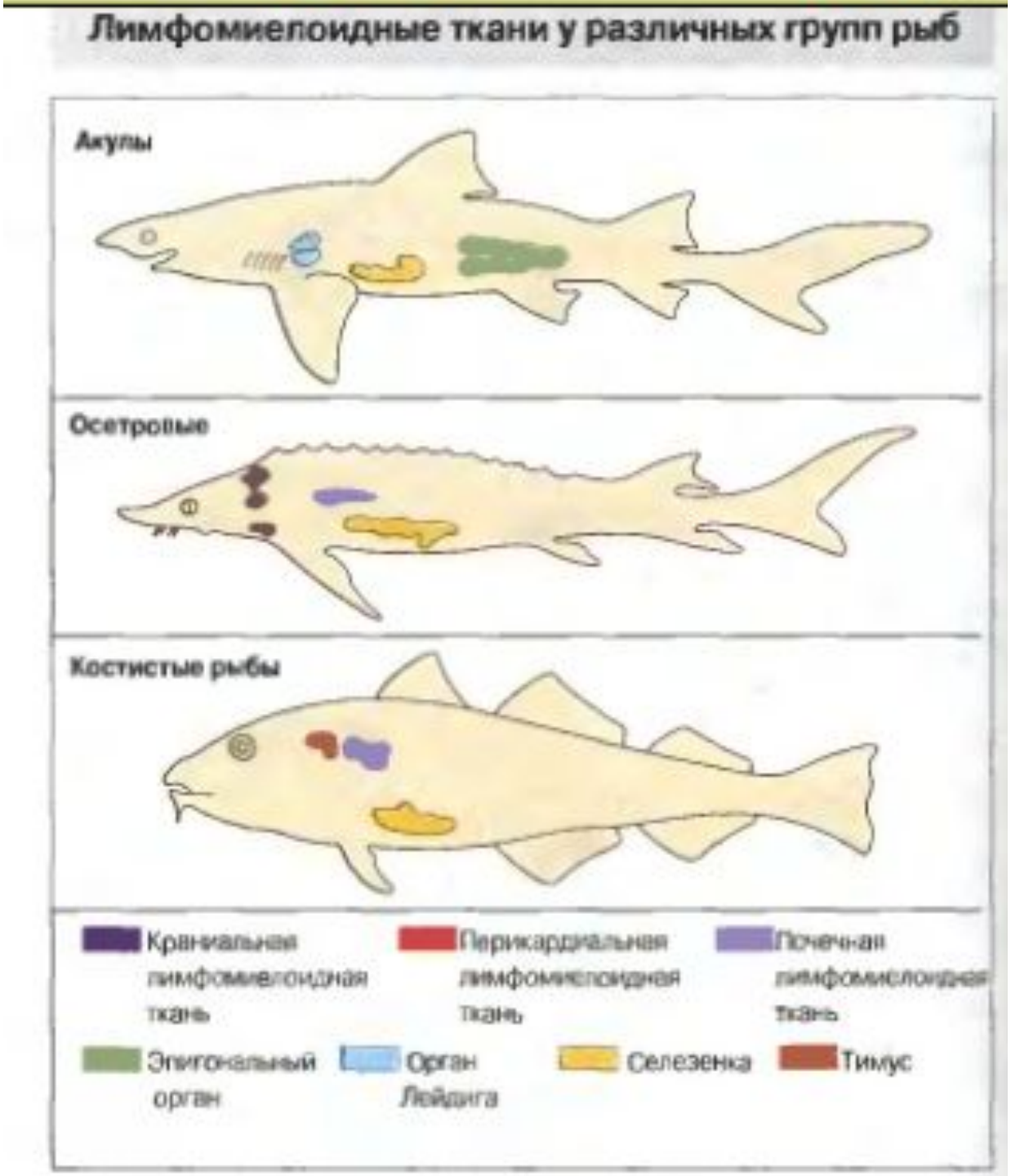
У мышей с заменой *foxn1* на *foxn4* наблюдается созревание В-клеток в тимусе



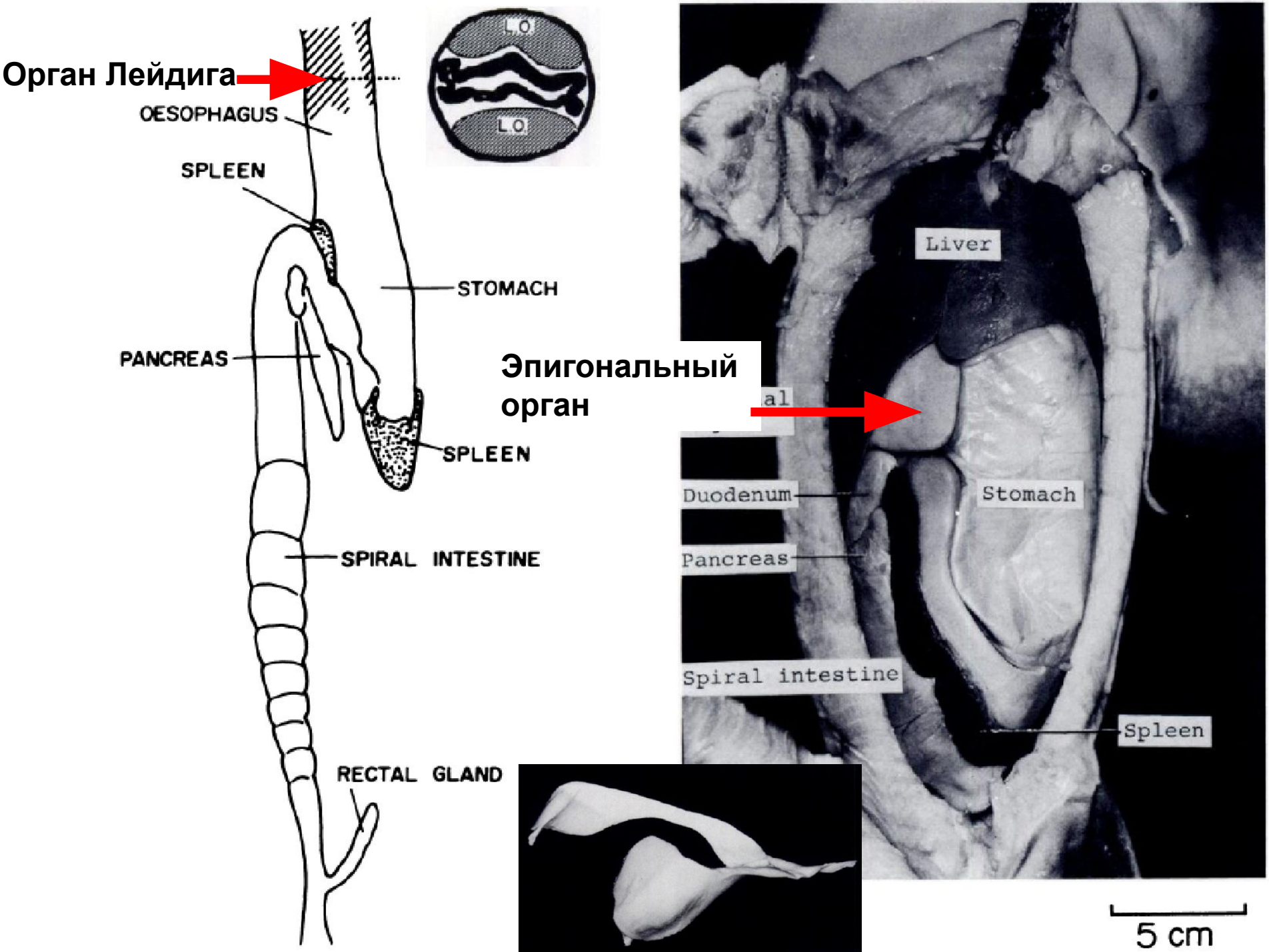








В связи с отсутствием лимфатических узлов лимфомиелоидные ткани у рыб часто оказываются локально расположенными в различных эпителиях, например, в эпителии жаберных лепестков


Орган Лейдига
OESOPHAGUS
SPLEEN
STOMACH
PANCREAS
SPLEEN
SPIRAL INTESTINE
RECTAL GLAND
L.O.
L.O.
Эпигональный орган
Liver
Duodenum
Stomach
Pancreas
Spiral intestine
Spleen
5 cm

Ультраструктура органа Лейдига схожа со структурой костного мозга млекопитающих

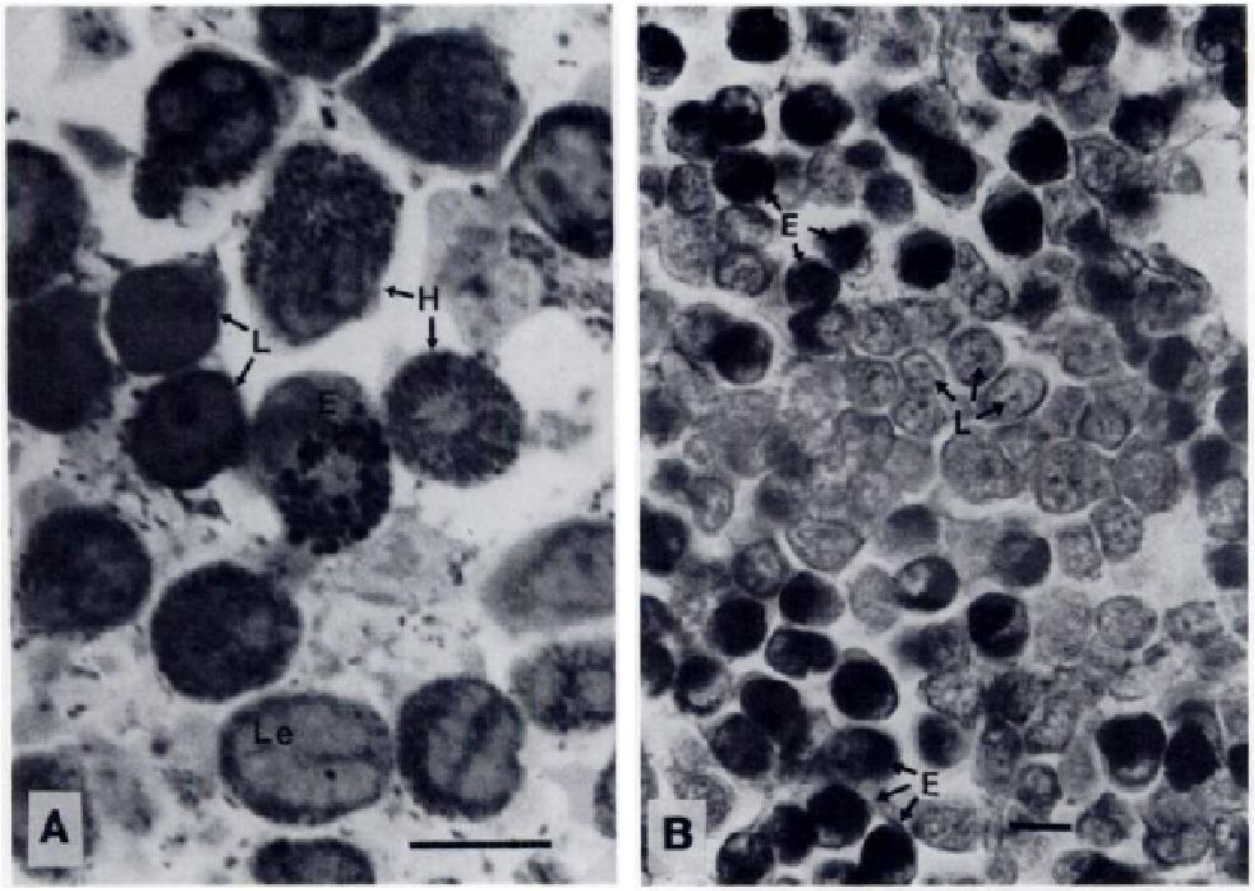


FIGURE 2. Light microscopy of the Leydig organ of *Etmopterus*.

(A): Epon-embedded section, about 1 μm thick and stained with toluidine blue. In the middle an eosinophilic granulocyte (E). Lymphocytes (L) and heterophilic granulocytes (H) are seen around the eosinophilic cell. At the bottom a typical leptomeric cell (Le) with invaginated nucleus. Oil immersion. Bar = 10 μm.

(B): Azan-stained paraffin section. In the middle of the figure a group of non-granulated cells, mainly lymphocytes. Above and below these cells there are eosinophilic granulocytes (dark cells). Bar = 10 μm.

Клетки органа Лейдига

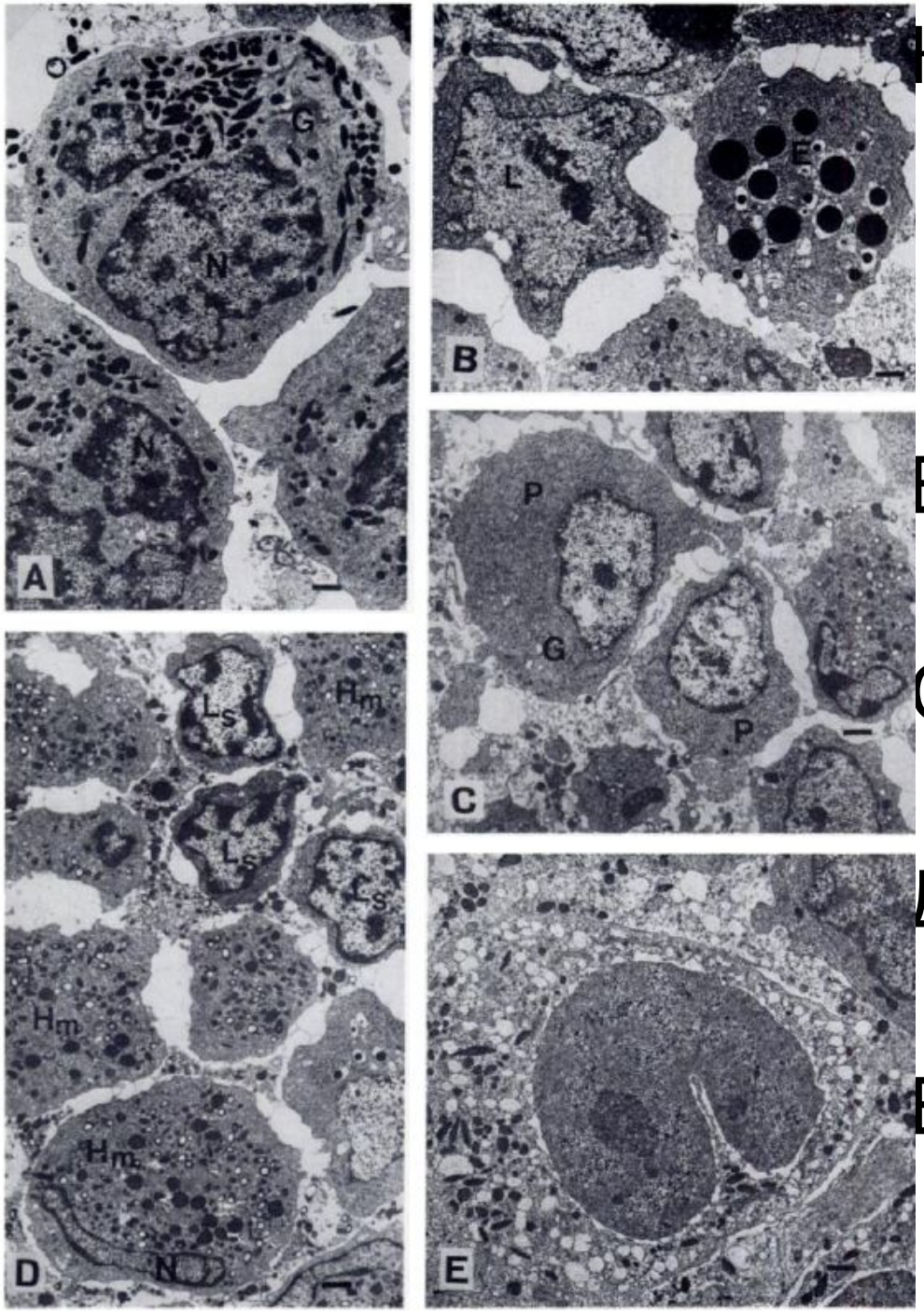


FIGURE 3. Electron microscopy of different cell types from the Leydig organ. Bar = 1 μm.

А – гранулоциты с дольчатым ядром,

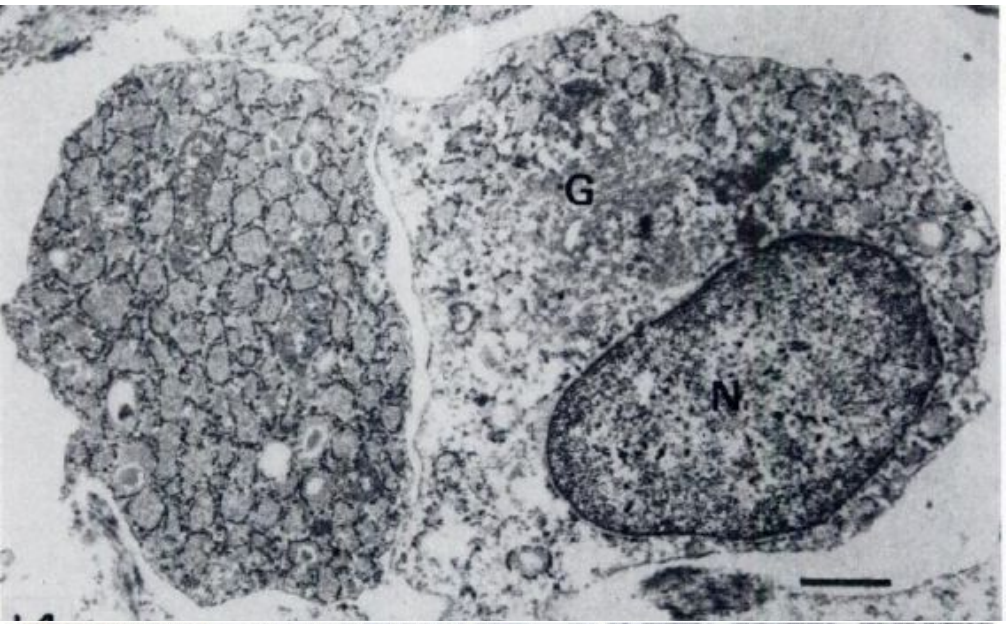
В – лимфоцит, справа от него – эозинофил

С – Две плазматические клетки

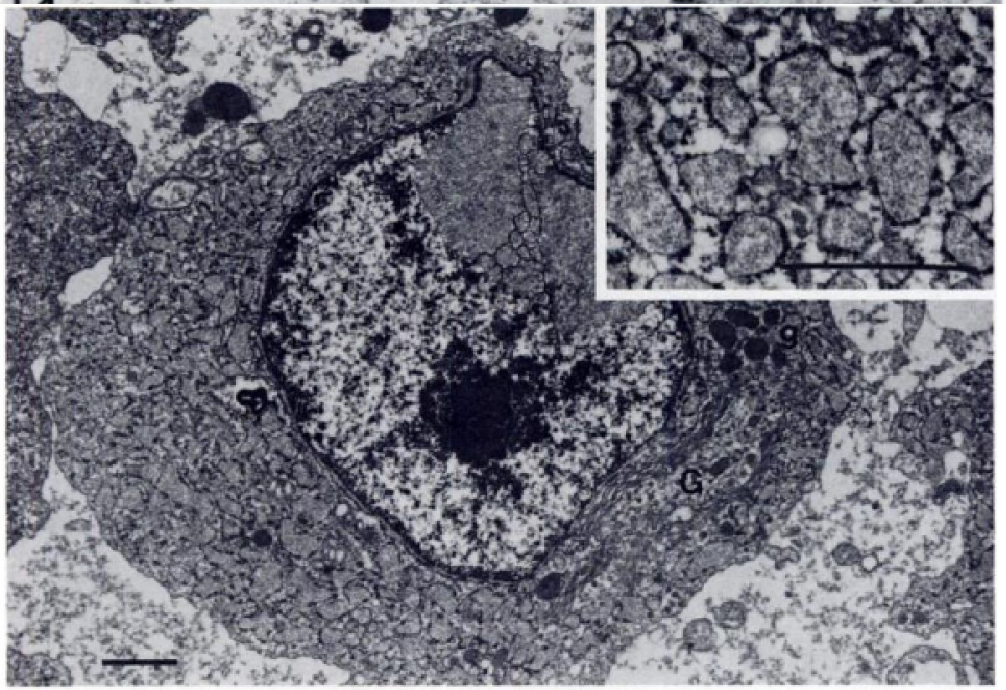
Д – Малые лимфоциты и гранулоцит

Е – Гетерофильный гранулоцит

Плазматические клетки акул

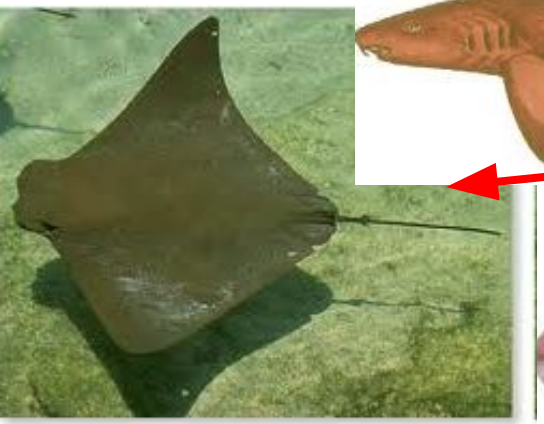


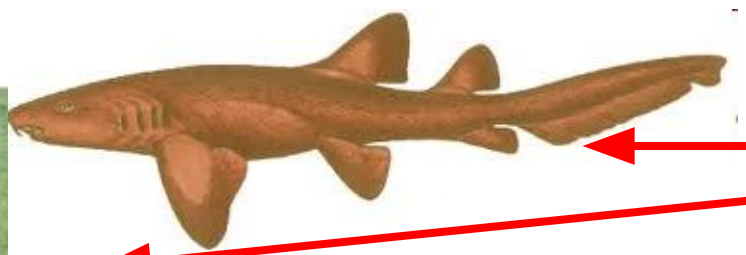
Из селезенки



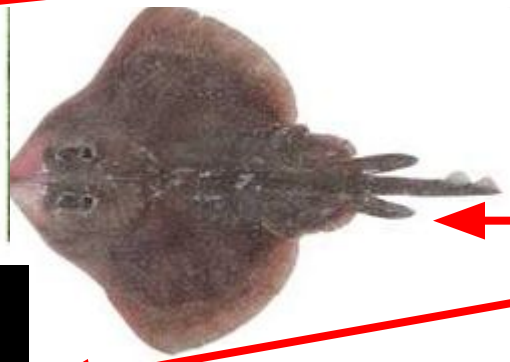
Из органа Лейдига

Важнейший вклад в изучение лимфоидных органов хрящевых и костистых рыб внесла в 50-80х годах группа доктора Рагнара Фанге

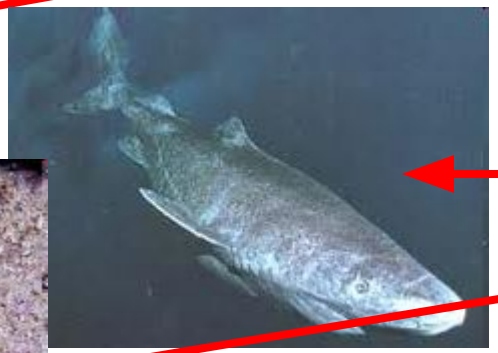




Только эпигональный орган, без органа Лейдига имеют акулы и скаты *Ginglymostoma cirratum, Rhinoptera bonasus, Heterodontus francisci* и *Negaprion brevirostris.*



Оба органа присутствуют у видов *Raja sp., Scyliorhinus canicula, Scyliorhinus stellaris* и *Sequalus acanthias.*



Только орган Лейдига остался у *Somniosus microcephalus, Etmopterus spinax* и *Torpedo sp.*

У некоторых хрящевых рыб, например у цельноголовых (химеры) отсутствуют оба этих органа.

Иммуногистохимическое окрашивание тканей тимуса акулы

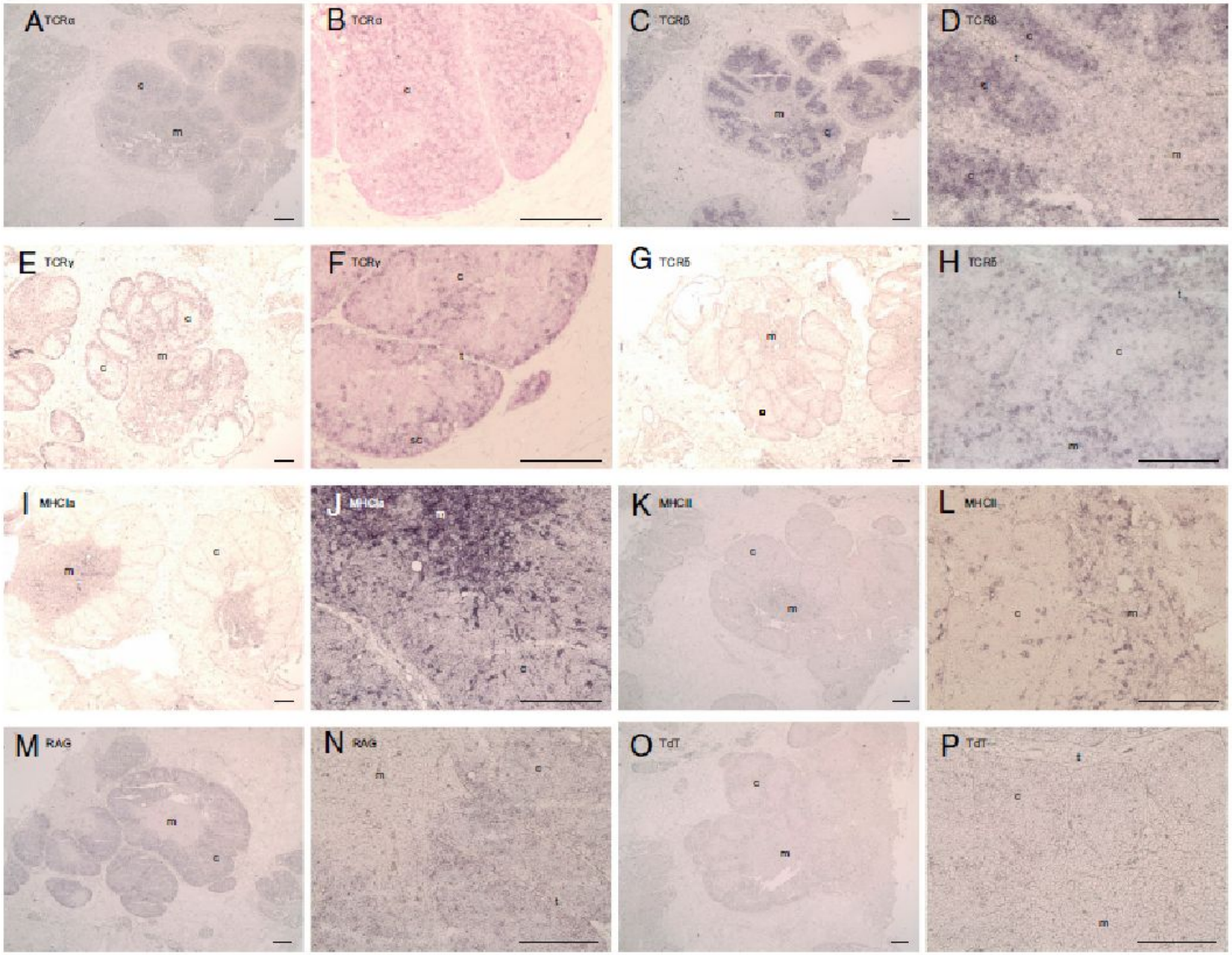


FIGURE 6. In situ hybridization of shark thymus. Positive hybridization is purple. TCRαC probe (*A*, *B*), TCRβC probe (*C*, *D*), TCRγC probe (*E*, *F*), TCRδC probe (*G*, *H*), MHC class Ia probe (*I*, *J*), MHC class IIb probe (*K*, *L*), RAG1 probe (*M*, *N*), and TdT probe (*O*, *P*). Scale bar, 100 μM; original magnification ×10 and ×20. Negative controls with sense probes (not shown) showed no staining. c, cortex; m, medulla; t, trabeculae.

Rag expression

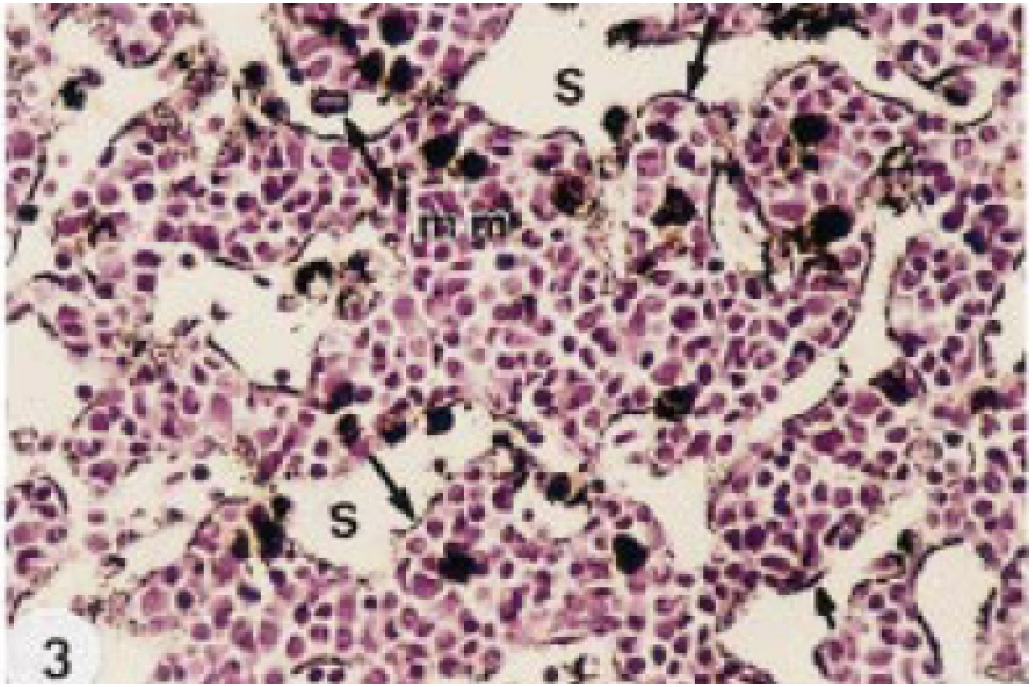
Species	Organ	Method	Day
Zebrafish	Kidney	wish	–
	Pronephros	ISH on tissue sections clusters 3–4 wpf	First cells 2 wpf
	Pronephros	ISH on tissue sections	2–3 wpf
	Pronephros	Rag-2,GFP	8 dpf
	Wholefish	RT-PCR	3 dpf
	Pancreas	wish	4 dpf
Rainbow trout	Wholefish	RT-PCR	10 dpf (before appearance of thymus and kidney)
	Pronephros	ISH on tissue sections	Hatching

Ig expression

Species	Organ	Evidence	Method
Zebrafish	Wholefish	VDJ(μ) rearrangements	PCR amplification of genomic DNA
	Wholefish	IgM expression	RT-PCR
	Wholefish	IgM expression	ISH on tissue sections
	Wholefish	IgLC isotype	RT-PCR
	Kidney	IgLC isotype	ISH on tissue sections
Rainbow trout	Wholefish	cIgH-cells	Immunohistochemistry
	Wholefish	IgL	ELISA
	Pronephros	SIgM+cells	Immunohistochemistry

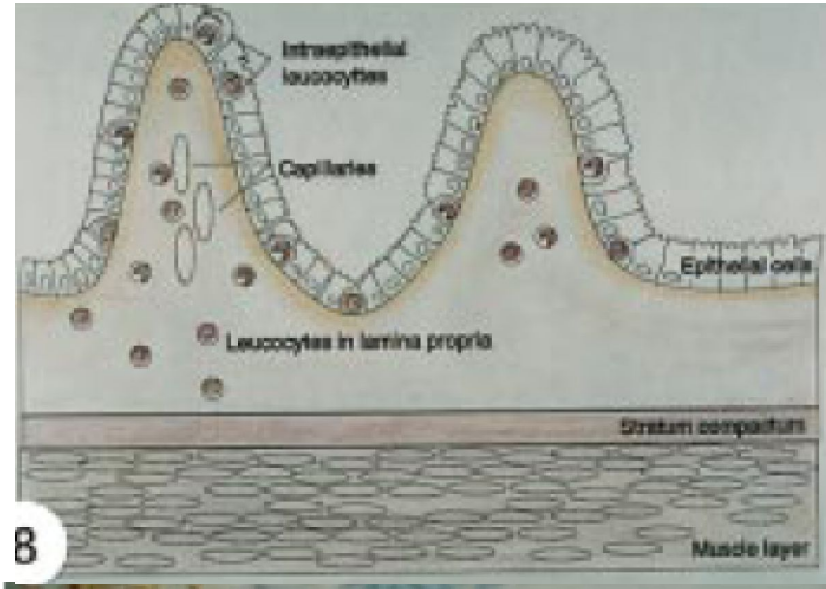
Лимфомиелоидные ткани костных рыб

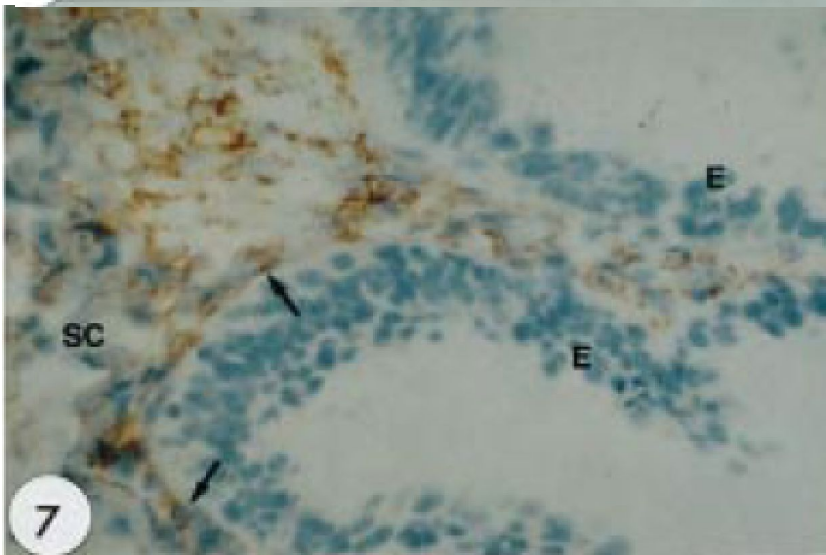
Головная почка – первичный источник иммунных клеток

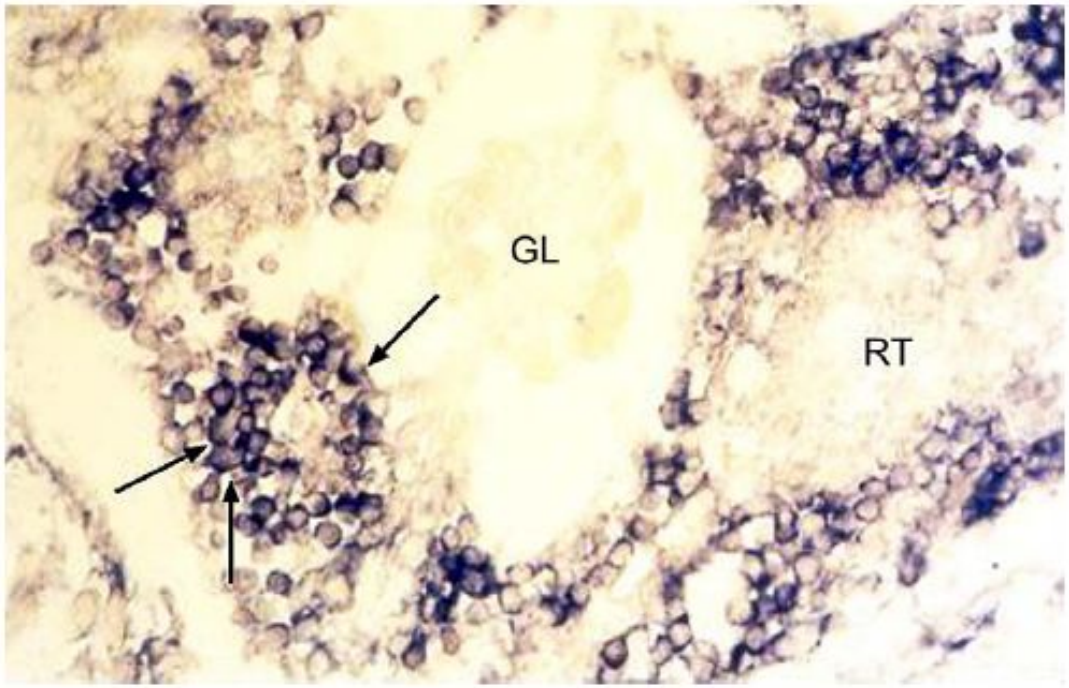


Стрелками отмечены частицы активированного угля, введенные форели в брюшную полость и сорбировавшиеся в почечных синусах. Также видны меланомакрофаги.

Стенка кишечника форели



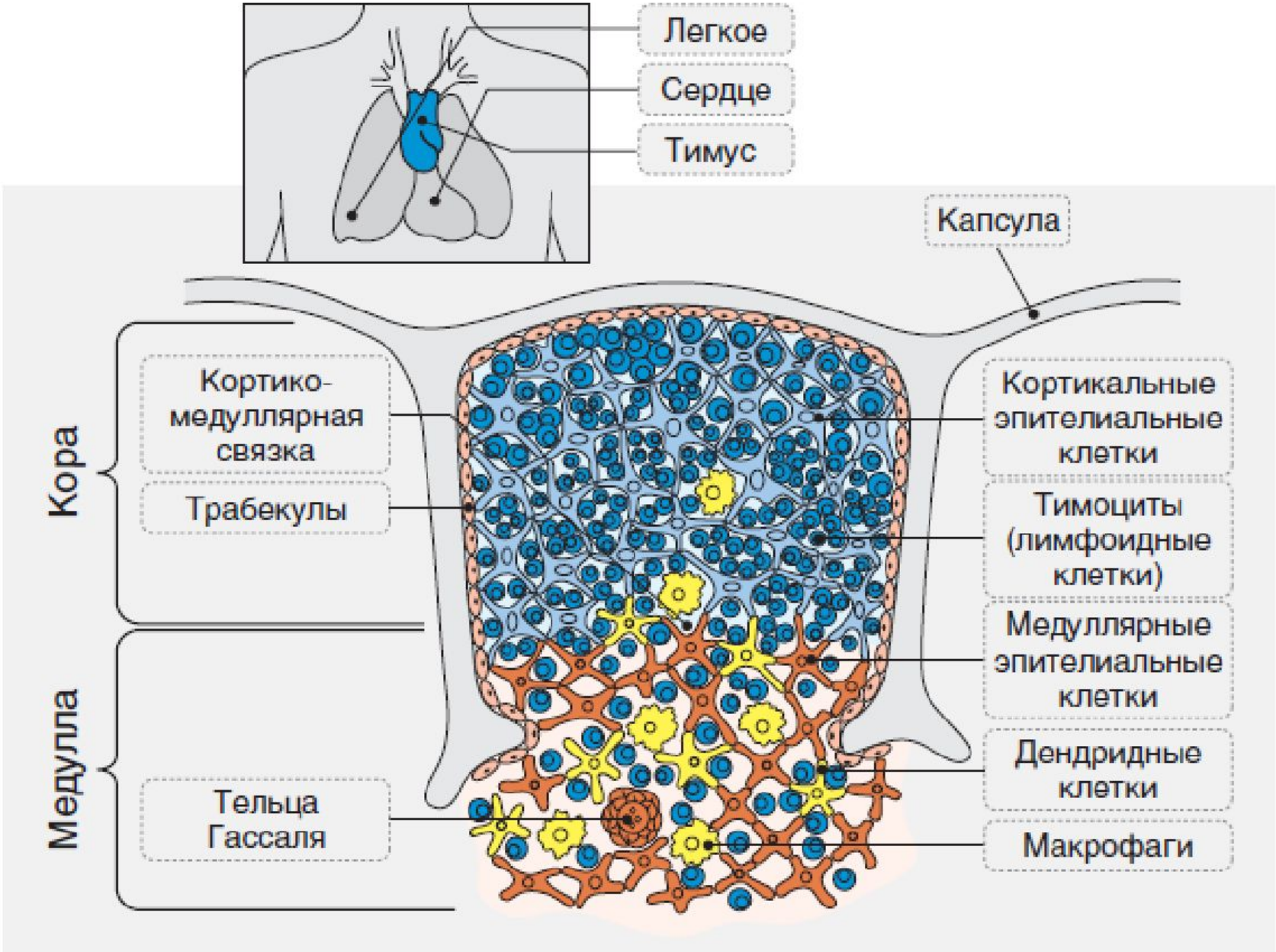




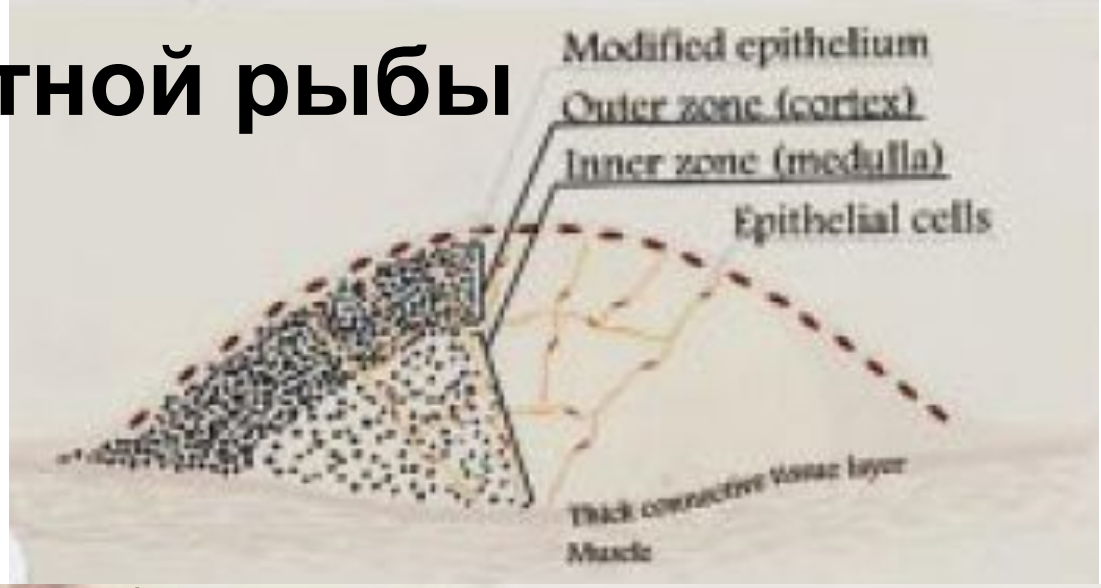
Maturation of immunological competence

Species	Lymphocytes in Lymphoid organs	Appearance of Ig+cells	Allograft rejection	Humoral immune response
Rainbow trout (14 °C)	Thymus (8 d pre-hatching or 3 days) Kidney (4–5 days) Spleen (6–14 days)	4 d (Kidney) 1 m (spleen)	14 days	8 w (HGC+FCA) *A. salmonicida*
Carp (22 °C)	Thymus (3 d–5 m) Kidney (6 d–6 m) Spleen (8 d–10 m)	1 m (spleen)	16 days	4 w (no response to SRBC) 8 w (response to both HGG+FCA and *A. salmonicida*)
Zebrafish	Thymus (3 dpf) Kidney (1–2 w) Spleen (more than one month)	2 w (kidney)		Secreted Ig against *A. hidrophila* HGG (6 w onward)

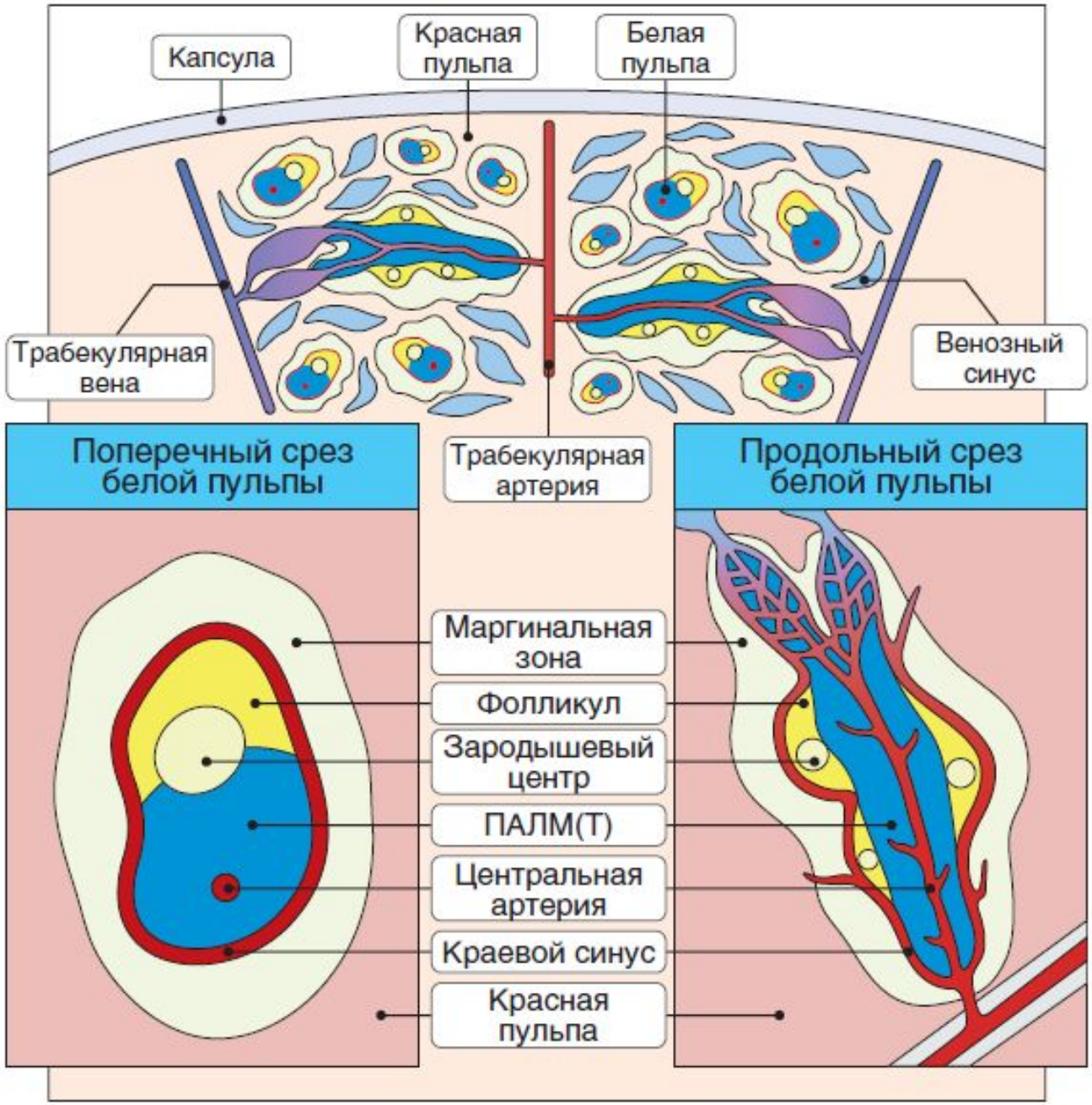
Тимус человека



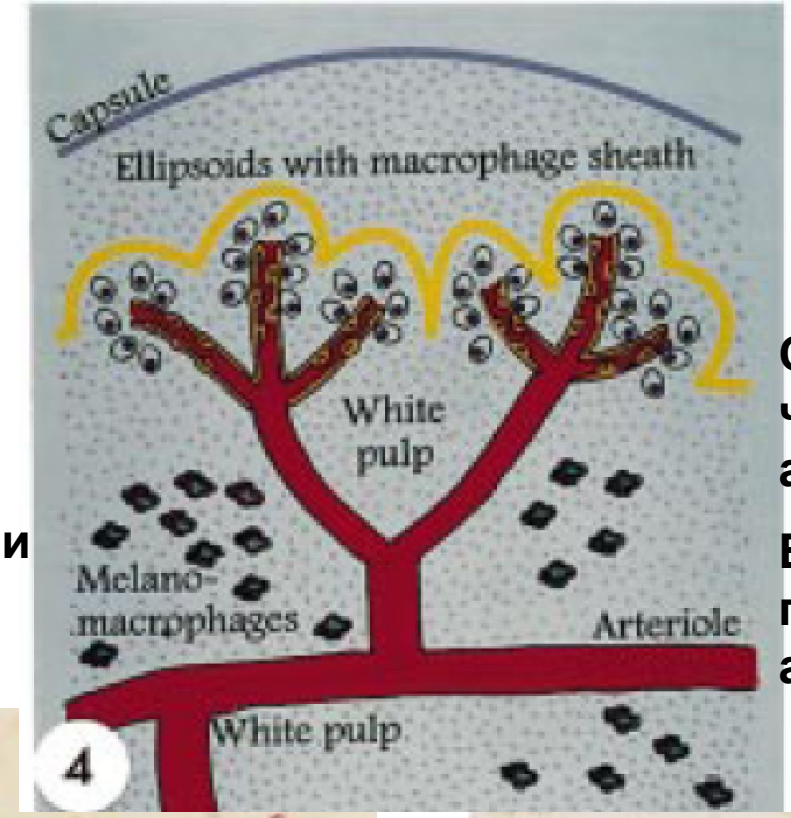
Тимус костной рыбы



Селезенка человека



Селезенка костной рыбы

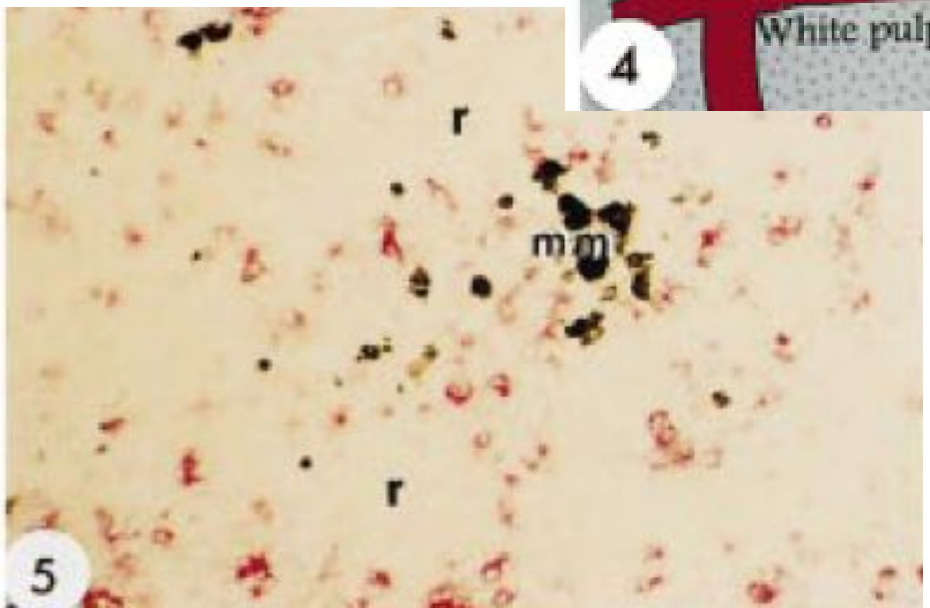


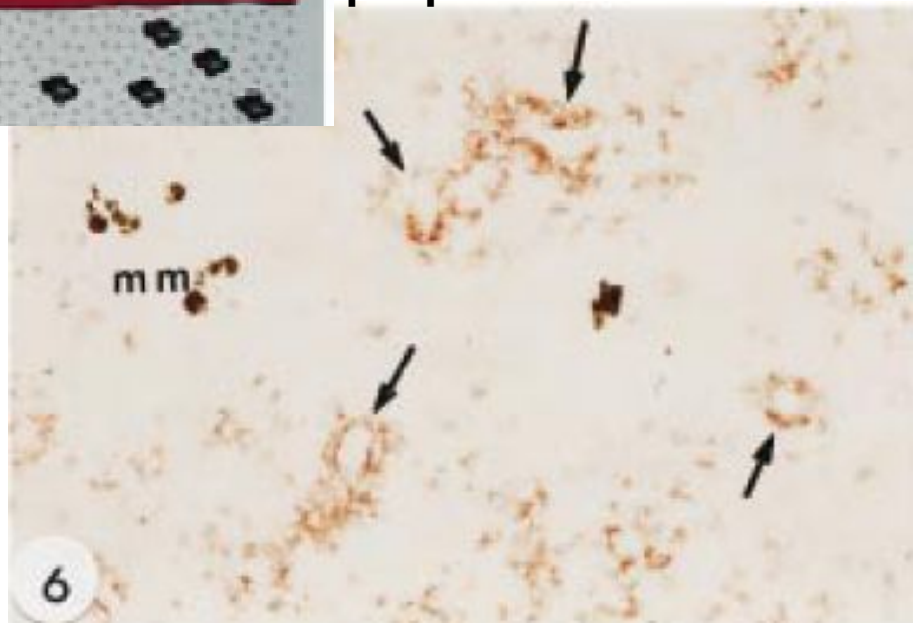
Селезенка лосося

Видны В-лимфоциты и меланомакрофаги

Селезенка лосося через час после иммунизации антигеном

В-лимфоциты перегруппировались к артериолам





Обзор иммунной системы у тетрапод

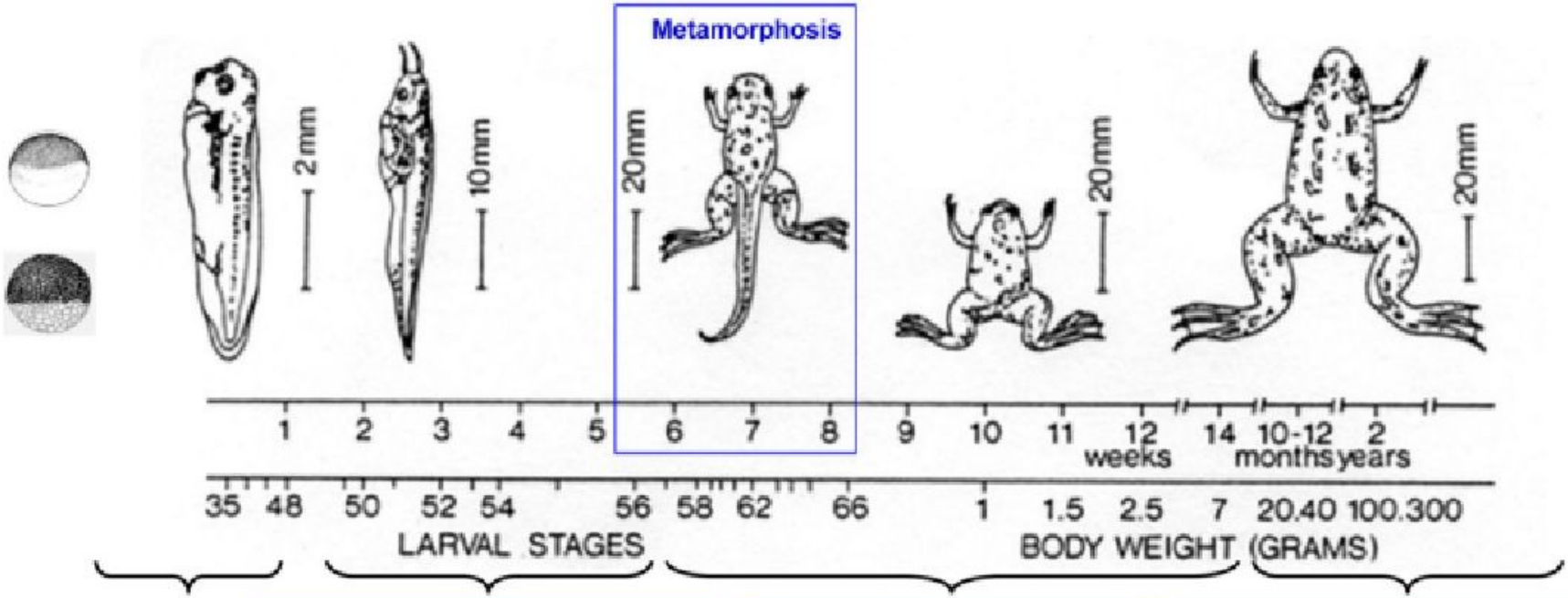
Table 1. Overview of the tetrapod immune system

	Amphibians[1]	Reptiles	Birds[2]	Mammals[3]
General				
Amniote	–	+	+	+
Endotherm	–	–	+	+
Ectotherm	+	+	–	–
Lymphoid tissue				
Thymus	+	+[4]	+	+
Spleen	+	+[5]	+	+
Bone marrow	+	+[6]	+	+
Bursa fabricii	–	–	+	–
GALT	+	+[7]	+	+
Peyer's patches	–	–[8]	+	+
Lymph nodes	–	–[9]	+	+
Germinal centers	–	–[9]	+	+
Innate				
Antimicrobial peptides	+	+[10]	+	+
Complement	+	+[11]	+	+
Non-specific leukocytes				
Macrophages	+	+[12]	+	+
Heterophils/neutrophils	+	+[13]	+	+
Basophils	+	+[14]	+	+
Eosinophils	+	+[4]	+	+
Inflammation				
Fever	+ (behavioral)[15]	+ (behavioral)[16]	+	+
Adaptive				
Cell-mediated				
MHC (Class I, II)	+	+[17]	+	+
TCR	+	+[17]	+	+
Humoral				
Antibody heavy chain isotypes	IgM, IgX, IgY, IgD, IgF	IgM, IgY, IgA? IgD?[18,19]	IgM, IgY, IgA, IgD	IgM, IgG, IgA, IgD, IgE
Light chain isotypes	λ, κ, σ	λ, κ[20]	λ	λ, κ
Phagocytic B cells	+[21]	+[22]	?	–
RAG	+	+[23]	+	+
Class switching	+	+[24]	+	+
Affinity maturation	–	–[24]	Poor	+
Somatic hypermutation	+	+[24]	+	+

Иммунитет амфибий

Components	Mammals	Amphibians
Soluble innate immune factors (complement, cytokines)	+	+
Antibody classes	IgA, IgD, IgE, IgG, IgM	IgA/X, IgD, IgF, IgM, IgY
Cells of the innate immune system (Gr[a], MPh, DC, NK)	+	+
Cells of the adaptive immune system (B and T cells)	+	+
Unconventional T cells		
iNKT	+	+[b]
MAIT	+	+[b]
ILCs	+	Few cells (NK); other have not been determined
Primary lymphoid organs		
Thymus	+	+
Bone marrow	+	+[c]
Secondary lymphoid organs		
Spleen	+	+[d]
Lymph nodes	+	–
Peyer's patches	+	–
GALT	+	+
Scattered lymphoid cells		
IEL[a]	+	+
Organized lymphoid tissues		
M cells	+	–
Peyer's patches	+	–
MLNs	+	–
ILF	+	+
Secretory Ig	IgA	IgX (functional analog of IgA)

Components	Mammals	Amphibians
Primary lymphoid organs		
Thymus	T-cell differentiation	T-cell differentiation
Bone marrow	B-cell differentiation	Neutrophil differentiation and presence of macrophage precursors
Secondary lymphoid organs		
Spleen	T- and B-cell activation	T- and B-cell activation B-cell differentiation in tadpole and adult (in tadpole, B cells differentiate also in liver)
Lymph nodes	T- and B-cell activation	Unknown
GALT	T- and B-cell activation	T- and B-cell activation (unclear)
MLNs	T- and B-cell activation	(Absent structures)
Peyer's patches	Mostly B-cell activation	(Absent structures)



Maternal Ab

Tailbud stage
Innate-type myeloid cells

Larvae: B & T cell Ag recognition but inefficient effector system

- Larval type BCR & TCR repertoires
- Very low or no surface MHC class I expression
- CD3ε+ cells but no B cells in GALT
- Weak B cell & Ab responses
- Poor switch IgM to IgY
- Incomplete skin graft rejection (e.g., tolerance to minor H-Ags)
- No detectable NK cell and NK cell activity
- CD8 T cells but no detectable CTL activity
- Weak T helper-like MLR

Metamorphic to 6 month-old adult: Down regulation of immune responses

- Thymic histolysis
- Thymus migrates towards the tympanum
- Incomplete skin graft rejection
- Weak anti-tumor responses
- NK cells but no NK cell killing activity
- T helper-like MLR impaired

One year-old adult or older: Efficient anti-viral & anti-tumor immune responses

- Adult type BCR & TCR repertoires
- MHC class I express on all cells
- B & T cells in GALT
- More efficient B cell & Ab responses
- Acute skin graft rejection including minor H-Ag-disparate
- Potent NK cell killing activity
- CTLs against major and minor H-Ag
- Stronger, MHC class II-dependent, T helper-like MLR

Long lasting immunological memory
DNP-KLH, minor H skin Ags, and Tumor Ags

Summary of the Main Developmental Steps of the *Xenopus* Immune System

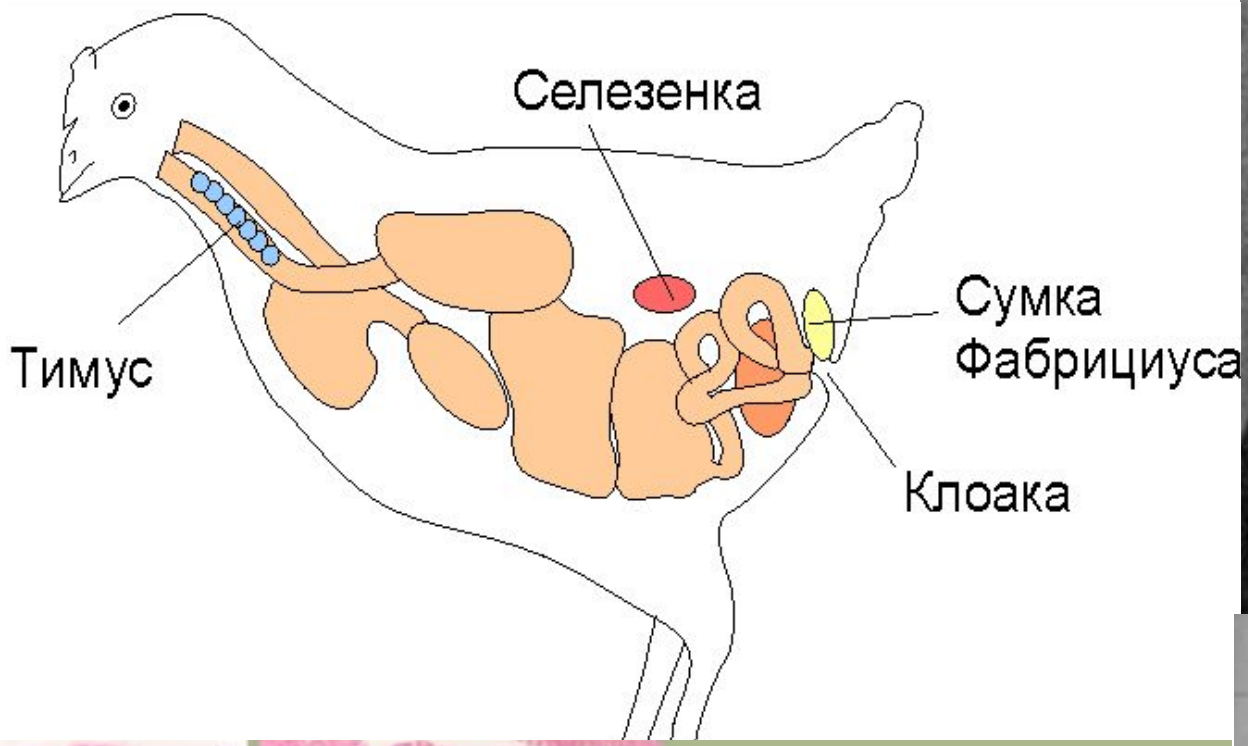
Devel. stages (days)	Liver	Thymus	Spleen	GALT
40 (d3)		Thymic epithelium buds from 2nd visceral pouch	Absent	Few scattered CD3ε Expressing leukocytes
46 (d4)	Lymphopoiesis in peripheral layer, Ig (μ)and sterile TCRβ RAG, AID	Epithelium, no precursors	Spleen anlage mesenchymal thickening in the mesogastrium	No B cells
47 (d4–5)	Lymphopoiesis, and B cell development in absence of Ag	Colonization by Lymphopoietic precursors from post-VBI (~100 cells) RC47$^+$	Blood cells (No Lymphopoiesis)	
48 (d6–7)		Cortex-meddula, full TCRβ mRNA, CD3ε CD8$^+$ Thym., class II$^+$ epithelial cells		
49 (d10–13)	IgL rearrangements	First CTX$^+$ thymocyte, more CD8$^+$	Spleen B cells (~200) and 1st detect Ab responses	
50 (d15)		Ongoing thymocyte differentiation (3×10^4 cells)		
56 (d38)		Ongoing thymocyte differentiation (9×10^5 cells)	Detectable T cell responses	
58 (d44)		Max. size of the thymus ($1–2\times10^6$ cells)	Max. larval T cell response (1×10^6 cells)	
Adult (>d60)	Adult-type leukocytes	Thymus move near tympanum New adult–type thymocyte differentiation	Adult T cell responses ($1–2 \times 10^7$ cells)	
Adult (> 1 yr)		Thymus progressively filled by fat tissues	($1–2 \times 10^7$ cells)	Many IgM$^+$ and IgX$^+$ B cells, as well as T cells (CD8$^+$ and CD8-)

Иммунитет у головастика и взрослой лягушки

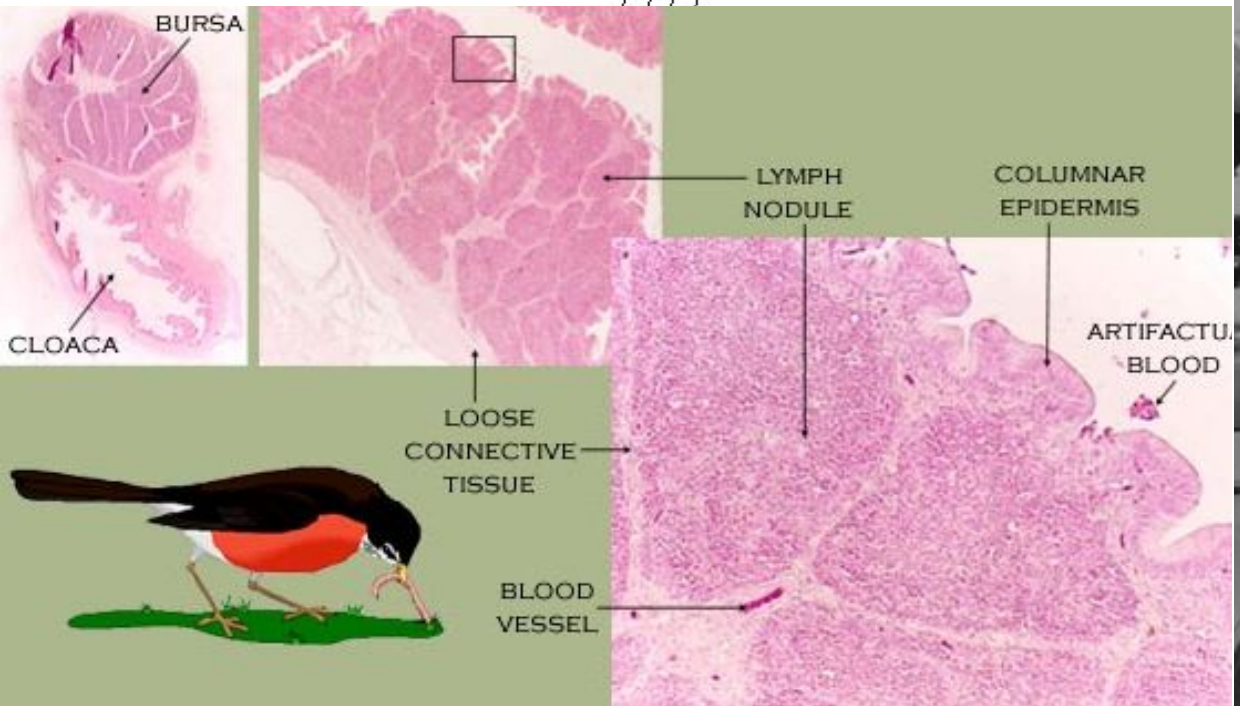
Table 1. Summary of immune responses to FV3 in larval and adult *X. laevis*.

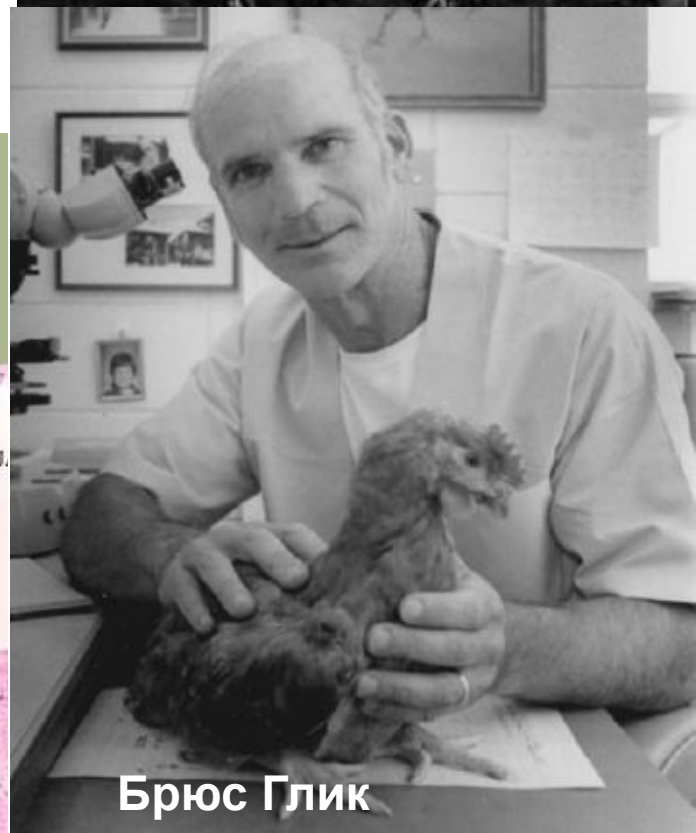
	Adults		
	Primary	**Secondary**	**Larvae**
Symptoms	2–3 weeks	3–5 days	Long lasting, >80% death
Virus Clearance	1 month	1 week	Ineffective
Innate Immunity			
Cells	1 dpi: Activated Mø 3 dpi: NK cells	Same + Mø as APC Similar to primary	Mø less resistant to FV3 No NK
Induced genes	1 dpi: TNF-α, IL-1β, IFN-γ, Mx1	Similar to primary	Delayed (3 dpi) and weaker
Adaptive T cell immunity			
Splenic CD8 T cell	Peak at 6 dpi	Peak at 3 dpi but lower expansion	?
CD8 T cell in kidneys	At 6 dpi	At 3 dpi but fewer	?
T cell memory	-	yes	?
Adaptive B cell immunity			
Anti-FV3 antibodies	Not detected	IgY from 10 dpi	Not detected
More IgY mRNAs	6 dpi	3dpi	6–7 dpi
AID up-regulation	9 dpi	3 dpi	6–7 dpi
B cell memory	-	Yes	Possibly

Abbreviations: AID: activation-induced cytidine deaminase; dpi: days post-infection; Mø: Macrophages.


Селезенка
Тимус
Сумка
Фабрициуса
Клоака

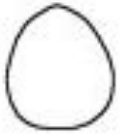
Иероним Фабрициус


BURSA
CLOACA
LYMPH
NODULE
COLUMNAR
EPIDERMIS
ARTIFACTU
BLOOD
LOOSE
CONNECTIVE
TISSUE
BLOOD
VESSEL

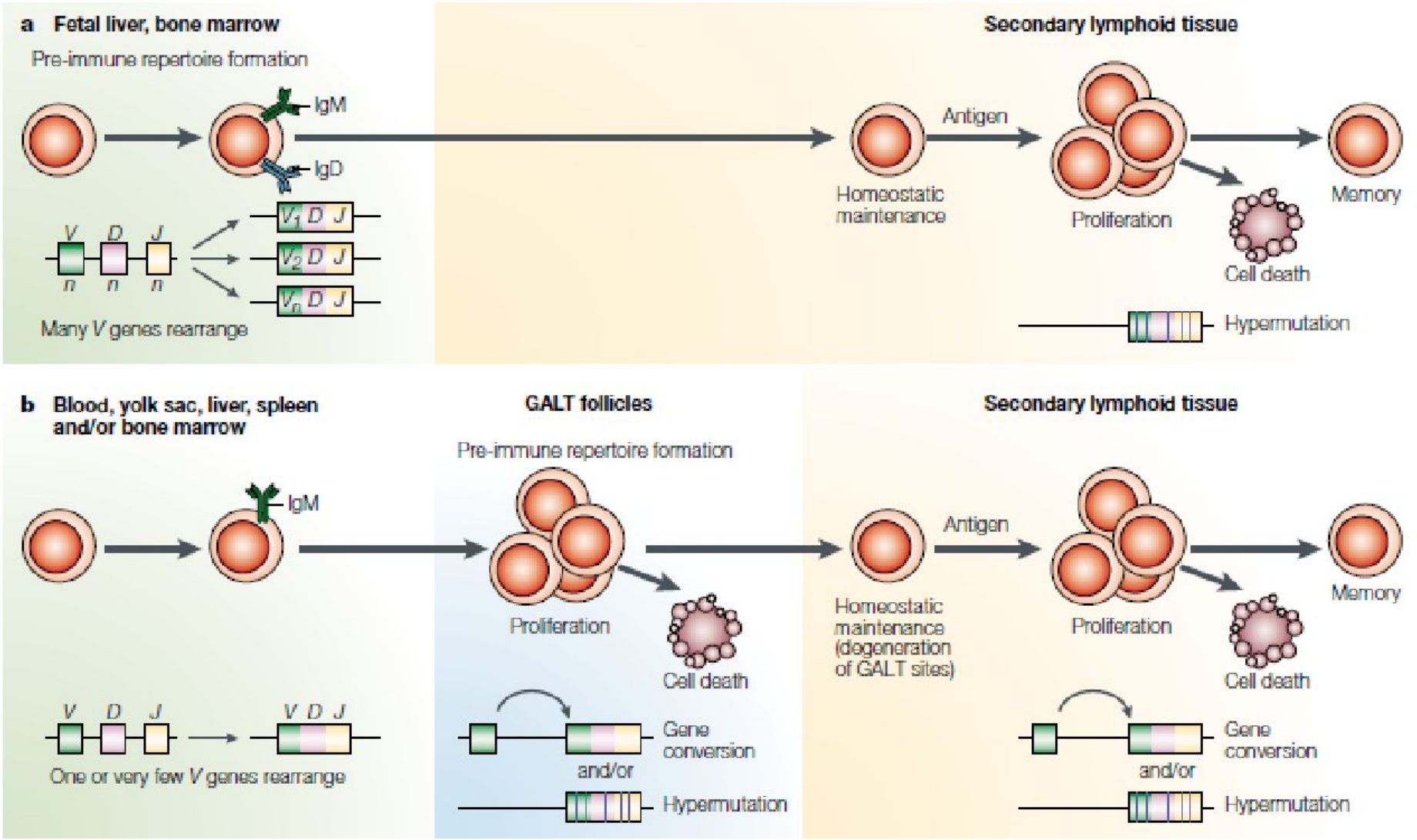

Брюс Глик

Созревание В-лимфоцитов у курицы

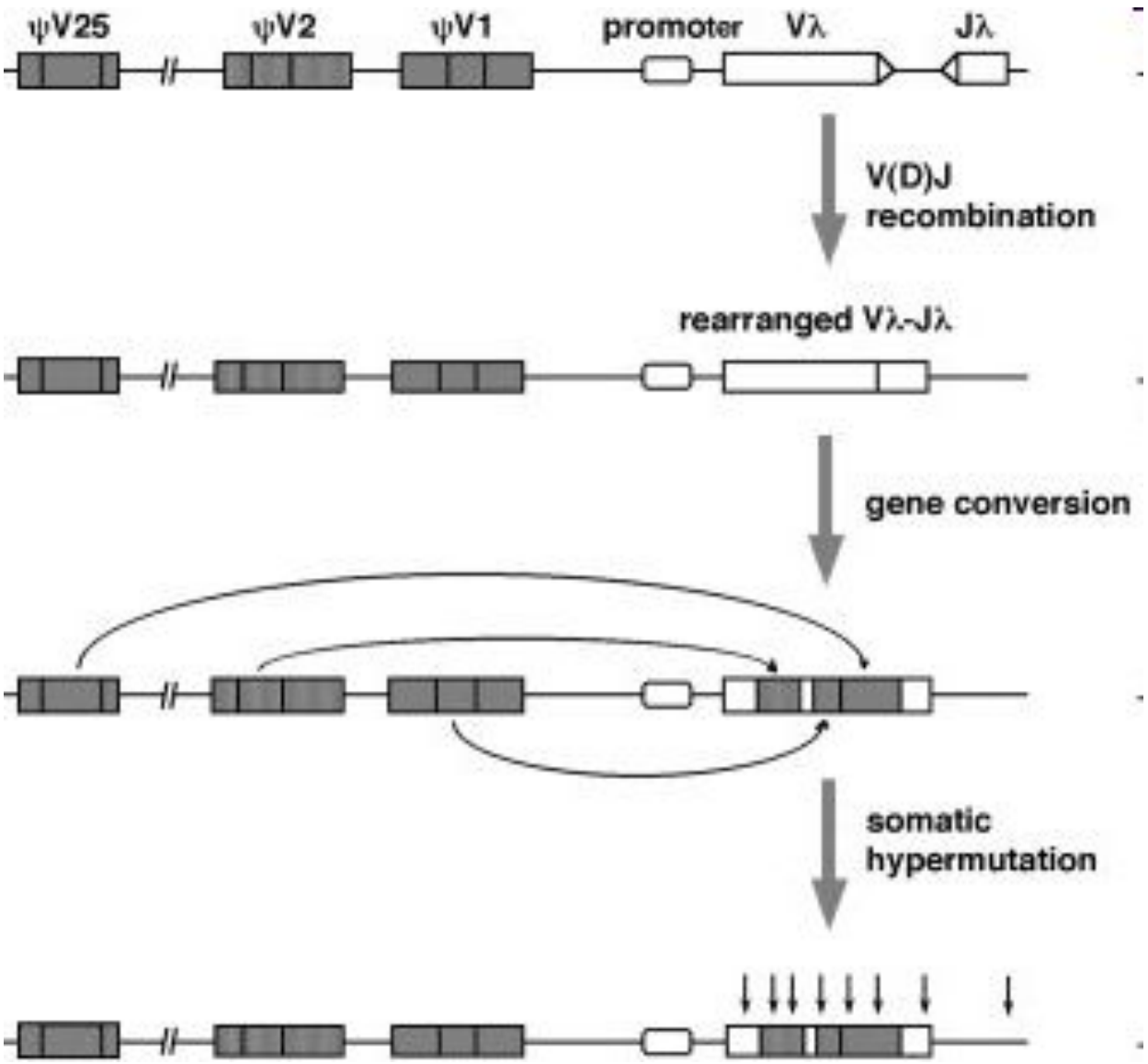
	Ig gene remodelling	organ	ontogeny
pre-bursal stage			
	D_H-J_H recombination	yolk sac	embryonic day 5-6
	completion of V(D)J recombination	spleen, thymus, blood etc.	embryonic day 7-15
bursal stage			
	gene conversion (high) somatic hypermutation (low)	the bursa of Fabricius	embryonic day 15 - after hatching (until involution of the bursa at 4-6 months after hatching)
post-bursal stage			
	gene conversion (high in early GC stage low in late GC stage) somatic hypermutation (high)	splenic germinal centers (GCs)	after 3 month old



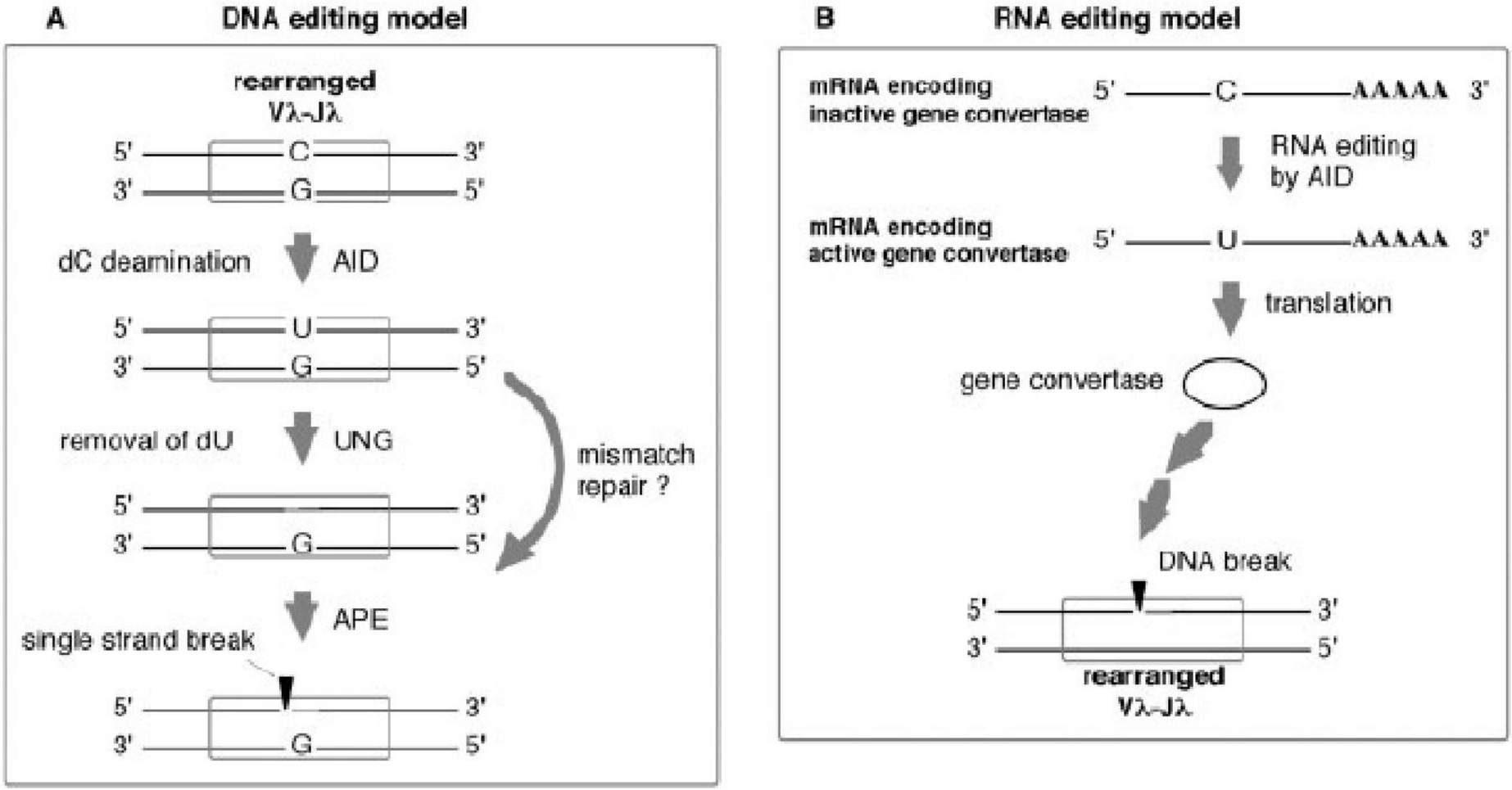
Rag-зависимая рекомбинация или конверсия генов?



Общая схема конверсии



После открытия дезаминазы AID предполагали, что она может редактировать РНК



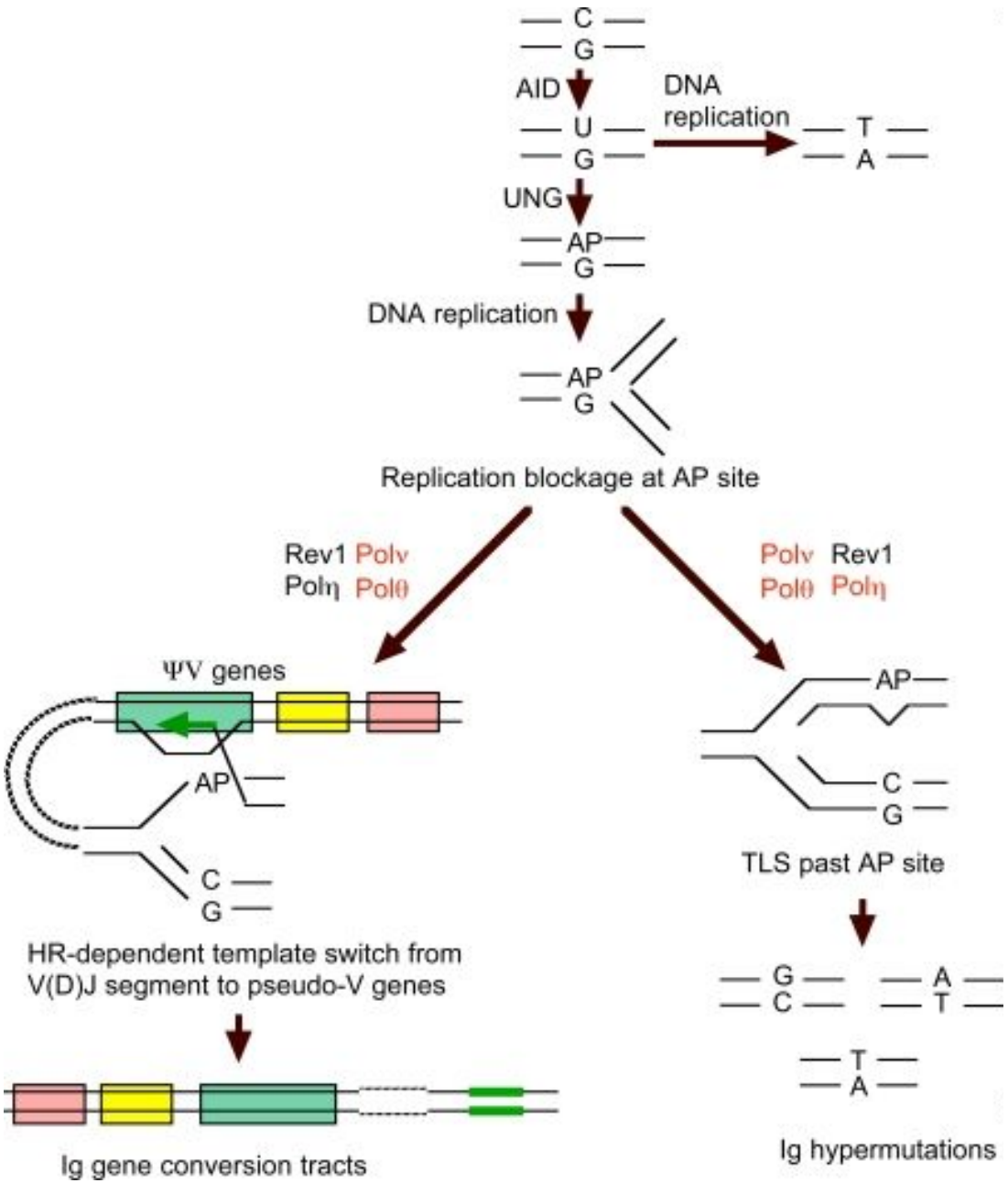

C
G
AID
U
G
DNA
replication
T
A
UNG
AP
G
DNA replication
AP
G
Replication blockage at AP site
Rev1 Polν
Polη Polθ
Polν Rev1
Polθ Polη
ΨV genes
AP
C
G
HR-dependent template switch from
V(D)J segment to pseudo-V genes
Ig gene conversion tracts
AP
C
G
TLS past AP site
G
C
A
T
T
A
Ig hypermutations

TABLE 1. Ig Gene Diversification System of Mammalian Species and Chicken

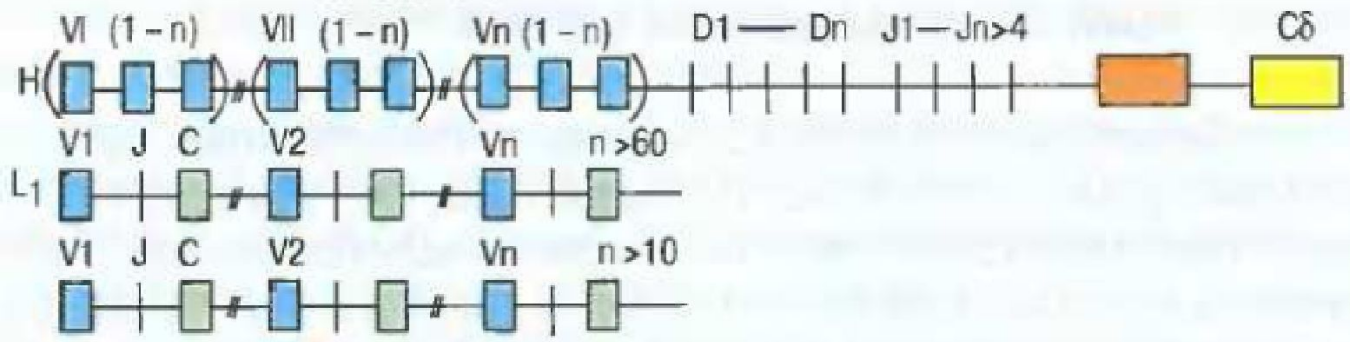
	Primary B cell repertoire formation	
	Mechanism	Organ
Human	V(D)J recombination	Bone marrow
Mouse	V(D)J recombination	Bone marrow
Rabbit	Gene conversion and somatic hypermutation	Appendix
Cattle	Gene conversion and somatic hypermutation	Illeal Peyer's partches
Swine	Gene conversion and somatic hypermutation	Illeal Peyer's partches
Horse	Gene conversion and somatic hypermutation	Illeal Peyer's partches
Chicken	Gene conversion and somatic hypermutation	The bursa of Fabricius

TABLE 2. DT40 Mutants Related to Gene Conversion and Somatic Hypermutation

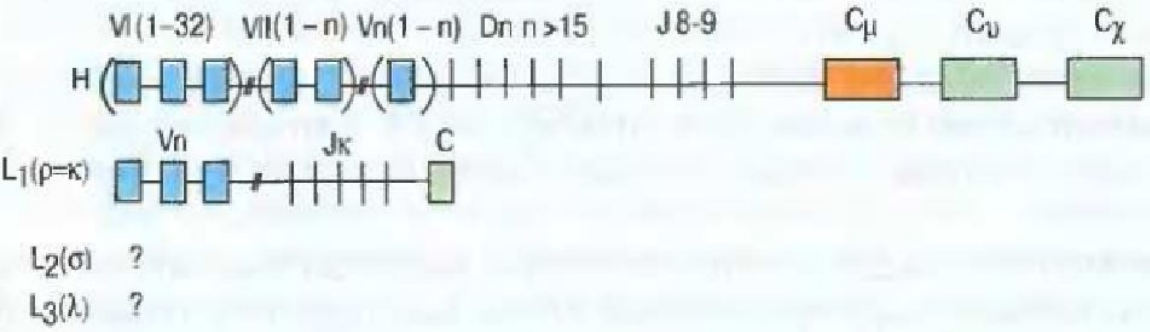
	Enzyme activity	DT40 mutant phenotype	References
RAG-2	V(D)J recombinase	No defect in gene conversion	Takeda et al., 1992
RAD51	Homologous recombination	Lethal	Sonoda et al., 1998
RAD52	Homologous recombination	Slight reduction of targeted integration	Yamaguchi-Iwai et al., 1998
RAD54	Homologous recombination	Reduction of gene conversion Reduction of targeted integration Hypersensitivity to DNA damaging reagent	Bezzubova et al., 1997
NBS1	Homologous recombination	Reduction of gene conversion Reduction of targeted integration	Tauchi et al., 2002
AID	Cytidine deaminase	No gene conversion activity	Arakawa et al., 2002
RAD51-paralogues (XRCC2, XRCC3, RAD51B)	Homologous recombination	Shift from gene conversion to somatic hypermutation	Sale et al., 2001
UNG	Uracil DNA glycosilase (base excision repair)	Shift of hypermutation pattern from transversion to transition	Di Noia and Neuberger, 2002
REV1	Deoxycytidyl transferase	Reduction of somatic hypermutation	Simpson and Sale, 2003

КОСТИСТЫЕ РЫБЫ

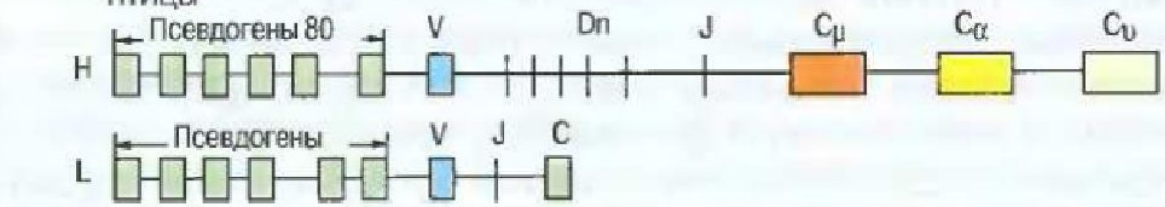
Число семейств >7



АМФИБИИ Число семейств 11

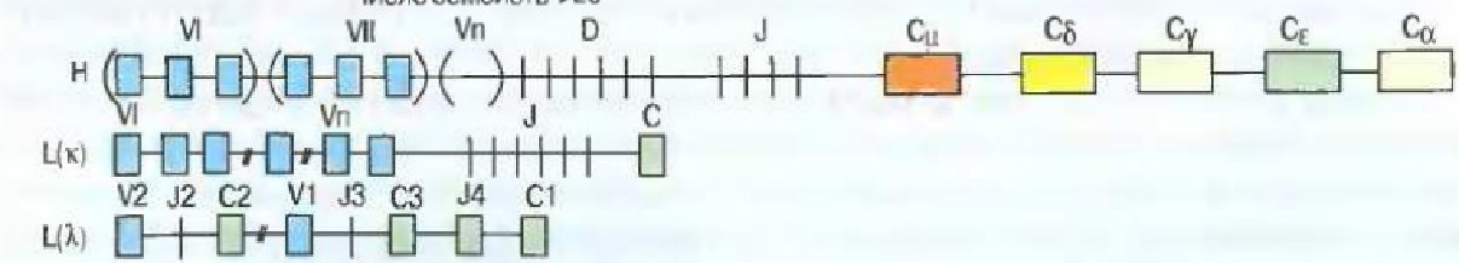


ПТИЦЫ

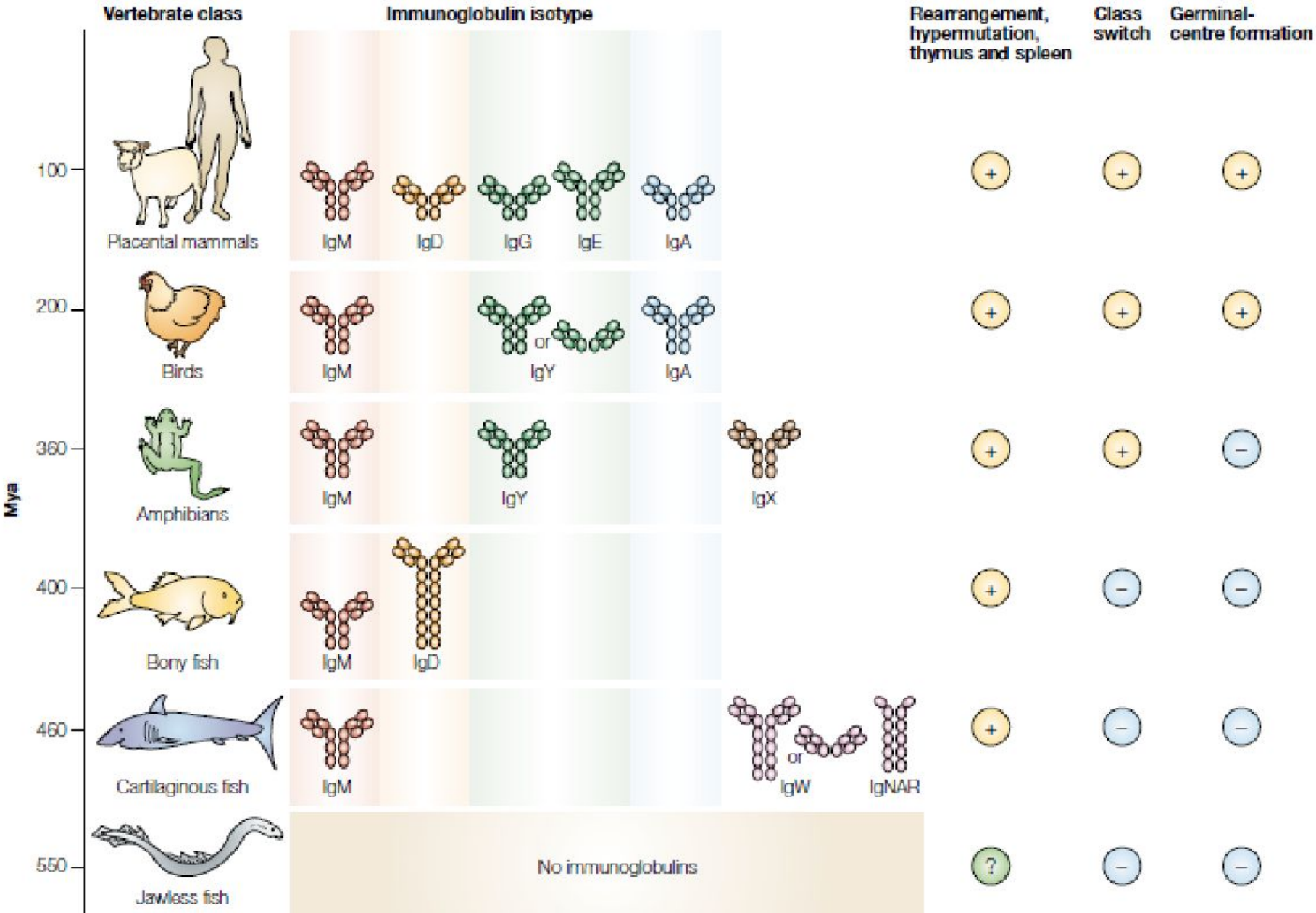


МЛЕКОПИТАЮЩИЕ

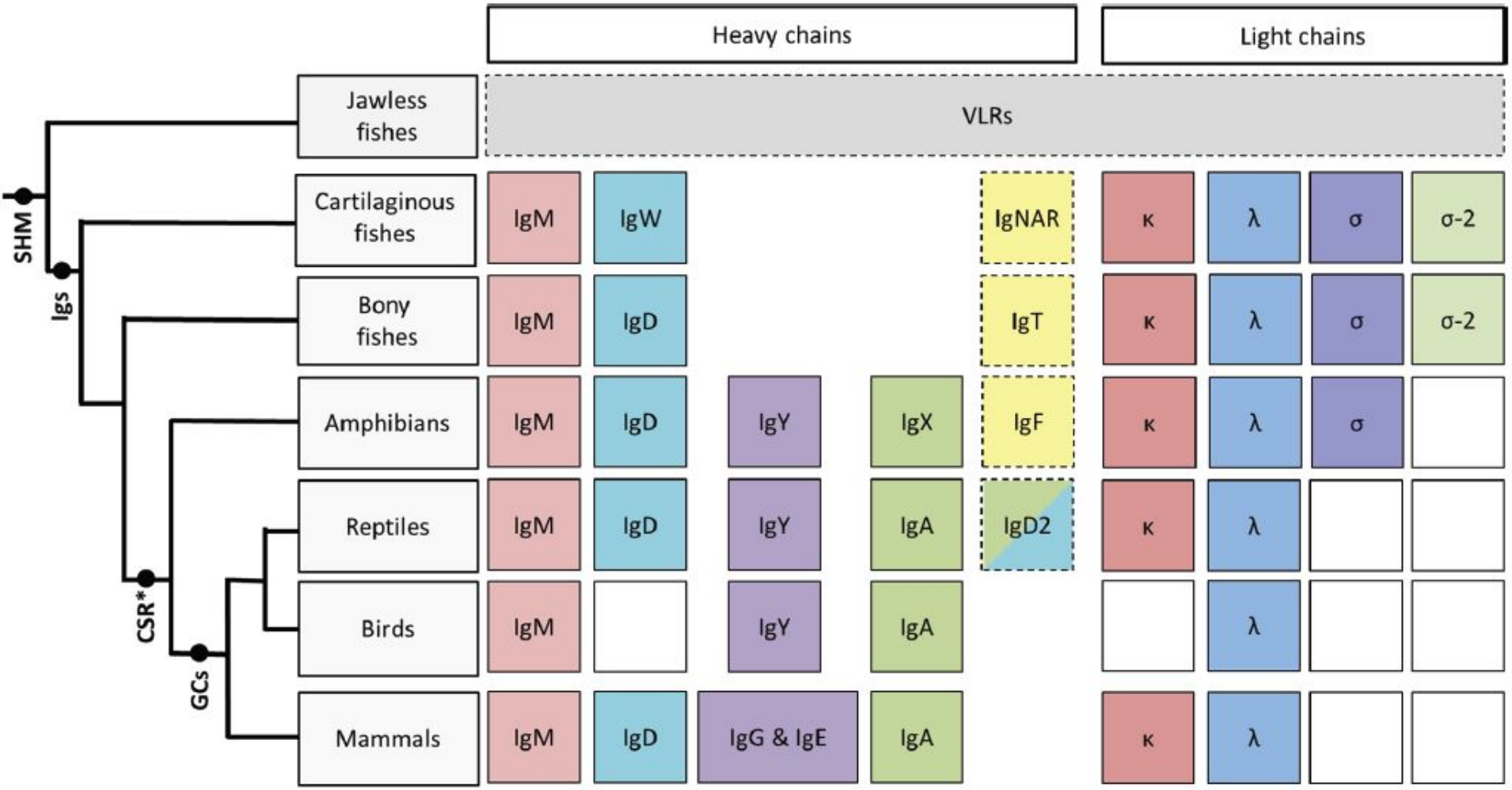
Число семейств >20



Разнообразие антител позвоночных



Наличие генов тяжелых и легких цепей антител у различных классов позвоночных



Антитела у акул

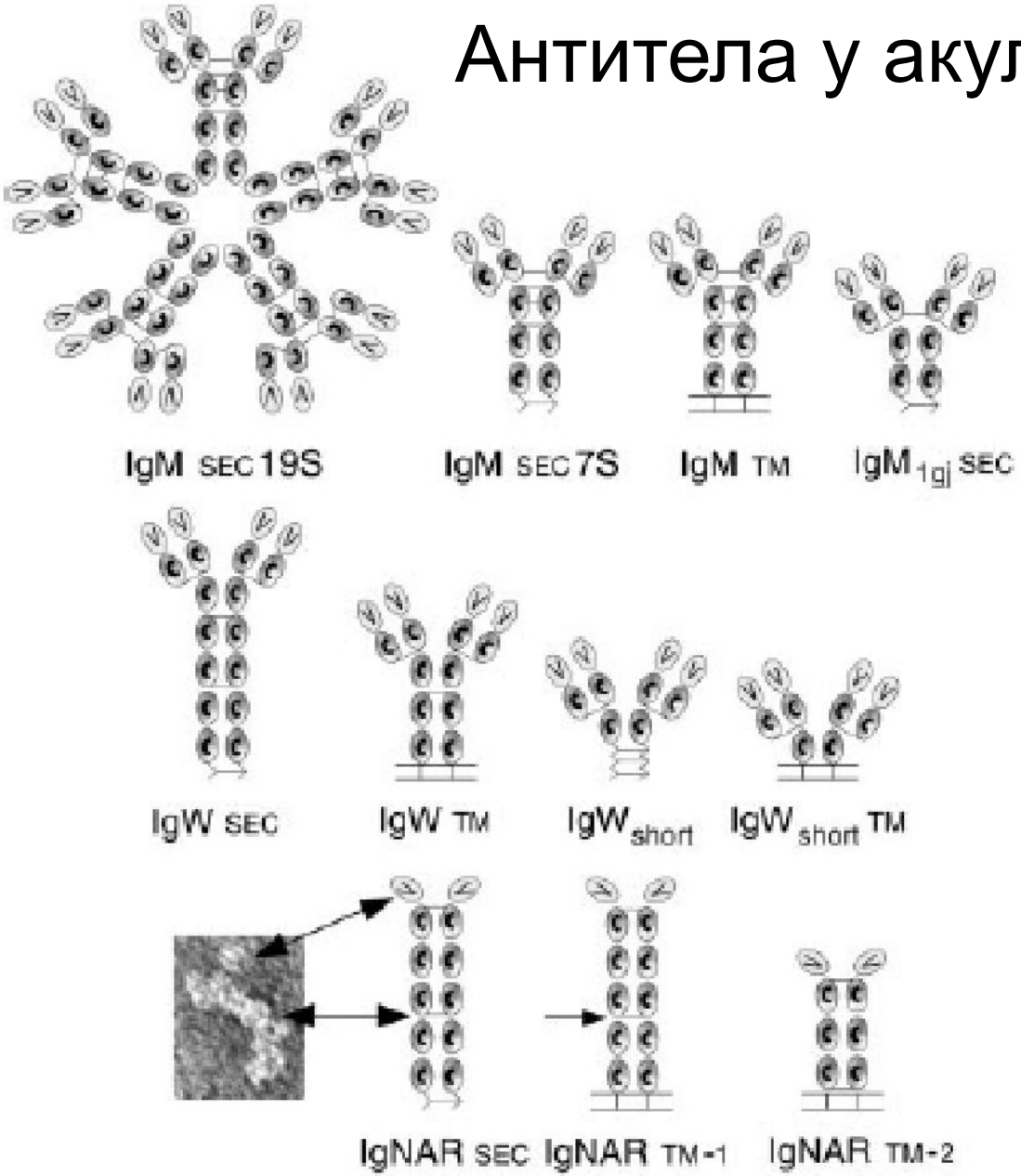
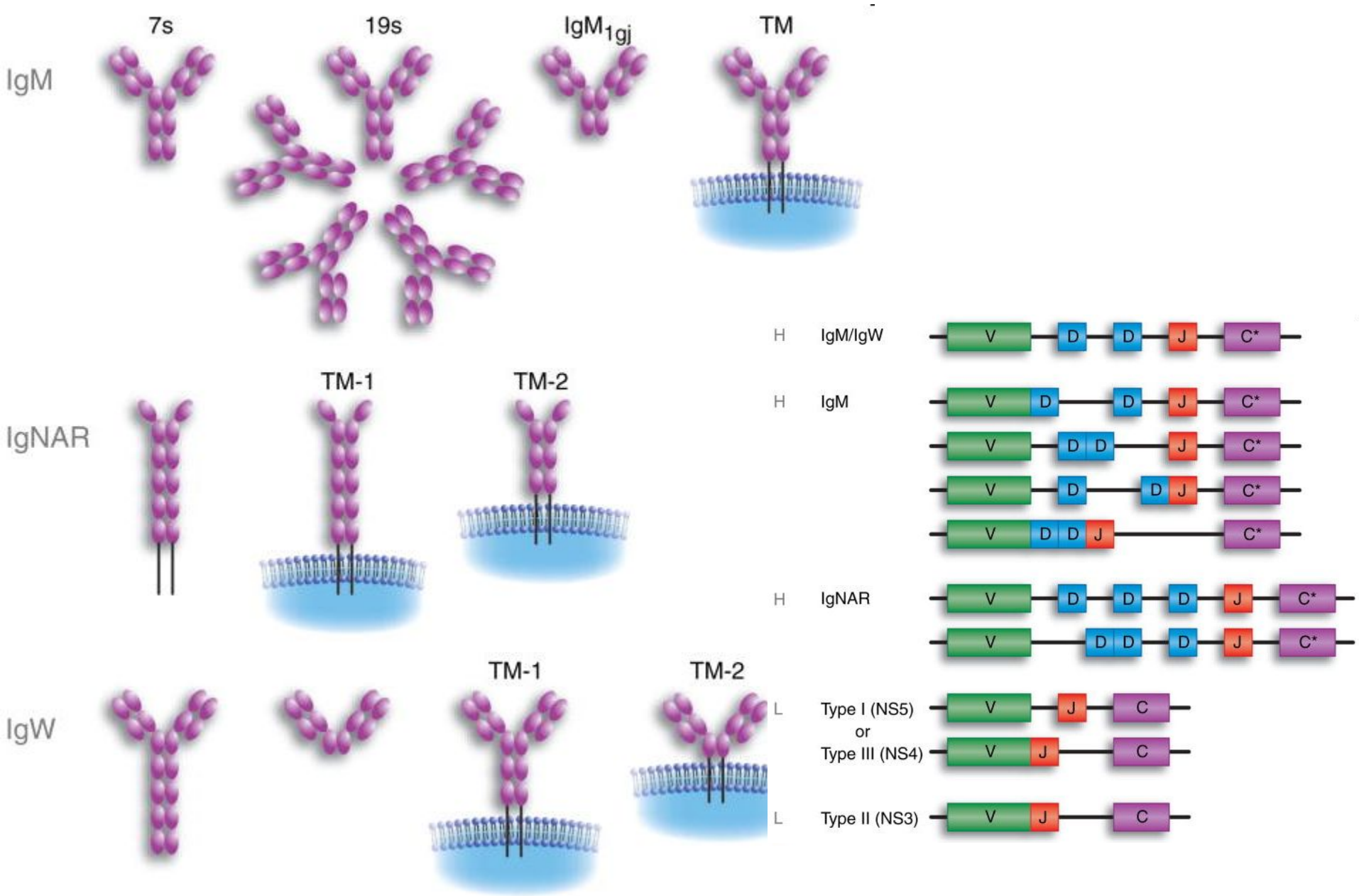
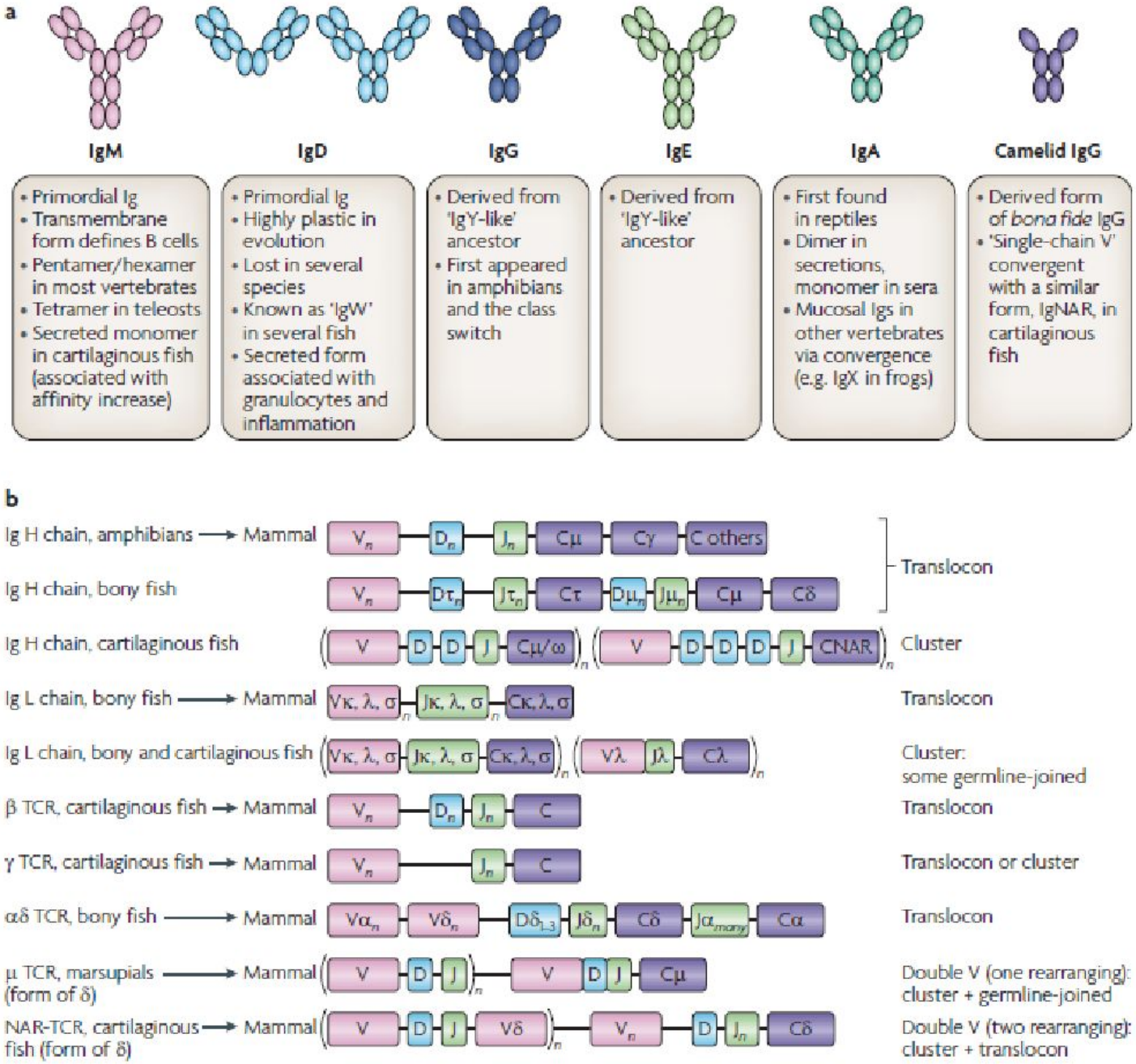


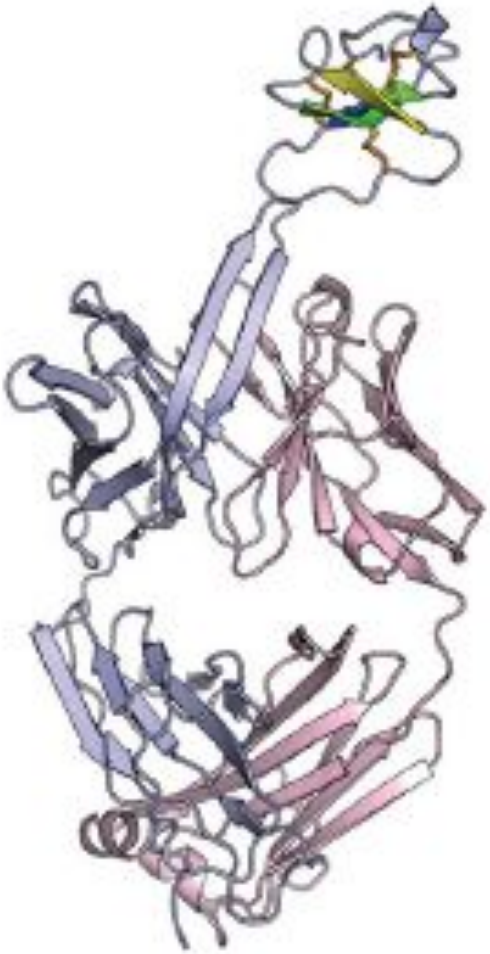
FIGURE 8. Cartilaginous fish Ig repertoire. Current molecular/protein models of Ig isotypes identified thus far in cartilaginous fish. See text for details.

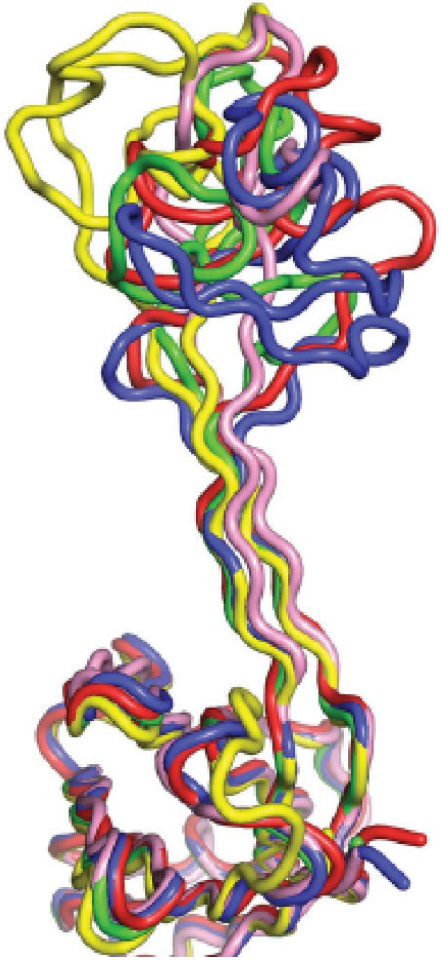

IgM
7s
19s
IgM1gj
TM
IgNAR
TM-1
TM-2
IgW
TM-1
TM-2
H IgM/IgW
V D D J C*
H IgM
V D D J C*
V D D J C*
V D D J C*
V D D J C*
H IgNAR
V D D D J C*
V D D D J C*
L Type I (NS5)
or
Type III (NS4)
V J C
V J C
L Type II (NS3)
V J C

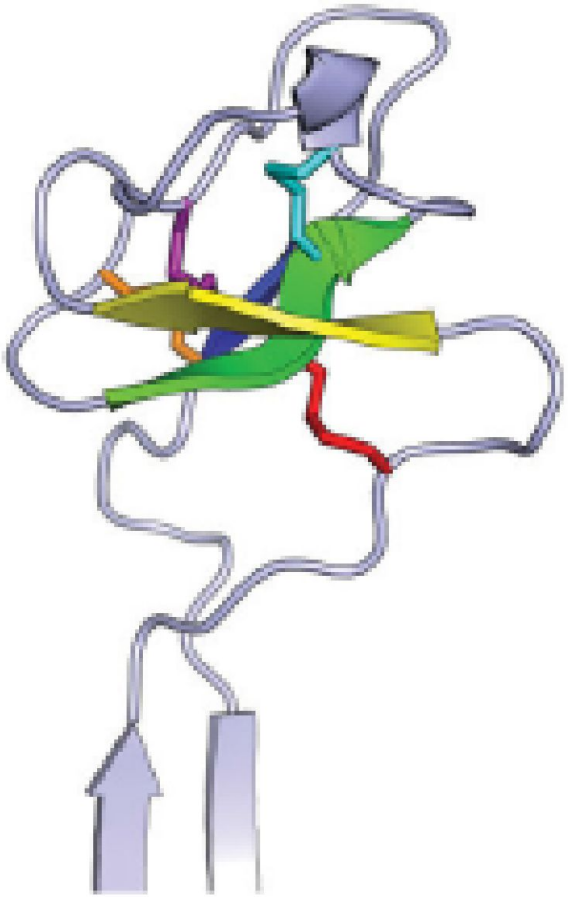
Характеристика антител млекопитающих



Бычьи антитела имеют длинный CDR3 V-домена тяжелой цепи



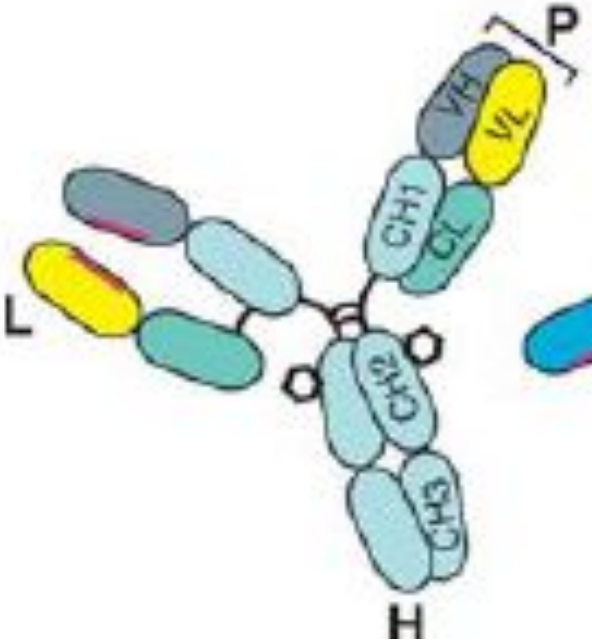


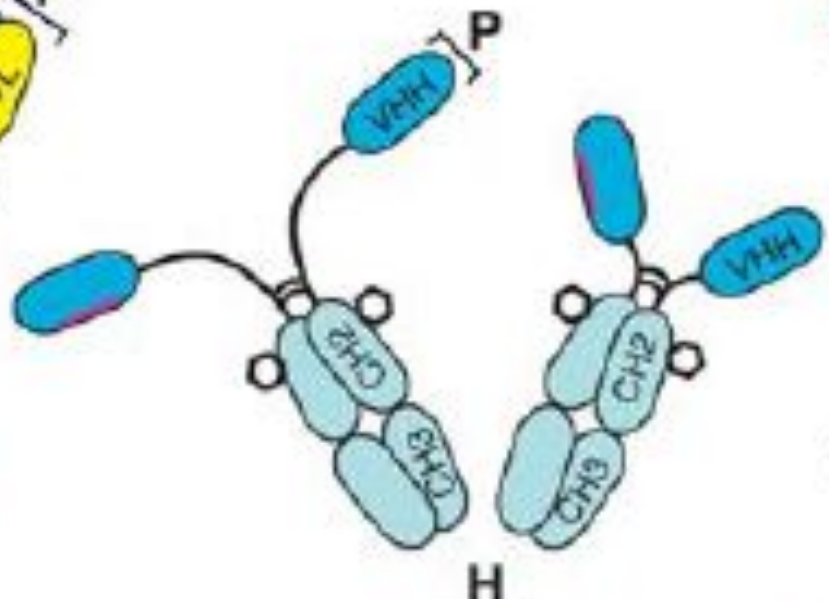


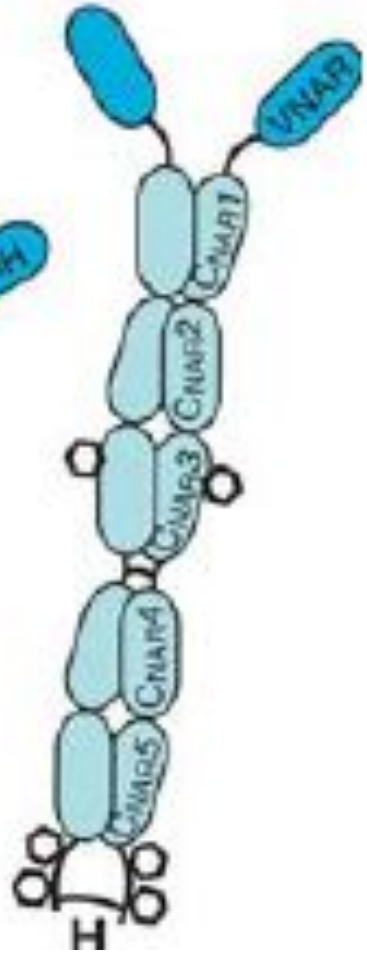
Одноцепочечные антитела – результат конвергентной эволюции

Обычное антитело

Антитела из тяжелых цепей





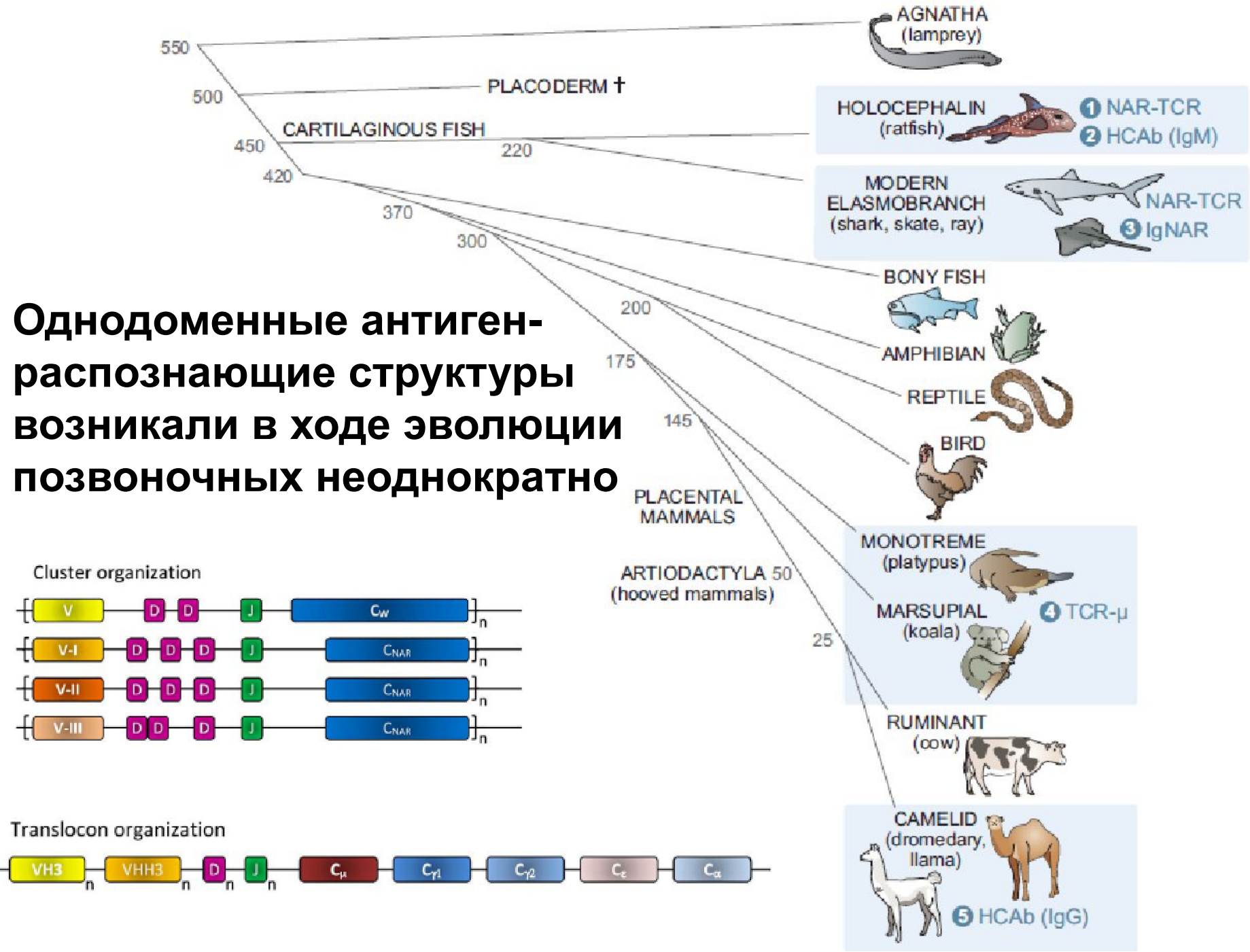


большинство Млекопитающих

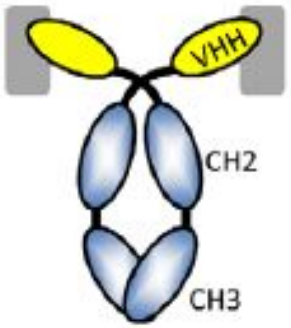
Мозоленогие

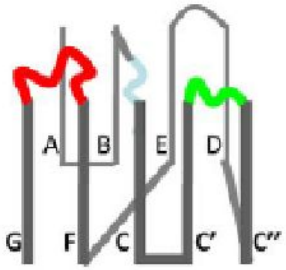
Акулы

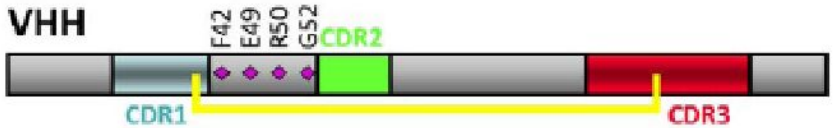
Однодоменные антиген-распознающие структуры возникали в ходе эволюции позвоночных неоднократно

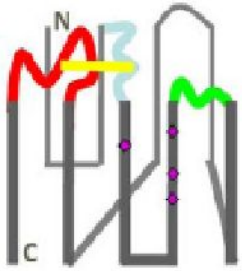


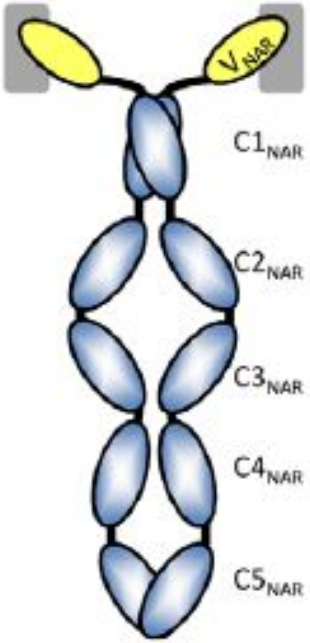
Топология однодоменных антител

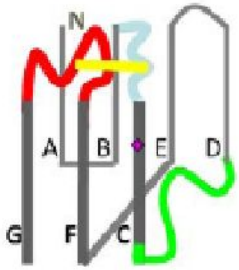


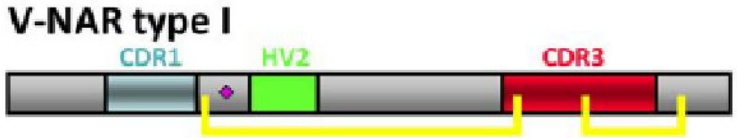


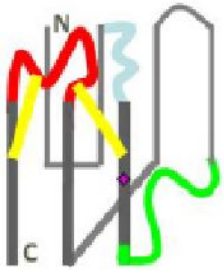




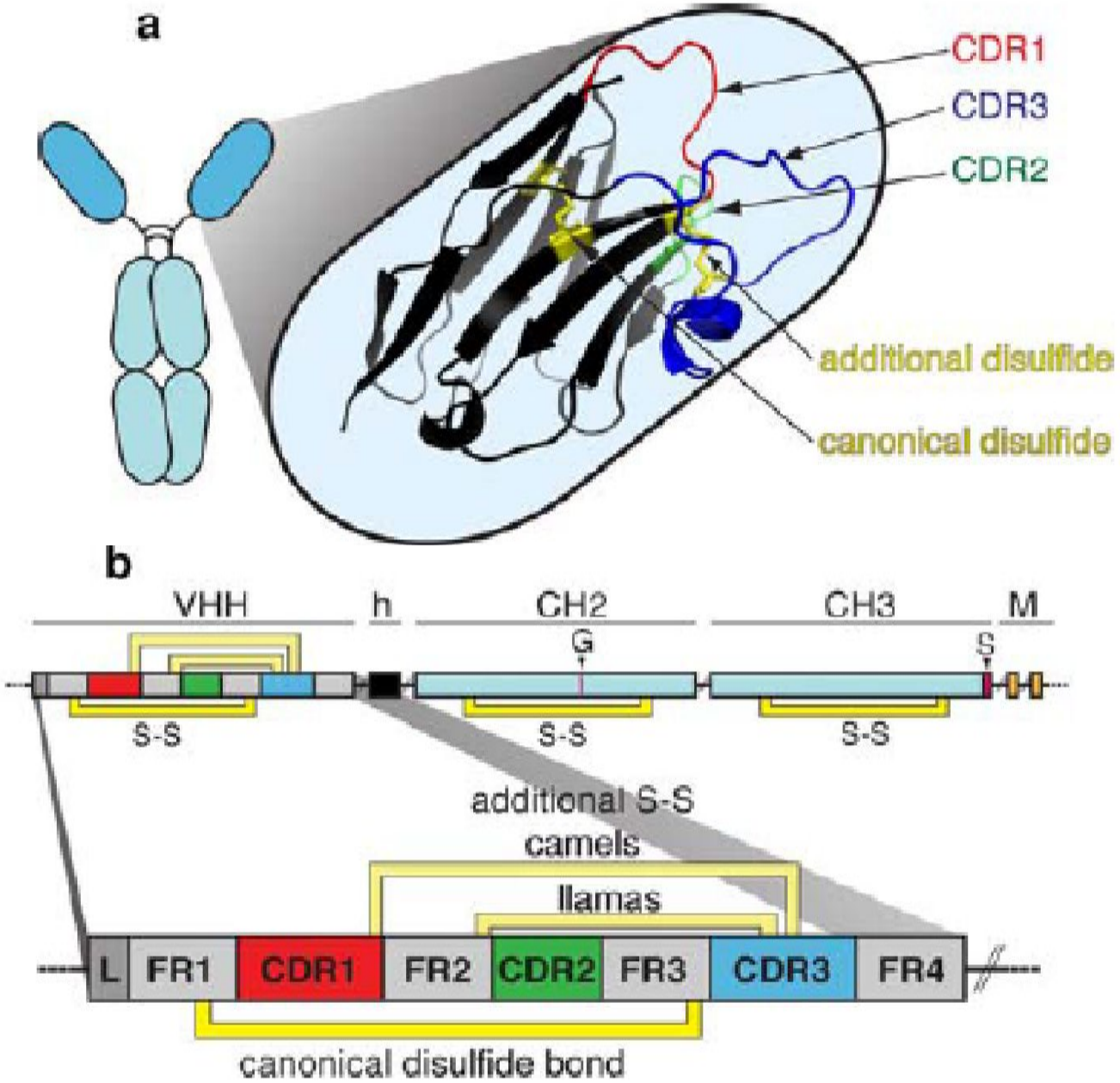




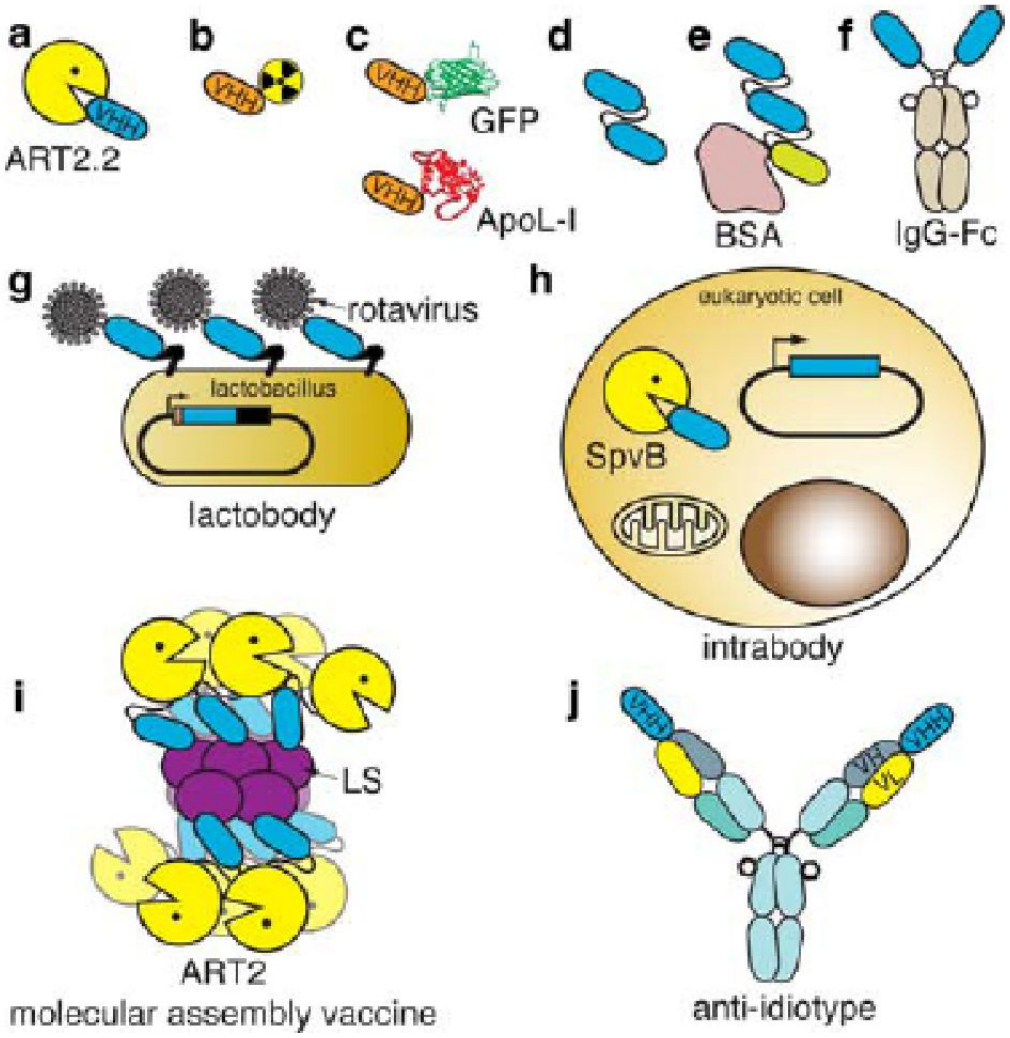




Структура однодоменных антител мозоленогих



Гипотетическое применение однодоменных антител



Моновалентные антитела (АТ)

a- ингибитор фермента с нежелательной активностью

b и c– носители радиоактивных, флуоресцентных и т.д. меток

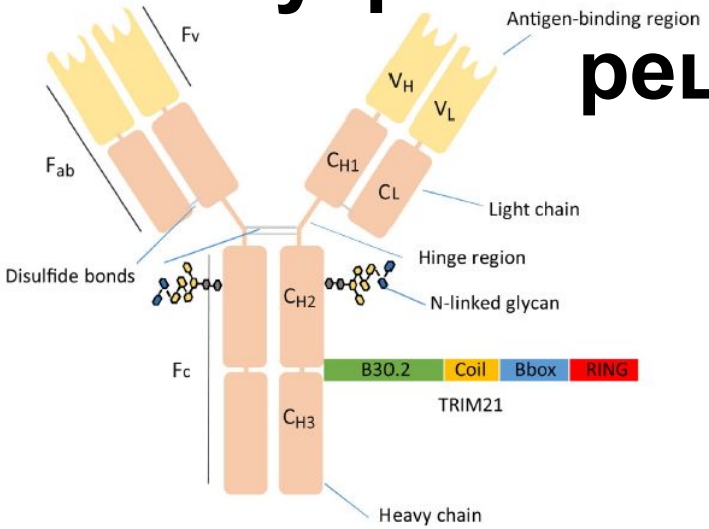
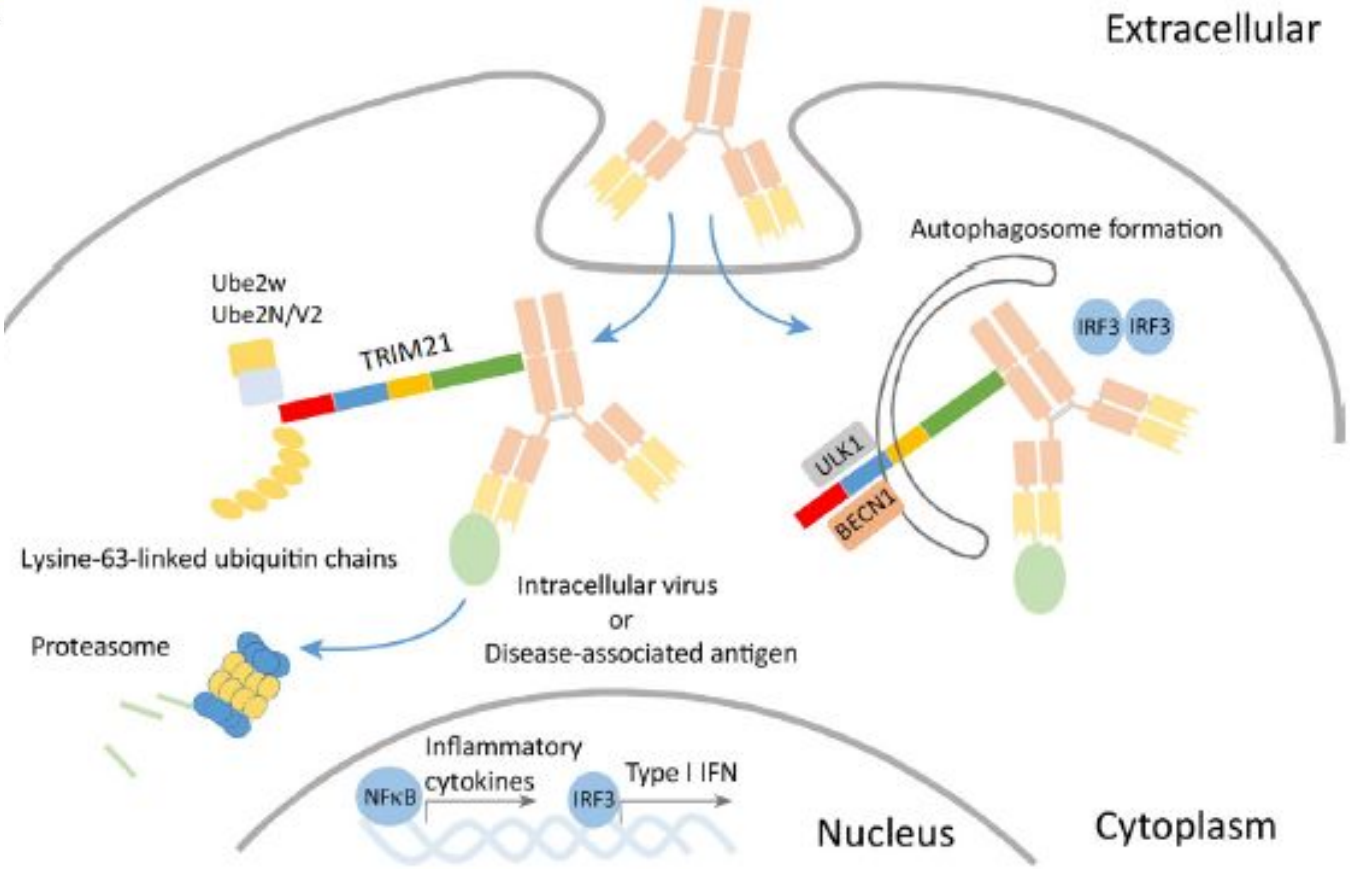
d- гомовалентное и e- гетеровалентное бивалентные АТ

f– рекомбинантное АТ с Fc-фрагментом человека

g– рекомбинатная лактобактерия, производящая АТ

h- генотерапия плазмидами, кодирующими АТ

i- вакцина- макромолекулярный комплекс из АТ и адъюванта

j- антиидиотипическое АТ

Внутриклеточный эффект антител и рецептор TRIM21