

RAG1的结构、功能和演化

G16: 郭潇潇 刘冰 晓娜 张剑寒

2017-01-14

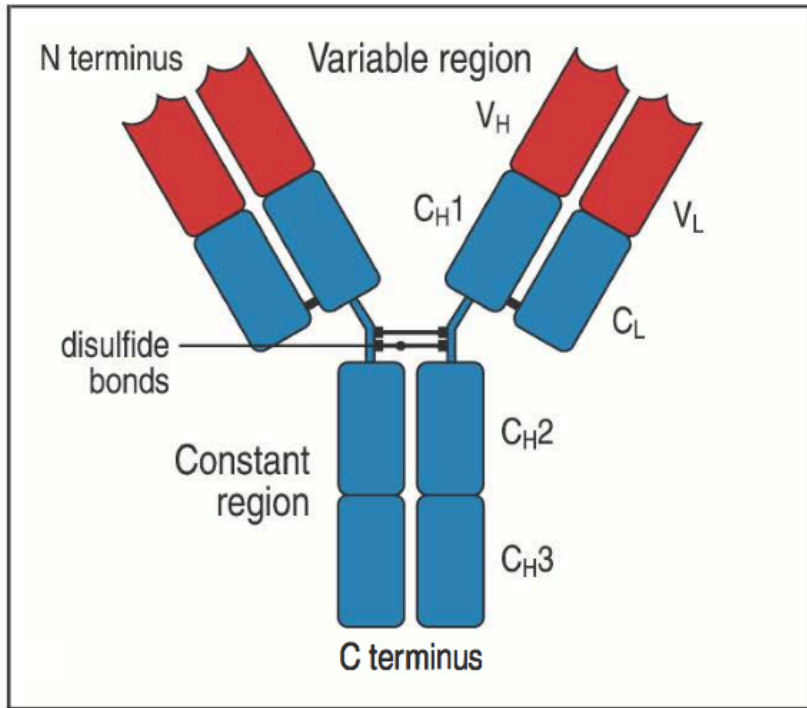
Outline

- 背景
- 结构与功能
- 演化分析
- 总结

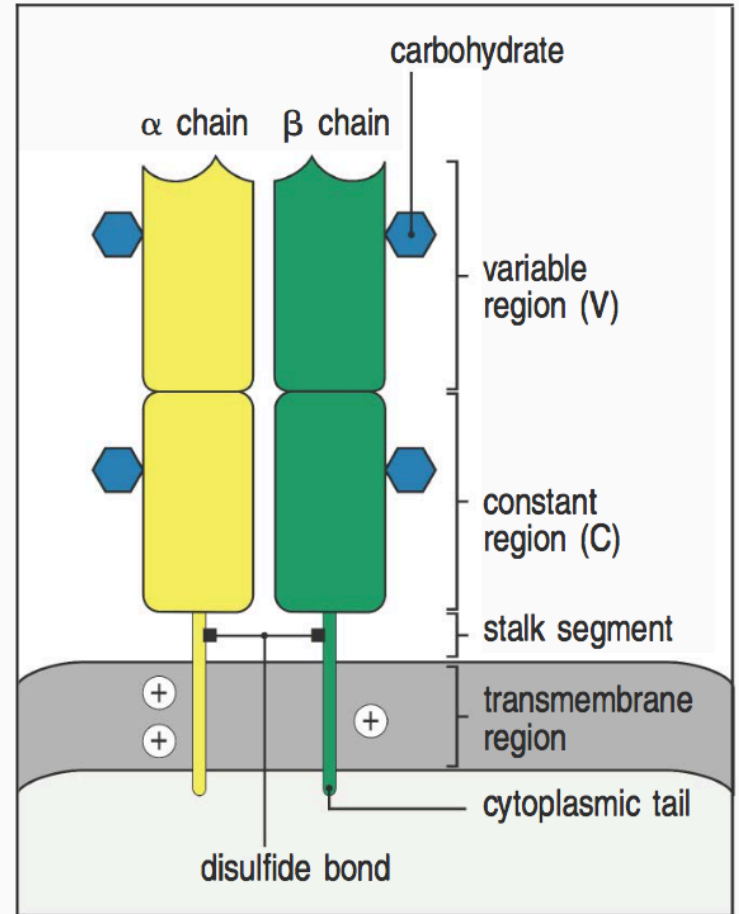
Outline

- 背景
 - RAG酶参与形成特异性免疫抗体受体多样性
- 结构与功能
- 演化分析
- 总结

特异性免疫系统受体和抗体的多样性

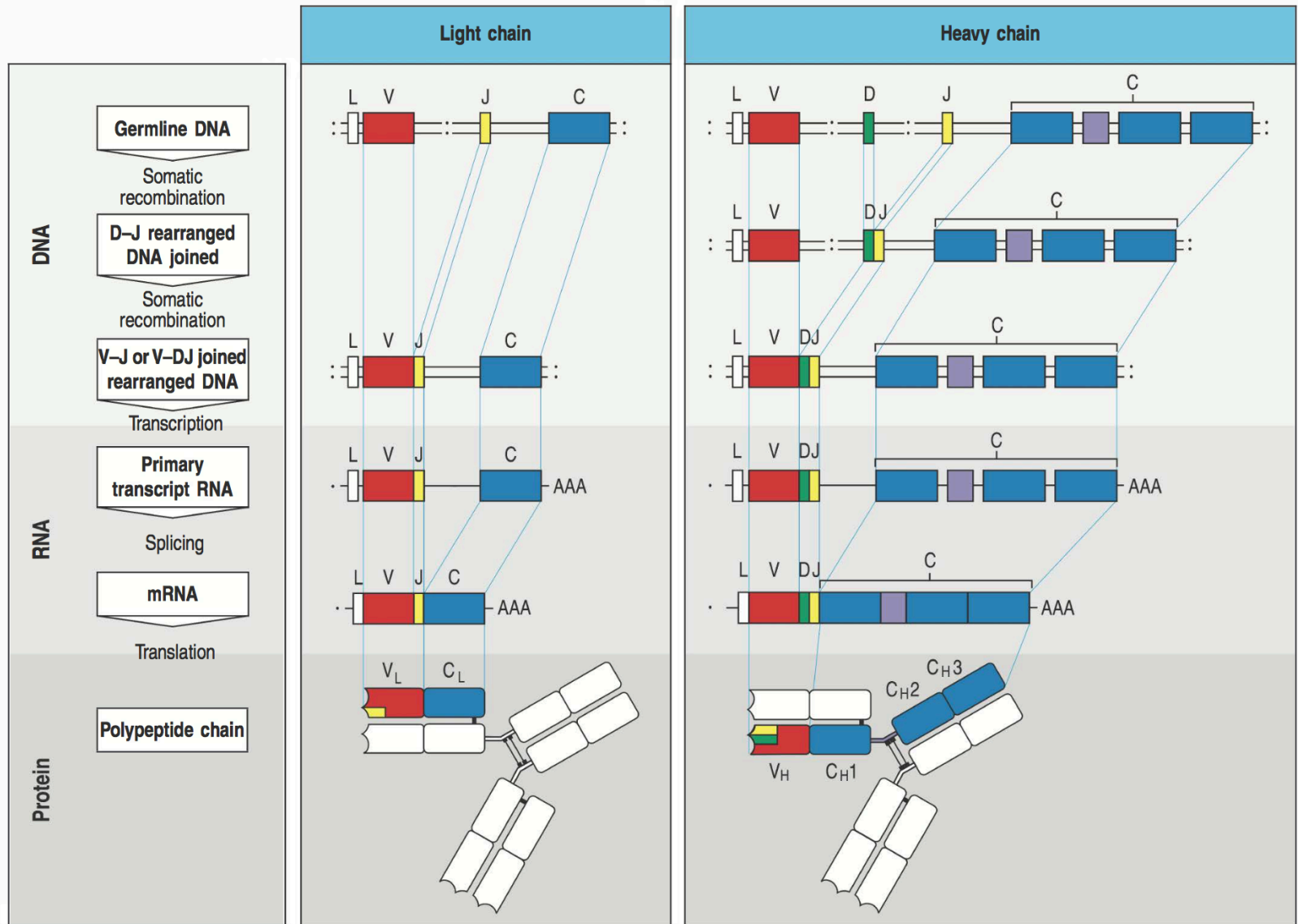


B细胞抗体



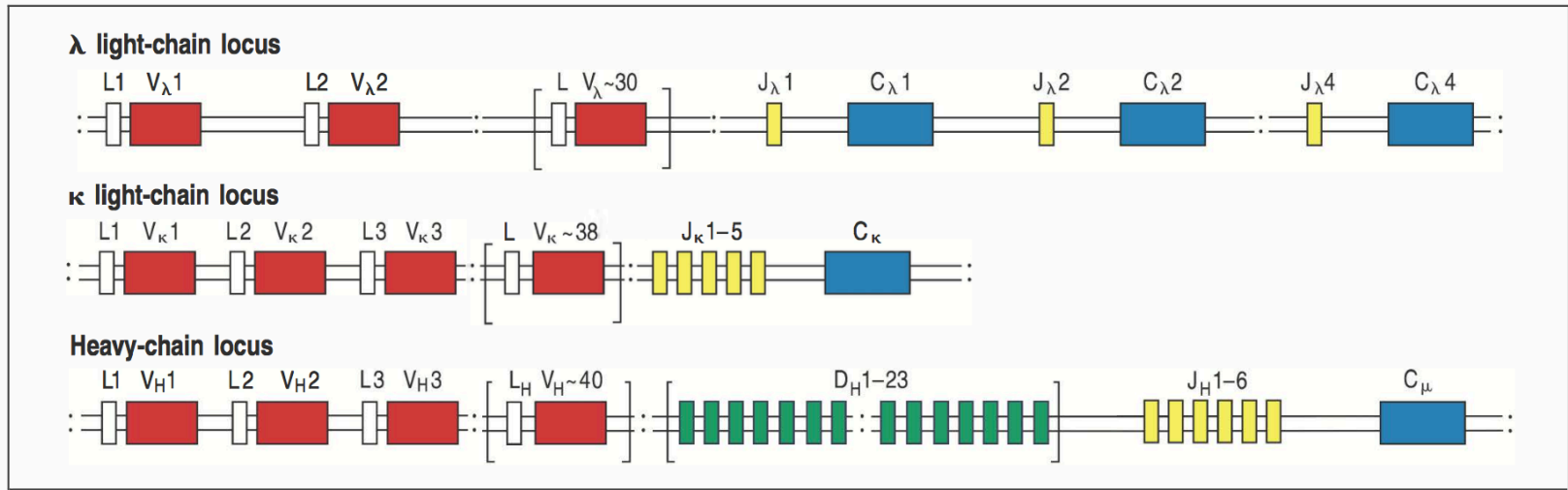
T细胞受体

B-cell Antibody的表达

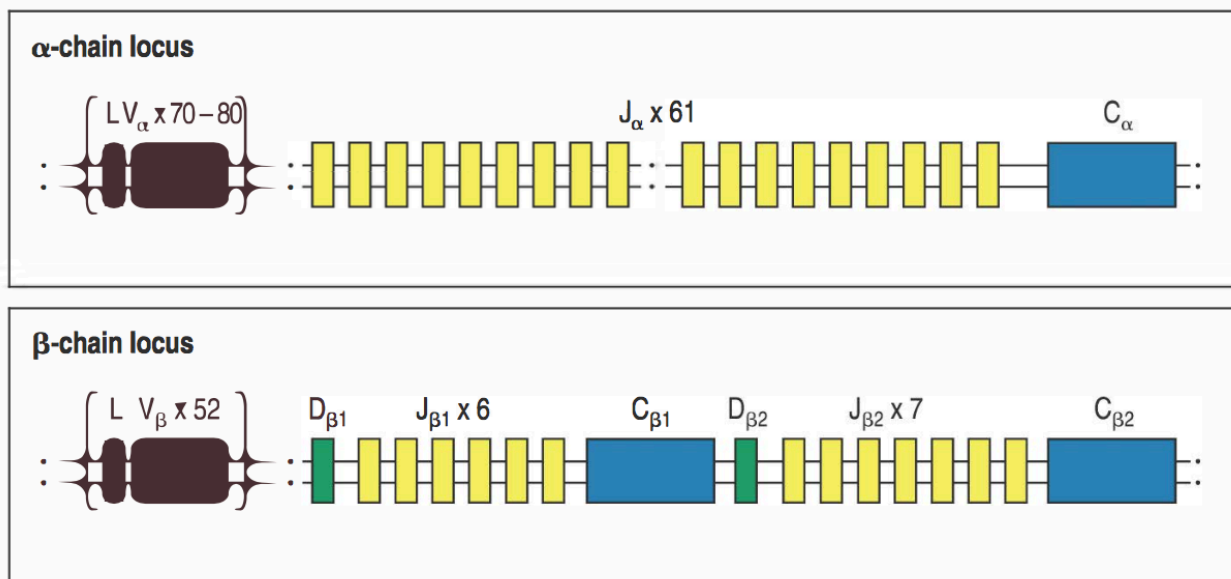


V-D-J的多样性

B细胞



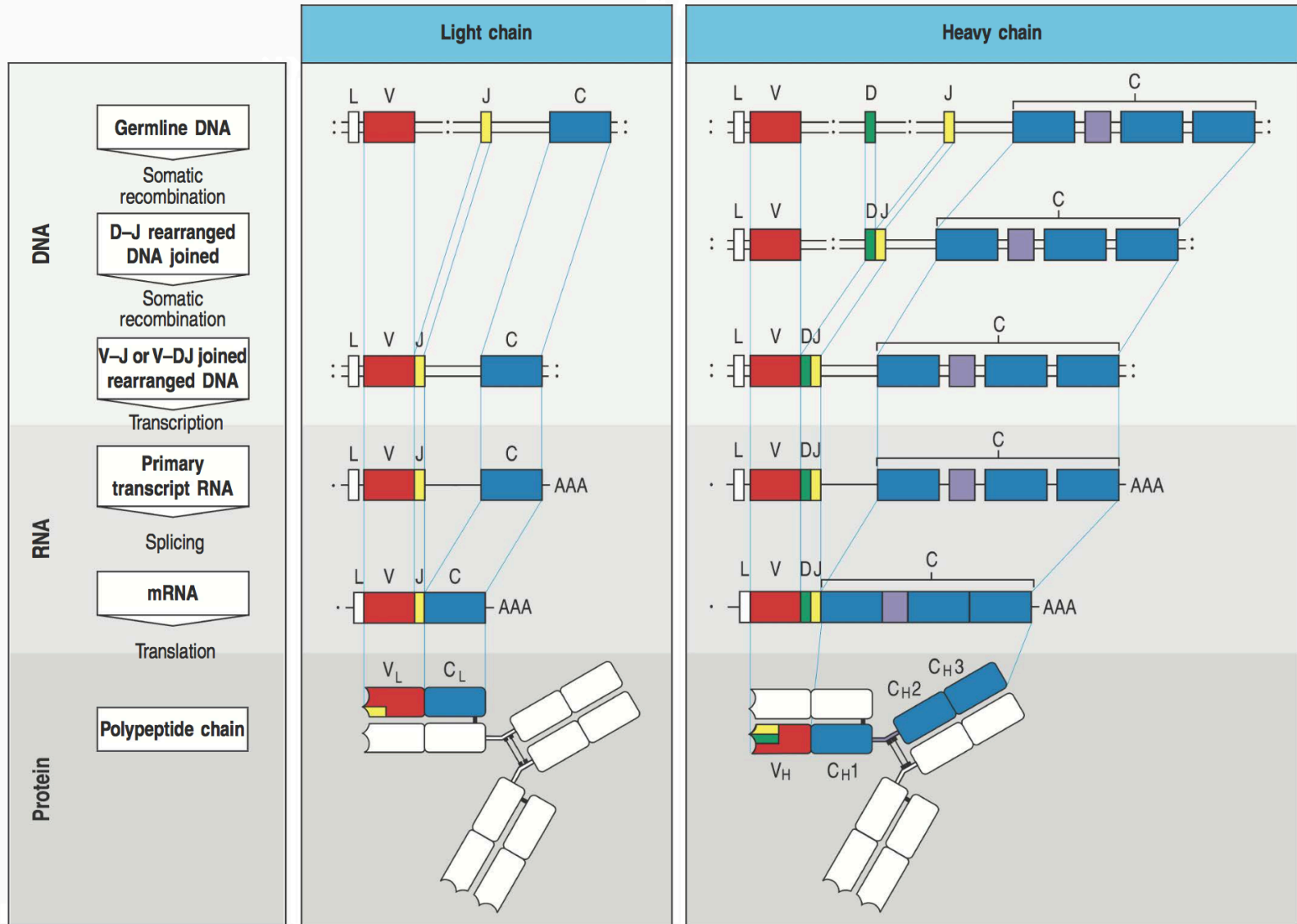
T细胞



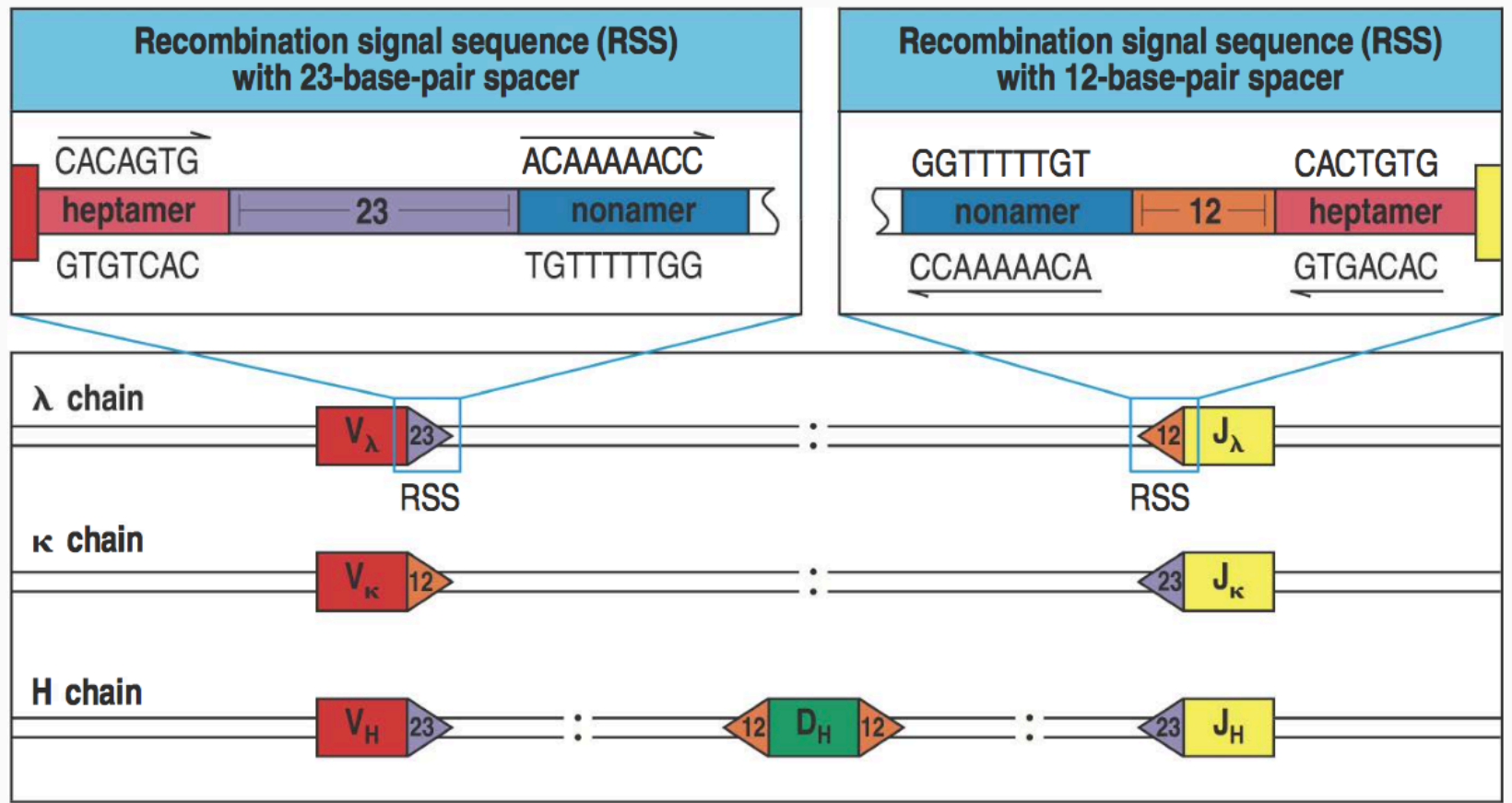
V-D-J区多样性导致抗体（受体）多样性

Element	Immunoglobulin		α : β T-cell receptors	
	H	κ + λ	β	α
Variable segments (V)	~40	~70	52	~70
Diversity segments (D)	23	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9 x 10 ⁶		5.8 x 10 ⁶	
Junctional diversity	~3 x 10 ⁷		~2 x 10 ¹¹	
Total diversity	~5 x 10 ¹³		~10 ¹⁸	

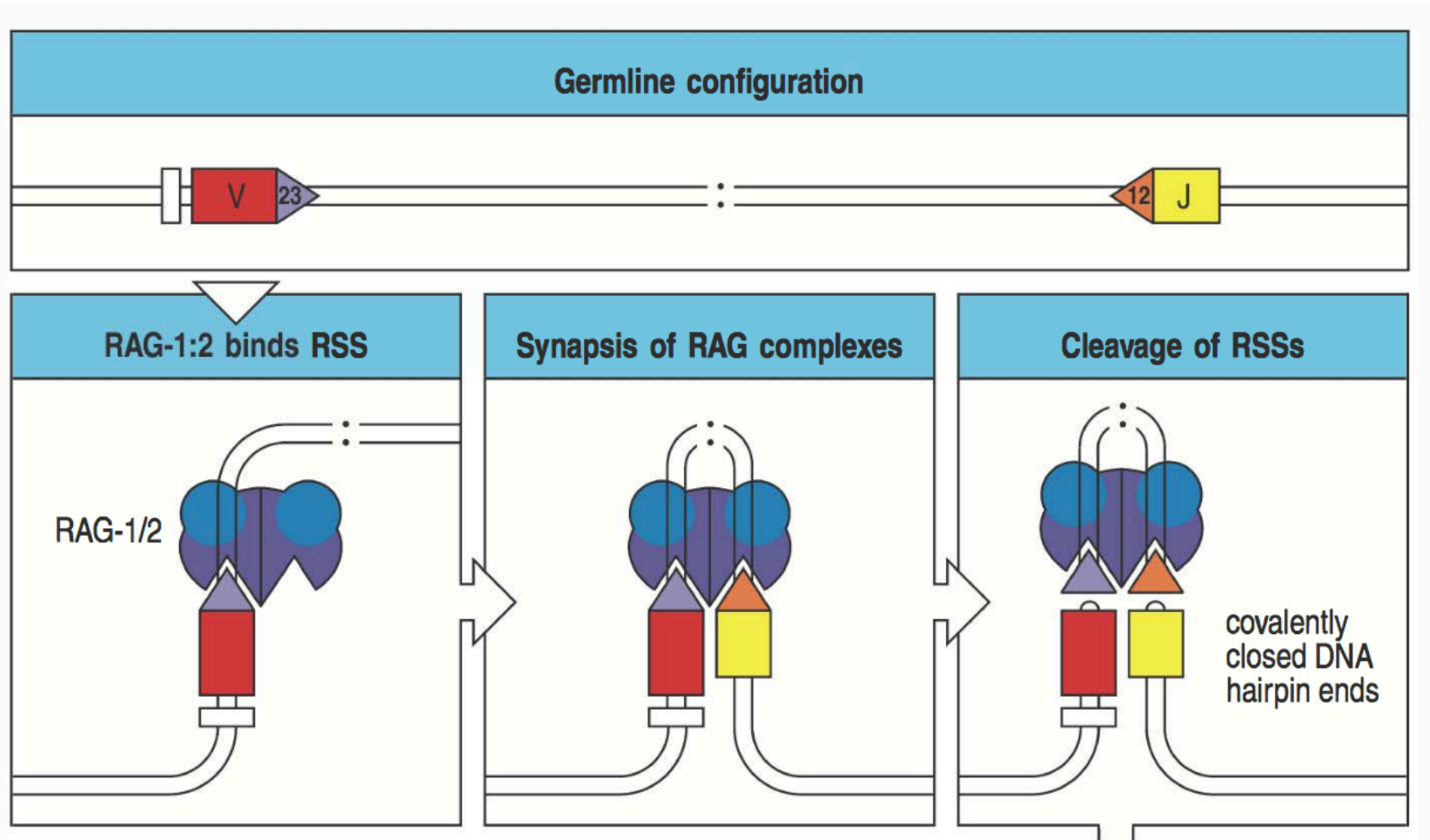
RAG酶参与的V-D-J区重组



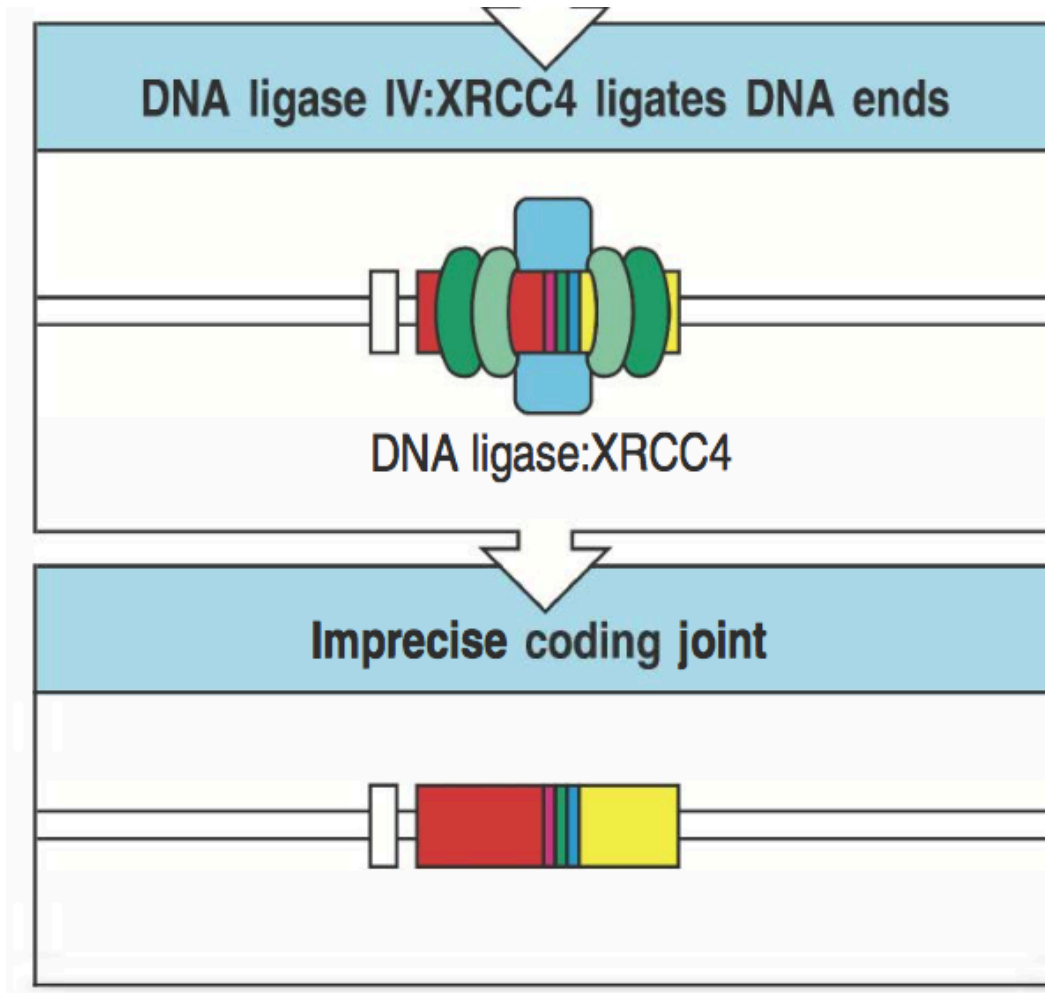
RAG酶识别RSS序列



RAG酶参与的V-D-J区重组



RAG酶参与的V-D-J区重组



Outline

- 背景

- RAG酶参与形成特异性免疫抗体受体多样性

- 结构与功能

- RAG1的序列、结构以及和功能的关系

- 演化分析

- 总结

Uniprot RAG1

Term
Protein name [DE] V(D)J recombination-ac

Term
AND Gene name [GN] RAG1

AND Reviewed Yes

Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/> P15919	RAG1_MOUSE	V(D)J recombination-activating prot...	Rag1	Mus musculus (Mouse)	1,040
<input type="checkbox"/> P15918	RAG1_HUMAN	V(D)J recombination-activating prot...	RAG1 RNF74	Homo sapiens (Human)	1,043
<input type="checkbox"/> Q867B5	RAG1_PIG	V(D)J recombination-activating prot...	RAG1	Sus scrofa (Pig)	1,043
<input type="checkbox"/> P34088	RAG1_RABIT	V(D)J recombination-activating prot...	RAG1	Oryctolagus cuniculus (Rabbit)	1,042
<input type="checkbox"/> O13033	RAG1_DANRE	V(D)J recombination-activating prot...	rag1	Danio rerio (Zebrafish) (Brachydanio rerio)	1,057
<input type="checkbox"/> Q91829	RAG1_XENLA	V(D)J recombination-activating prot...	rag1	Xenopus laevis (African clawed frog)	1,045
<input type="checkbox"/> P24271	RAG1_CHICK	V(D)J recombination-activating prot...	RAG1	Gallus gallus (Chicken)	1,041
<input type="checkbox"/> Q91187	RAG1_ONCMY	V(D)J recombination-activating prot...	rag1	Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)	1,073
<input type="checkbox"/> Q90523	RAG1_GINCI	V(D)J recombination-activating prot...	rag1	Ginglymostoma cirratum (Nurse shark) (Squalus cirratus)	106

Uniprot RAG1_HUMAN (1043)

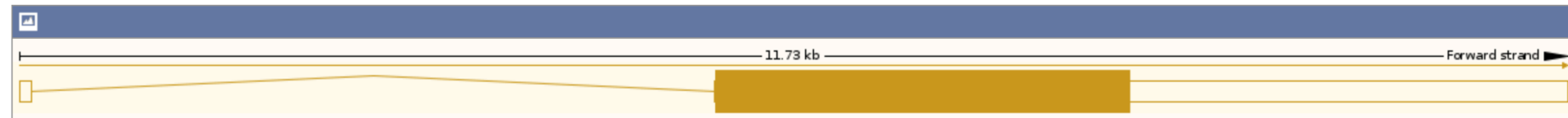
```
>sp|P15918|RAG1_HUMAN V(D)J recombination-activating protein 1
MAASFPPTLGLSSAPDEIQHPHIKFSEWKFKLFRVRSFEKTPEEAQKEKKDSFEGKPSLE
QSPAVLDKADGQKPVPTQPLLKAHPKFSKKFHDNEKARGKAIHQANLRHLCRICGNSFRA
DEHNRRYPVHGPVDGKTLGLLRKKKEKRATSWPDLIAKVFRIDVKADVDSIHPTEFCHNCW
SIMHRKFSSAPCEVYFPRNVTMEWHPHTPSCDICNTARRGLKRKSLQPNLQLSKKLKTVL
DQARQARQHKRRAQARISSKDVMKKIANCSKIHLSTKLLAVDFPEHFVKSISQCICEHIL
ADPVETNCKHVFCRVCILRCLKVMGSYCPSCRYPCFPTDLESPVKSFLSVLNSLMVKCPA
KECNEEVSLEKYNHHISSHKEKEIFVHINKGGRPRQHLLSLTRRAQKHRLRELKLQVKA
FADKEEGGDVKSVCMTLFLALRARNEHRQADELEAIMQGKGSGLQPAVCLAIRVNTFLS
CSQYHKMYRTVKAITGRQIFQPLHALRNAEKVLLPGYHHFEWQPPLKNVSSSTDVGIIDG
LSGLSSSVDDYPVDTIAKRFRYDSALVSALMDMEEDILEGMRSQDLDDYLNQPFTVVVKE
SCDGMGDVSEKHGSGPVVPEKAVRFSFTIMKITIAHSSQNVKVFEEAKPNSELCKPLCL
MLADESDHETLTAILSPLIAERAMKSSELMLELGGILRTFKFIFRGTGYDEKLVREVEG
LEASGSVYICTLCDATRLEASQNLVFHSITRSHAENLERYEVWRSNPYHESVEELRDRVK
GVSAPKFIETVPSIDALHCDIGNAAEFYKIFQLEIGEVYKNPNASKEERKRWQATLDKHL
RKKMNLKPIMRMNGNFARKLMTKETVDAVCELIPSEERHEALRELMPLYLKMKPVWRSSC
PAKECPESLCQYSFNSQRFAELLSTKFKYRYEGKITNYFHKTLAHVPEIIERDGSIGAWA
SEGNESGNKLFRRFRKMNARQSKCYEMEDVLKHHWLYTSKYLQKFMNAHNALKTSGFTMN
PQASLGDPLGIEDSLESQDSMEF
```


Ensemble Transcript RAG1-002

Transcript: RAG1-002 ENST00000299440.5

Description	recombination activating 1 [Source:HGNC Symbol;Acc: HGNC:9831]
Synonyms	RNF74, MGC43321, RAG-1
Location	Chromosome 11: 36,568,025-36,579,756 forward strand.
About this transcript	This transcript has 2 exons , is annotated with 18 domains and features , is associated with 1036 variations and maps to 21 oligo probes .
Gene	This transcript is a product of gene ENSG00000166349 Show transcript table

Summary



Statistics	Exons: 2, Coding exons: 1, Transcript length: 6,564 bps, Translation length: 1,043 residues
CCDS	This transcript is a member of the Human CCDS set: CCDS7902
Uniprot	This transcript corresponds to the following Uniprot identifiers: P15918
Transcript Support Level (TSL)	TSL:1
Version	ENST00000299440.5
Type	Known protein coding
Annotation Method	Transcript where the Ensembl genebuild transcript and the Vega manual annotation have the same sequence, for every base pair. See article .
Alternative transcripts	This transcript corresponds to the following database identifiers: Havana transcript: OTTHUMT00000389535
GENCODE basic gene	This transcript is a member of the Gencode basic gene set.

RAG1结构域

Family & Domainsⁱ

Domainⁱ

The RING-type zinc finger mediates the E3 ubiquitin-protein ligase activity. [1 Publication](#)

The NBD (nonamer binding) DNA-binding domain mediates the specific binding to the nonamer RSS motif by forming a tightly interwoven homodimer that binds and synapses 2 nonamer elements, with each NBD making contact with both DNA molecules. Each RSS is composed of well-conserved heptamer (consensus 5'-CACAGTG-3') and nonamer (consensus 5'-ACAAAAACC-3') sequences separated by a spacer of either 12 bp or 23 bp. [PROSITE-ProRule annotation](#) [1 Publication](#)

Sequence similaritiesⁱ

Belongs to the [RAG1 family](#). [PROSITE-ProRule annotation](#)

Contains 1 [NBD \(nonamer binding\) DNA-binding domain](#). [PROSITE-ProRule annotation](#)

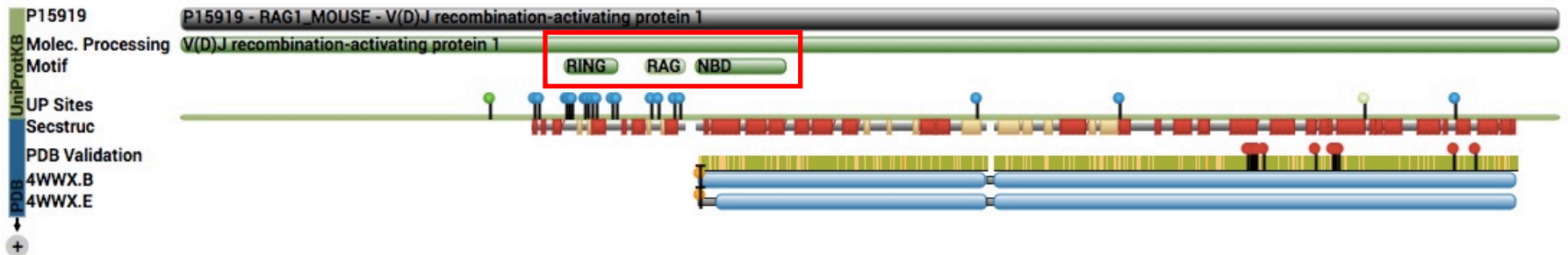
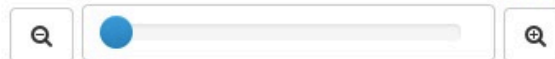
Contains 1 [RAG1-type zinc finger](#). [PROSITE-ProRule annotation](#) [Curated](#)

Contains 1 [RING-type zinc finger](#). [PROSITE-ProRule annotation](#)

Protein Feature View - UniProtKB AC: [P15919](#) [UniProt](#)

[Full Protein Feature View for P15919](#)

Find similar proteins by: [Sequence](#) | [Structure](#)



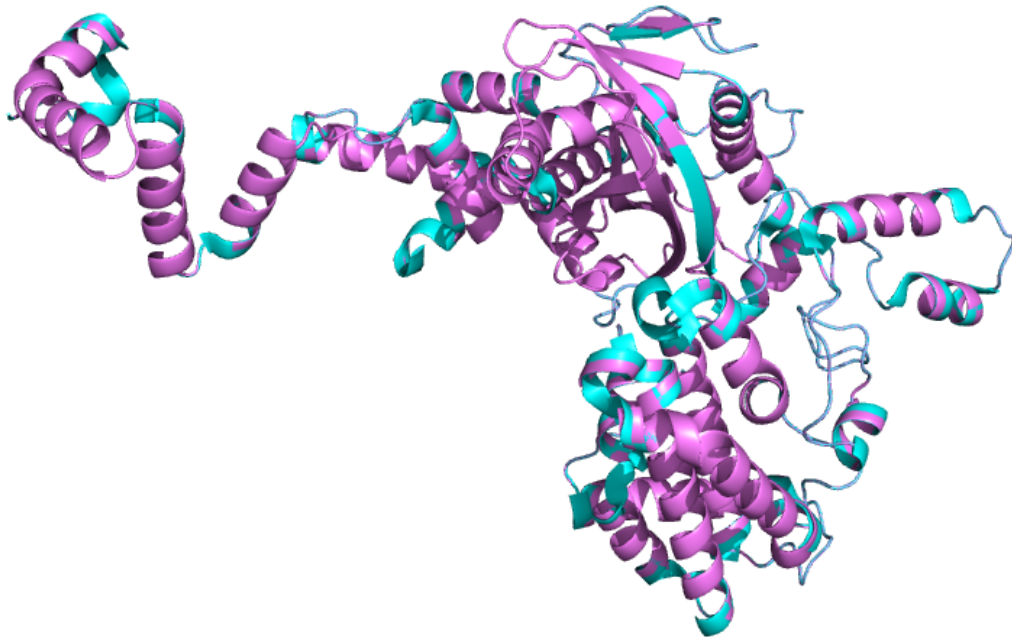
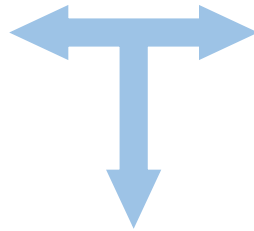
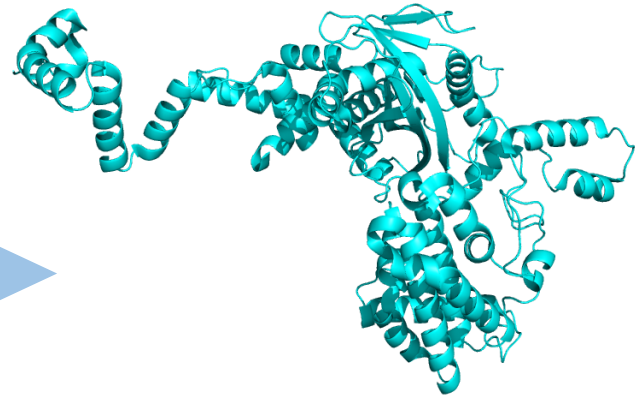
RAG1_HUMAN同源建模

Phyre2



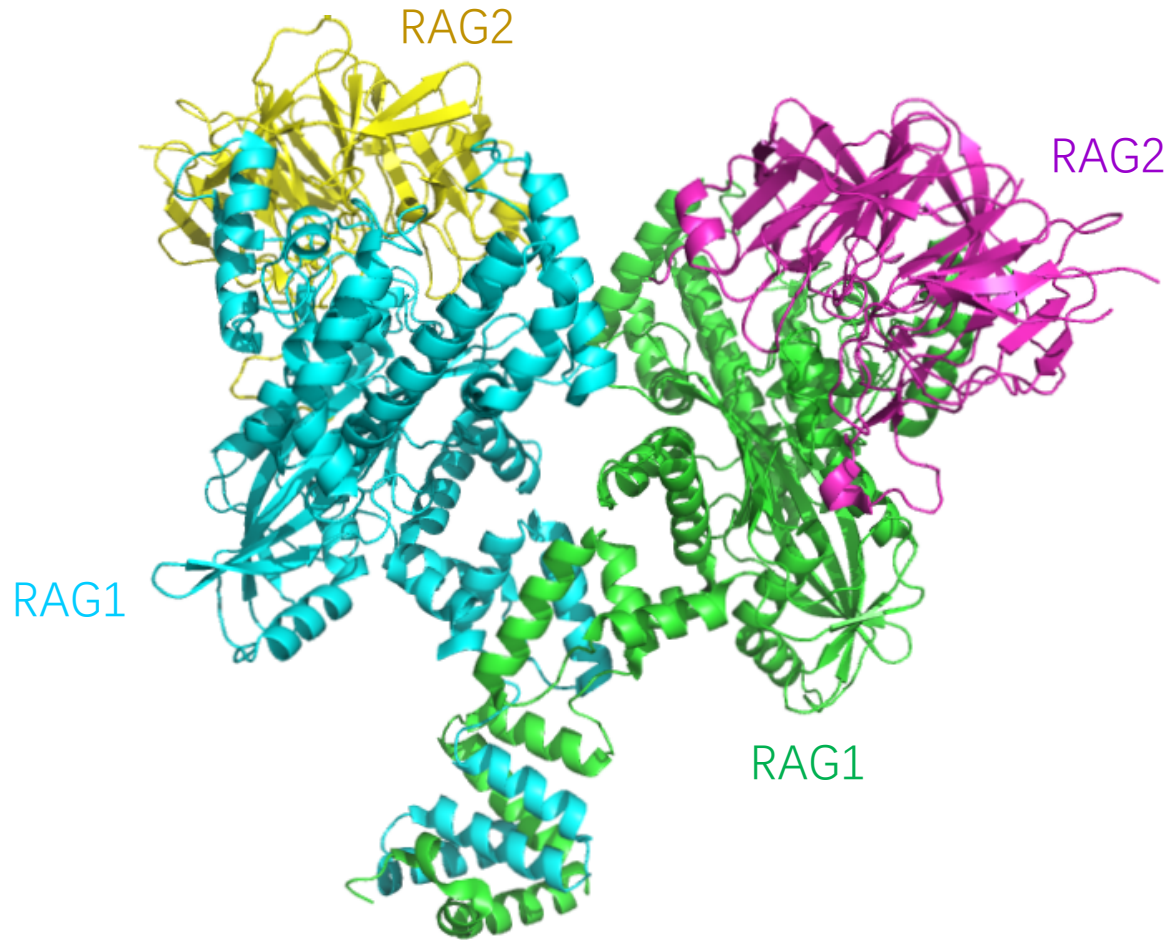
Phyre2

4wwx



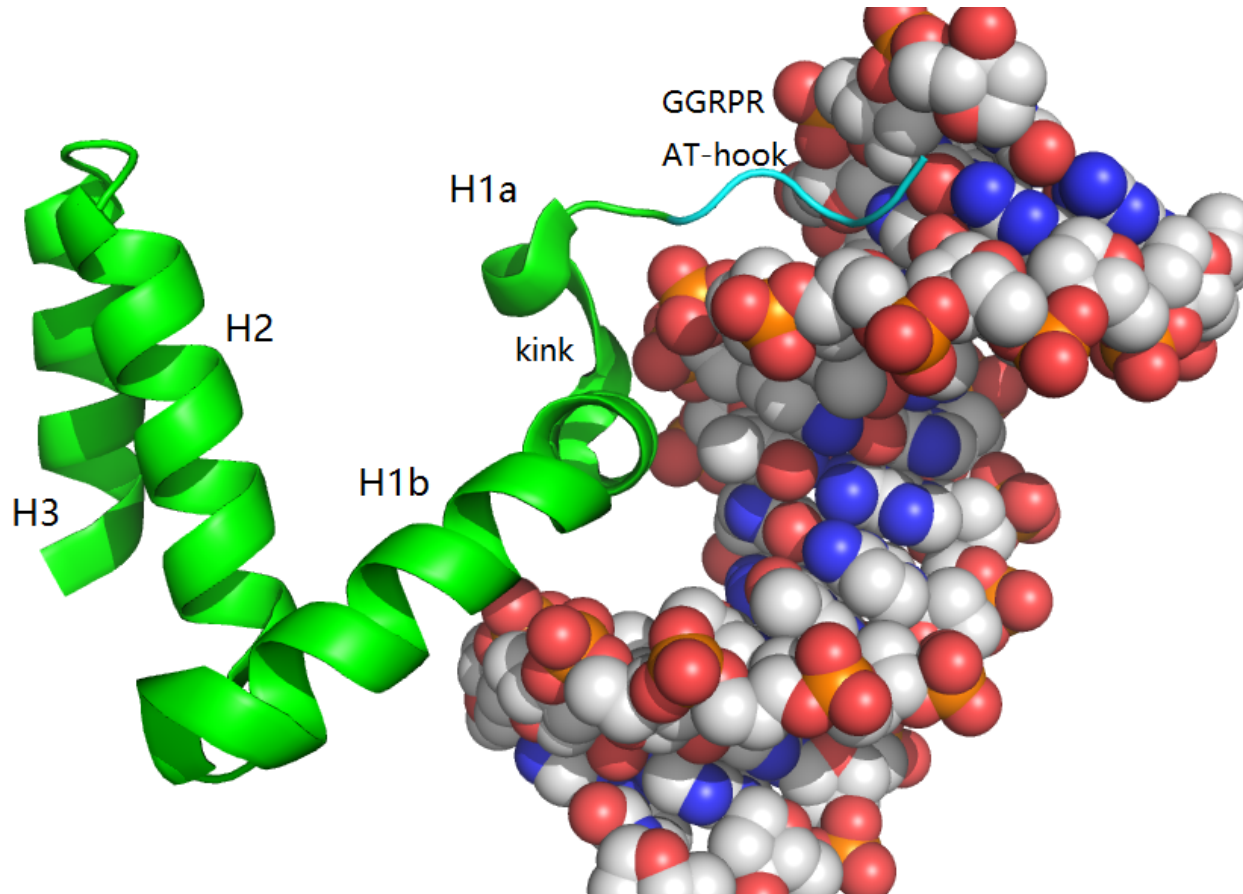
RAG1/2复合体结构

4wwx

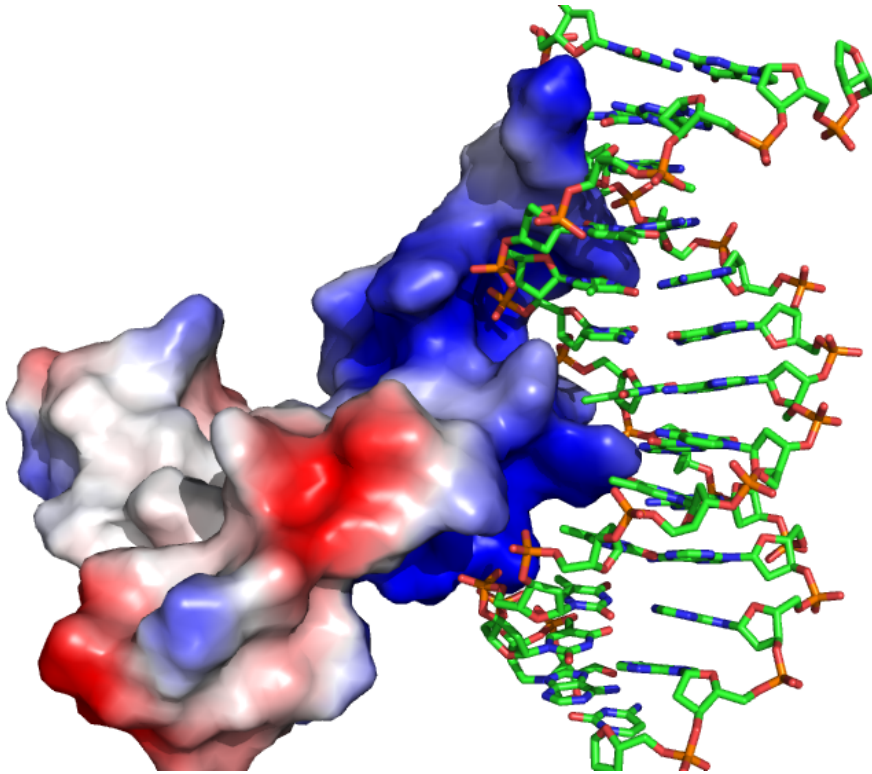


RAG1 NBD区与DNA的相互作用

3gnb

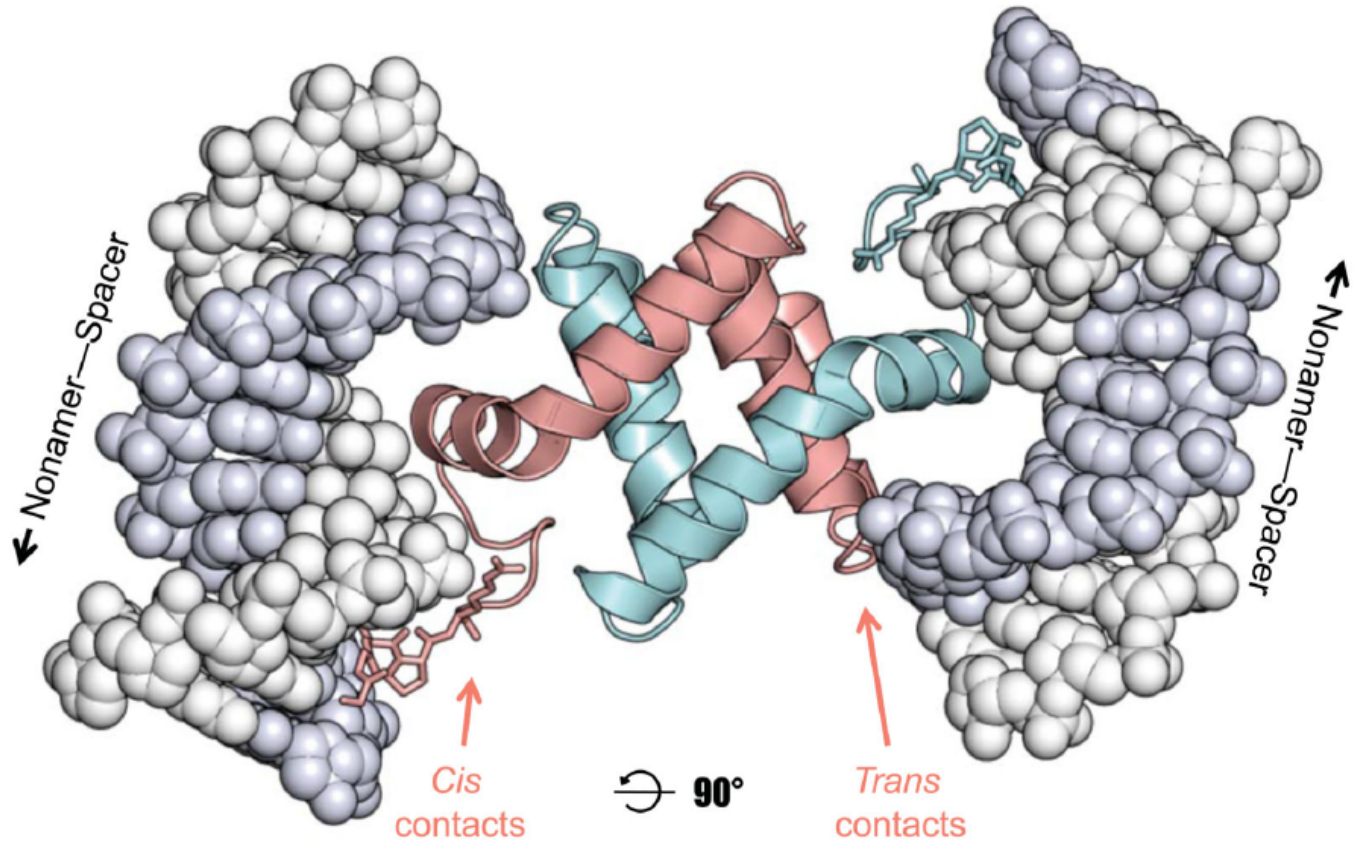


RAG1 NBD区与DNA的相互作用



Charge	Residue	Proportion
Positively charged (35%)	H	10%
	K	5%
	R	25%
Non-Charged (65%)	A	5%
	G	10%
	L	20%
	P	5%
	Q	10%
	S	5%
	T	5%

RAG1-NBD的二聚



Outline

- 背景

- RAG酶参与形成特异性免疫抗体受体多样性

- 结构与功能

- RAG1的序列、结构以及和功能的关系

- 演化分析

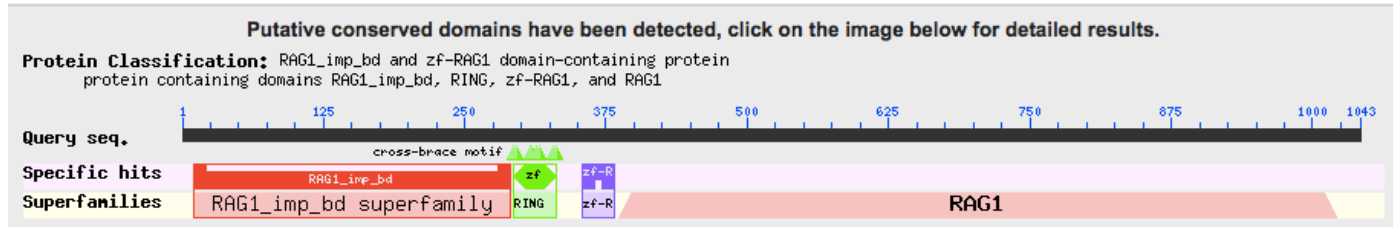
- 从分子进化的角度研究RAG1的演化

- 总结

NCBI Blastp

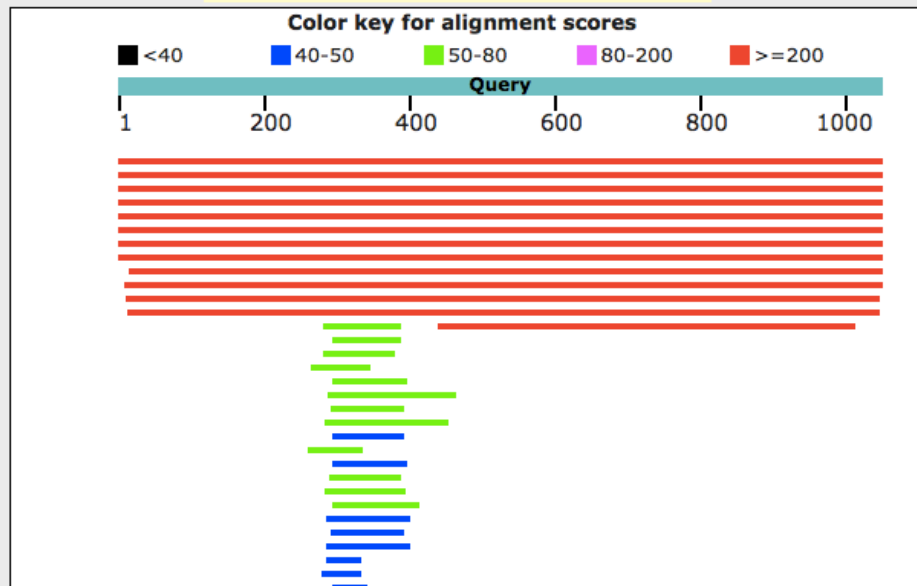
检索序列: RAG1_HUMAN

数据库: refseq_protein



Distribution of the top 100 Blast Hits on 100 subject sequences

Mouse over to see the title, click to show alignments



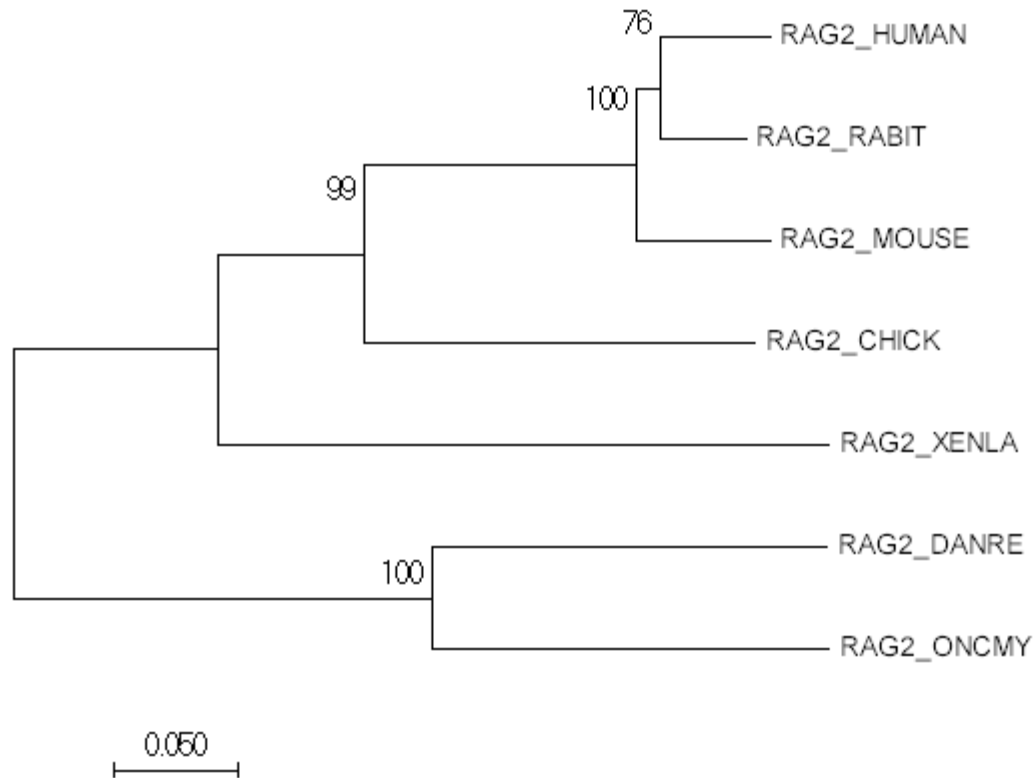
NCBI Blastp

检索序列: RAG1_HUMAN

数据库: refseq_protein

<input type="checkbox"/> V(D)J recombination-activating protein 1 [Homo sapiens]	2174	2174	100%	0.0	99%	NP_000439.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Macaca mulatta]	2137	2137	100%	0.0	98%	NP_001253701.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Equus caballus]	2033	2033	100%	0.0	93%	NP_001243830.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Oryctolagus cuniculus]	2002	2002	100%	0.0	93%	NP_001164611.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Rattus norvegicus]	1966	1966	100%	0.0	90%	NP_445920.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Mus musculus]	1966	1966	100%	0.0	90%	NP_033045.2
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Sus scrofa]	1964	1964	100%	0.0	92%	NP_001116656.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Ornithorhynchus anatinus]	1785	1785	100%	0.0	82%	NP_001229683.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Gallus gallus]	1663	1663	98%	0.0	76%	NP_001026359.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Xenopus laevis]	1558	1558	99%	0.0	71%	NP_001165554.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Danio rerio]	1249	1249	98%	0.0	59%	NP_571464.1
<input type="checkbox"/> V(D)J recombination-activating protein 1 [Oncorhynchus mykiss]	1239	1239	98%	0.0	58%	NP_001118209.1
<input type="checkbox"/> recombination activating protein 1-like [Strongylocentrotus purpuratus]	208	208	54%	7e-52	29%	NP_001028179.1
<input type="checkbox"/> E3 ubiquitin-protein ligase RNF138 [Xenopus tropicalis]	57.4	57.4	10%	1e-05	31%	NP_001016429.2
<input type="checkbox"/> E3 ubiquitin-protein ligase RNF138 [Xenopus laevis]	54.7	54.7	9%	9e-05	33%	NP_001089974.1
<input type="checkbox"/> zinc finger protein [Ciona intestinalis]	56.2	56.2	9%	1e-04	35%	NP_001071979.1
<input type="checkbox"/> ATP-dependent helicase [Schizosaccharomyces pombe 972h-]	55.8	55.8	7%	2e-04	31%	NP_587701.1
<input type="checkbox"/> zinc finger protein 313 [Oncorhynchus mykiss]	52.4	52.4	9%	6e-04	30%	NP_001117990.1

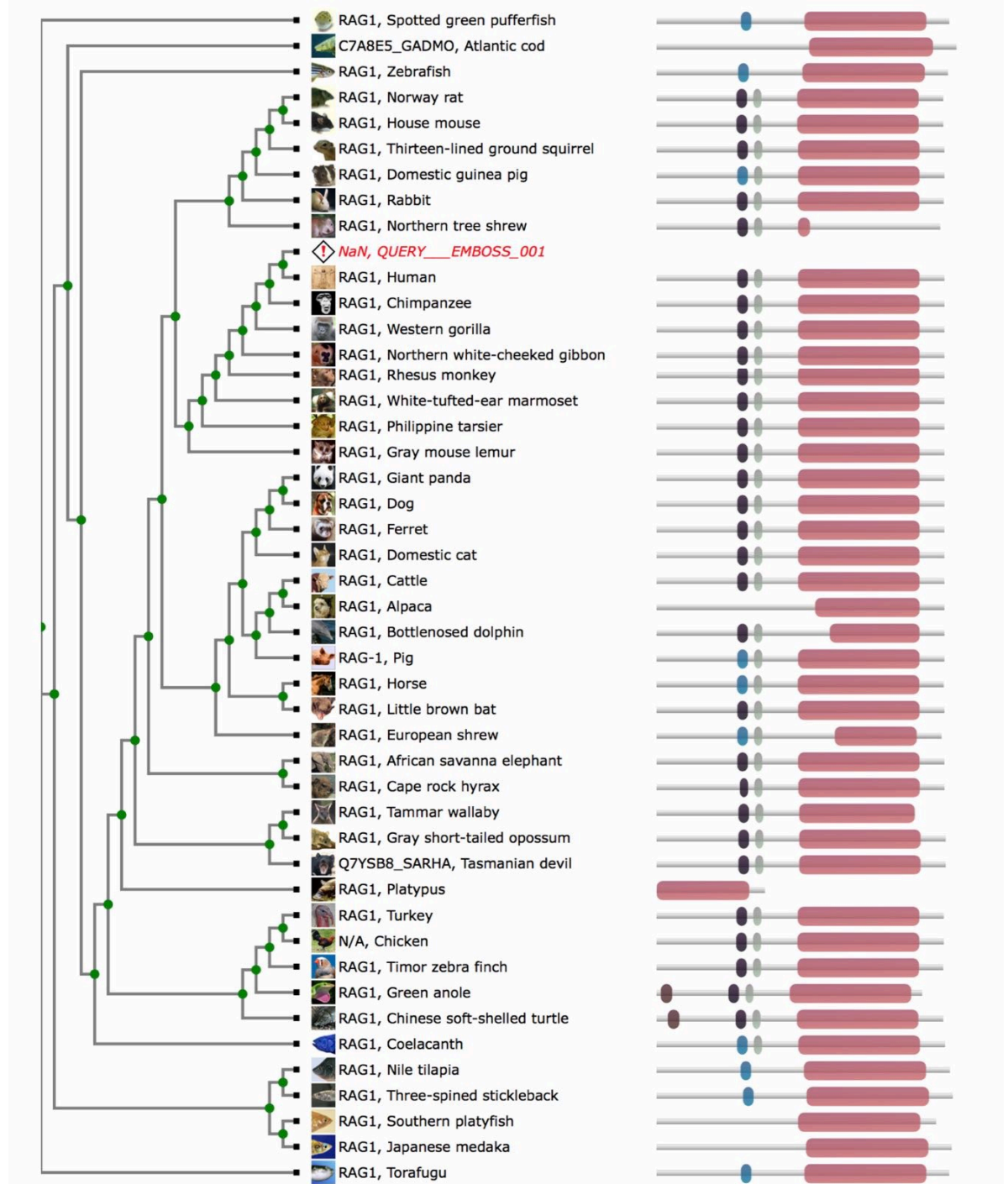
MEGA RAG1 Phylogeny



TreeFam

RAG1 Phylogeny

RAG1最早出现
于软骨鱼中



Uniprot NBD domain

[BLAST](#)
[Align](#)
[Download](#)
[Add to basket](#)
[Columns](#)

1 to 8 of 8 Show 25

Suggest adding column(s): Domains

Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q91187	RAG1_ONCMY	V(D)J recombination-activating prot...	rag1	Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)	1,073
<input type="checkbox"/> P34088	RAG1_RABIT	V(D)J recombination-activating prot...	RAG1	Oryctolagus cuniculus (Rabbit)	1,042
<input type="checkbox"/> O13033	RAG1_DANRE	V(D)J recombination-activating prot...	rag1	Danio rerio (Zebrafish) (Brachydanio rerio)	1,057
<input type="checkbox"/> Q91829	RAG1_XENLA	V(D)J recombination-activating prot...	rag1	Xenopus laevis (African clawed frog)	1,045
<input type="checkbox"/> P15919	RAG1_MOUSE	V(D)J recombination-activating prot...	Rag1	Mus musculus (Mouse)	1,040
<input type="checkbox"/> P24271	RAG1_CHICK	V(D)J recombination-activating prot...	RAG1	Gallus gallus (Chicken)	1,041
<input type="checkbox"/> P15918	RAG1_HUMAN	V(D)J recombination-activating prot...	RAG1 RNF74	Homo sapiens (Human)	1,043
<input type="checkbox"/> Q867B5	RAG1_PIG	V(D)J recombination-activating prot...	RAG1	Sus scrofa (Pig)	1,043

RAG1-type zinc finger

[BLAST](#)
[Align](#)
[Download](#)
[Add to basket](#)
[Columns](#)

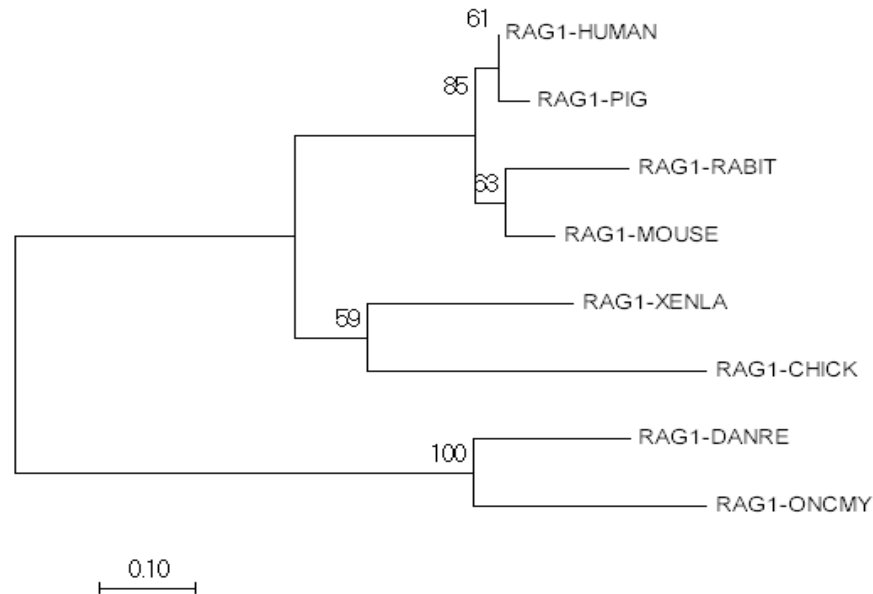
1 to 8 of 8 Show 25

Suggest adding column(s): Domains

Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/> Q91187	RAG1_ONCMY	V(D)J recombination-activating prot...	rag1	Oncorhynchus mykiss (Rainbow trout) (Salmo gairdneri)	1,073
<input type="checkbox"/> P34088	RAG1_RABIT	V(D)J recombination-activating prot...	RAG1	Oryctolagus cuniculus (Rabbit)	1,042
<input type="checkbox"/> O13033	RAG1_DANRE	V(D)J recombination-activating prot...	rag1	Danio rerio (Zebrafish) (Brachydanio rerio)	1,057
<input type="checkbox"/> Q91829	RAG1_XENLA	V(D)J recombination-activating prot...	rag1	Xenopus laevis (African clawed frog)	1,045
<input type="checkbox"/> P15919	RAG1_MOUSE	V(D)J recombination-activating prot...	Rag1	Mus musculus (Mouse)	1,040
<input type="checkbox"/> P24271	RAG1_CHICK	V(D)J recombination-activating prot...	RAG1	Gallus gallus (Chicken)	1,041
<input type="checkbox"/> P15918	RAG1_HUMAN	V(D)J recombination-activating prot...	RAG1 RNF74	Homo sapiens (Human)	1,043
<input type="checkbox"/> Q867B5	RAG1_PIG	V(D)J recombination-activating prot...	RAG1	Sus scrofa (Pig)	1,043

MEGA RAG1-ZF ALIGNMENT & PHYLOGENY

Species/Abbrev	Group Name	*				*					*									*			*								
1. RAG1-MOUSE		L	M	V	K	C	P	A	Q	D	C	N	E	E	V	S	L	E	K	Y	N	H	H	V	S	S	H	K	E	S	K
2. RAG1-HUMAN		L	M	V	K	C	P	A	K	E	C	N	E	E	V	S	L	E	K	Y	N	H	H	I	S	S	H	K	E	S	K
3. RAG1-RABBIT		L	I	V	K	C	S	A	P	E	C	N	E	E	V	S	L	E	K	Y	N	H	H	V	S	S	H	K	E	S	R
4. RAG1-PIG		L	M	V	K	C	P	A	K	E	C	N	E	E	I	S	L	E	K	Y	N	H	H	I	S	S	H	K	E	S	K
5. RAG1-DANRE		L	P	L	L	C	P	S	E	E	C	S	D	W	V	R	L	D	S	F	R	E	H	C	L	N	H	Y	R	E	K
6. RAG1-XENLA		L	L	L	K	C	T	V	S	G	C	D	E	E	I	S	L	G	K	Y	S	H	H	I	S	K	H	K	E	T	K
7. RAG1-CHICK		L	S	I	R	C	P	V	P	E	C	D	E	E	I	L	H	G	K	Y	Q	H	F	S	N	H	K	E	M	K	
8. RAG1-ONCMY		L	P	L	L	C	P	R	E	S	C	G	E	Q	V	R	L	D	S	F	R	A	H	C	L	G	H	L	E	E	



Ring-type Zinc Finger

UniProtKB results

Filter byⁱ

[BLAST](#)
[Align](#)
[Download](#)
[Add to basket](#)
[Columns](#)

[Reviewed \(387\)](#)
✕

Popular organisms

- Human (95)
- Mouse (75)
- Rat (29)
- A. thaliana (22)
- Slime mold (17)
- Other organisms

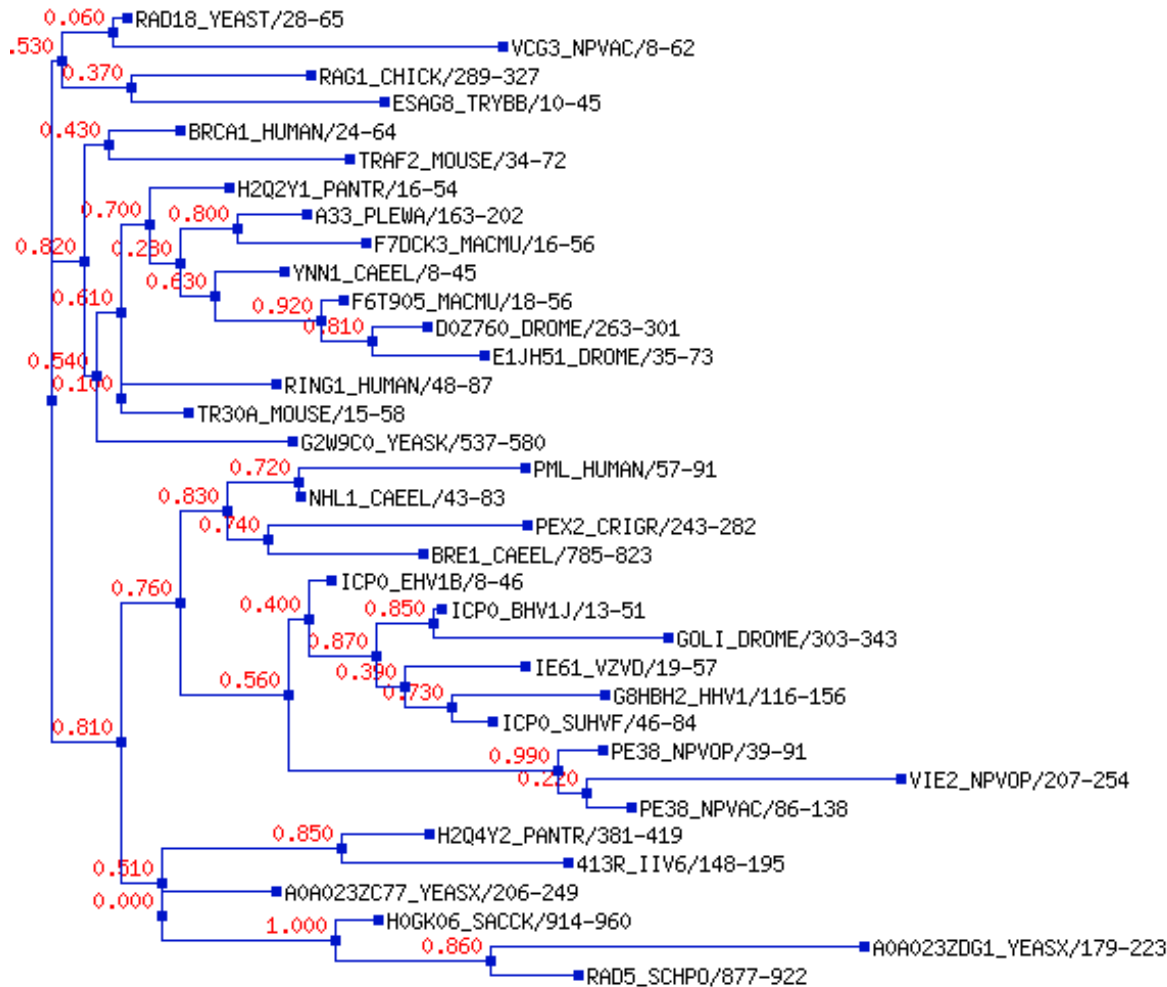
View by

- Results table
- Taxonomy
- Keywords
- Gene Ontology
- Enzyme class

Suggest adding column(s): Domains ✕

<input type="checkbox"/>	Entry	Entry name	Protein names	Gene names	Organism	Length
<input type="checkbox"/>	Q05AK5	MYLIB_DANRE	E3 ubiquitin-protein ligase MYLIB-B	mylibb zgc:153767	Danio rerio (Zebrafish) (Brachydanio rerio)	464
<input type="checkbox"/>	Q8BM54	MYLIP_MOUSE	E3 ubiquitin-protein ligase MYLIP	Mylip	Mus musculus (Mouse)	445
<input type="checkbox"/>	D3ZDI6	MYLIP_RAT	E3 ubiquitin-protein ligase MYLIP	Mylip	Rattus norvegicus (Rat)	445
<input type="checkbox"/>	Q6TEM9	MYLIA_DANRE	E3 ubiquitin-protein ligase MYLIP-A	mylipa mir, mylip, si:ch211-266j17.1	Danio rerio (Zebrafish) (Brachydanio rerio)	472
<input type="checkbox"/>	Q8WY64	MYLIP_HUMAN	E3 ubiquitin-protein ligase MYLIP	MYLIP BZF1, IDOL, BM-023, PP5242	Homo sapiens (Human)	445
<input type="checkbox"/>	Q8CJC5	NEUL3_MOUSE	E3 ubiquitin-protein ligase NEURL3	Neurl3 LincR	Mus musculus (Mouse)	254
<input type="checkbox"/>	Q91431	NF7O_XENLA	Nuclear factor 7, ovary		Xenopus laevis (African clawed frog)	610
<input type="checkbox"/>	Q5M870	NEUL3_RAT	E3 ubiquitin-protein ligase NEURL3	Neurl3 LincR	Rattus norvegicus (Rat)	254
<input type="checkbox"/>	Q12986	NFX1_HUMAN	Transcriptional repressor NF-X1	NFX1 NFX2	Homo sapiens (Human)	1,120
<input type="checkbox"/>	Q96EH8	NEUL3_HUMAN	E3 ubiquitin-protein ligase NEURL3	NEURL3 LINCRL	Homo sapiens (Human)	262

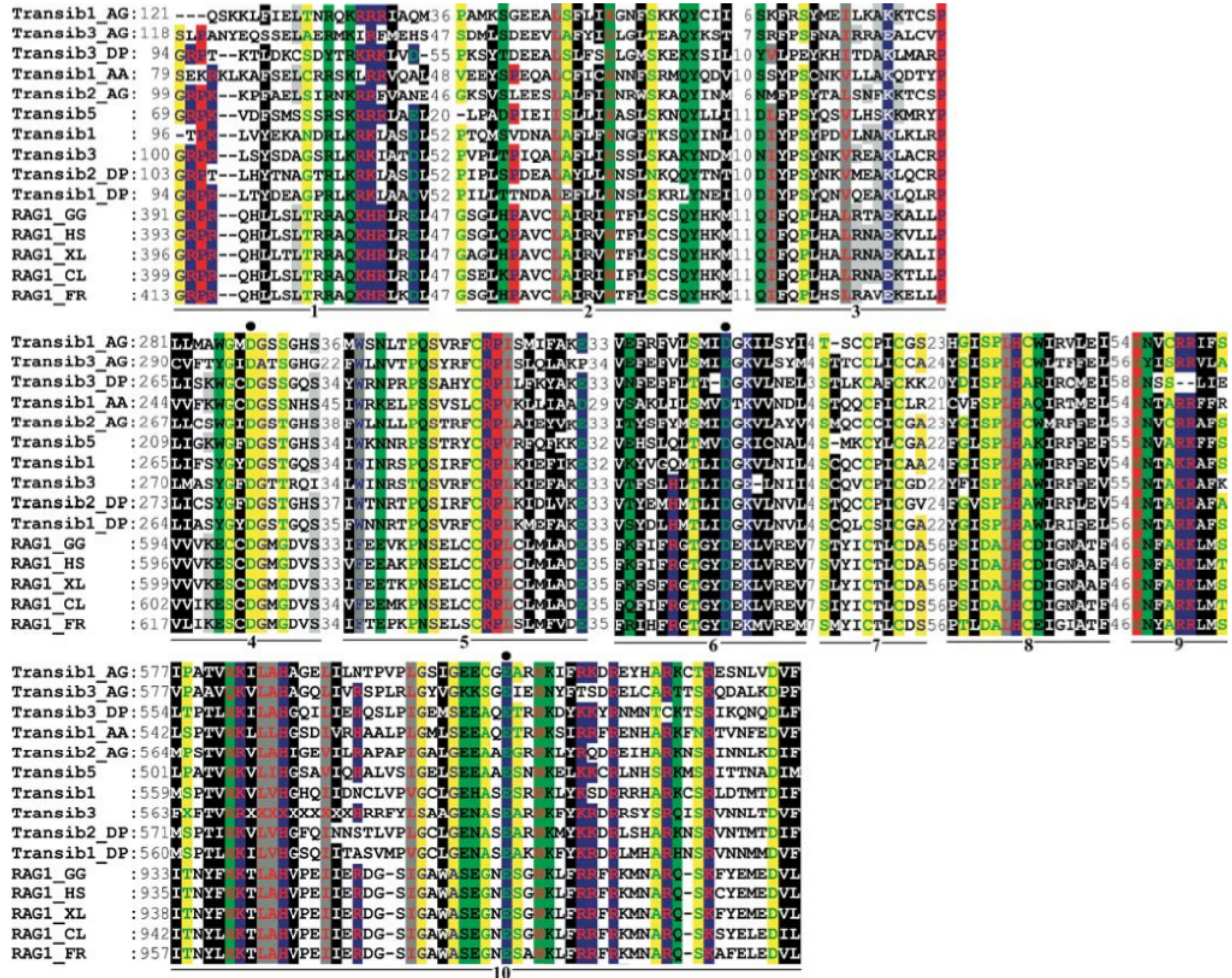
Pfam RING-ZF 结构域



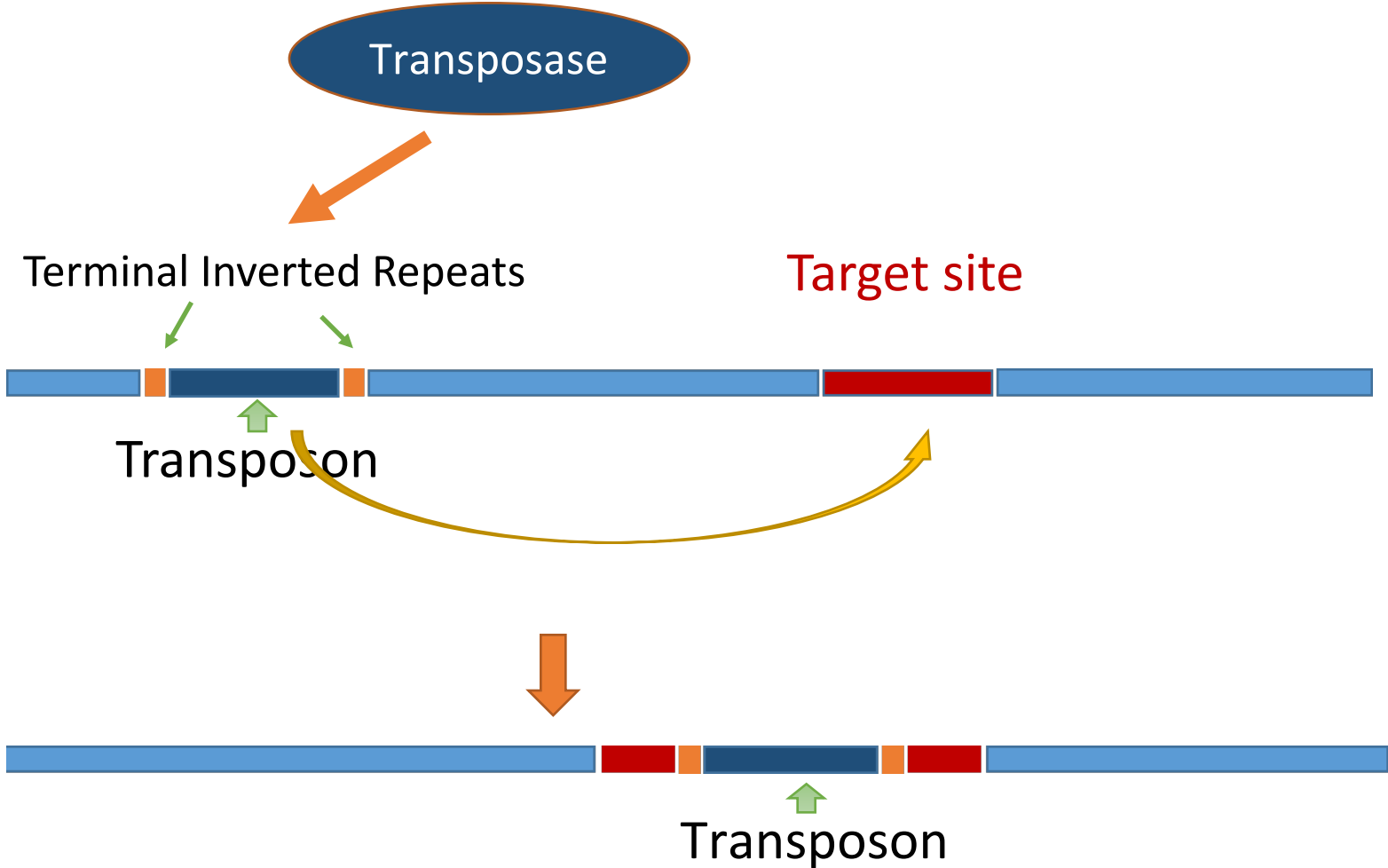
多为泛素化
相关的酶

RAG1核心区和Transib转座酶

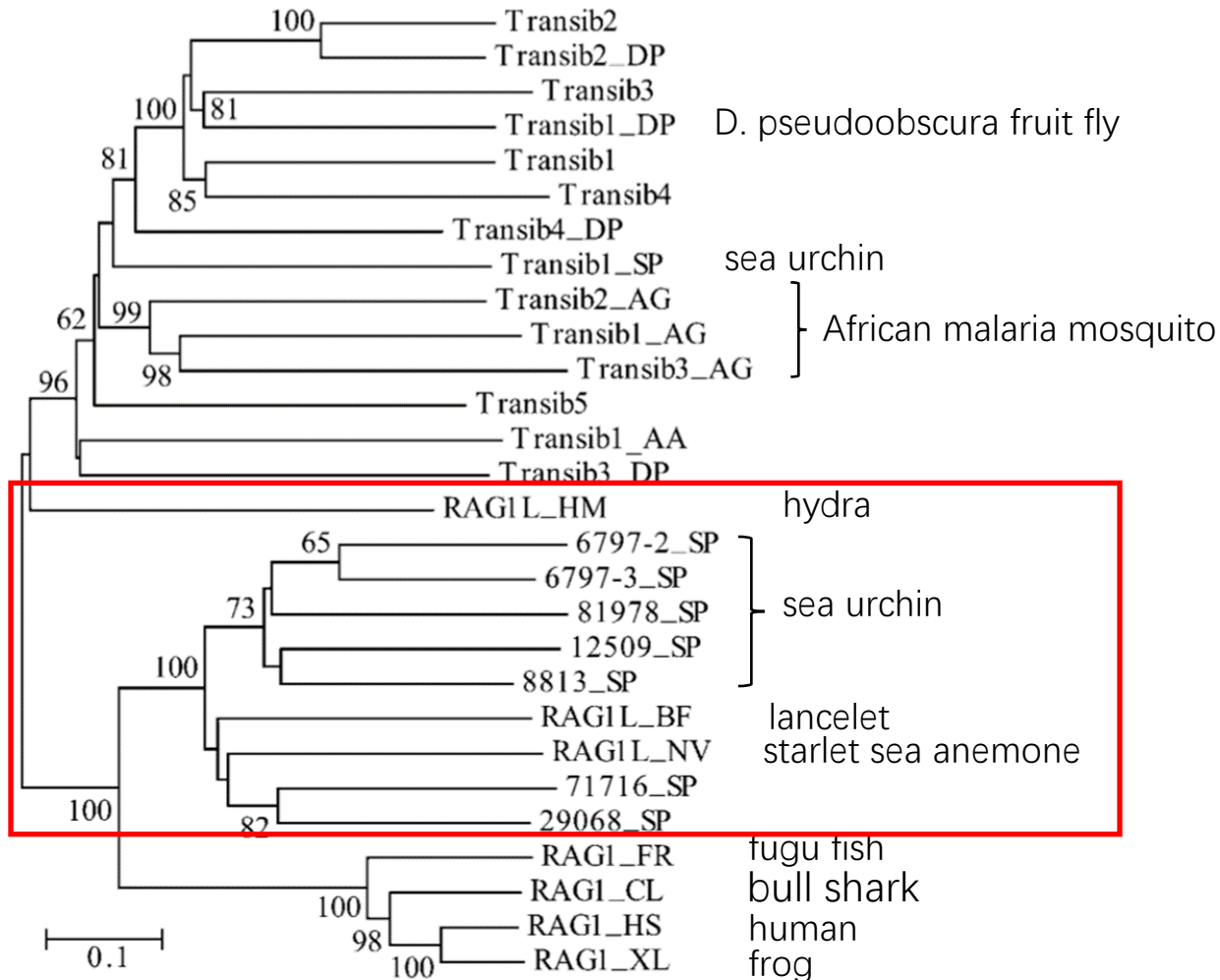
- RAG1 的核心区与Transib转座酶具有相似度很高的保守位点。



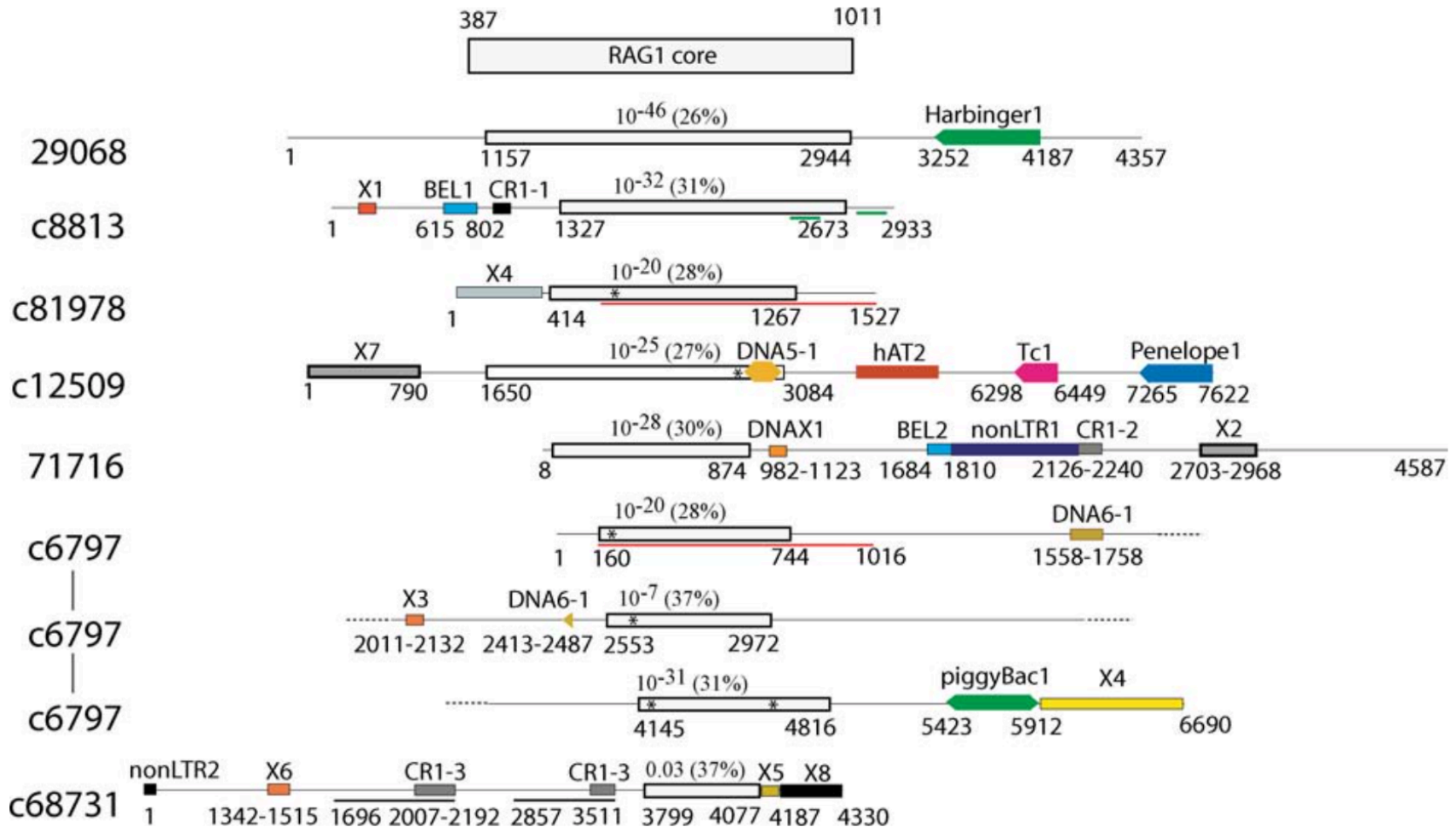
转座机制



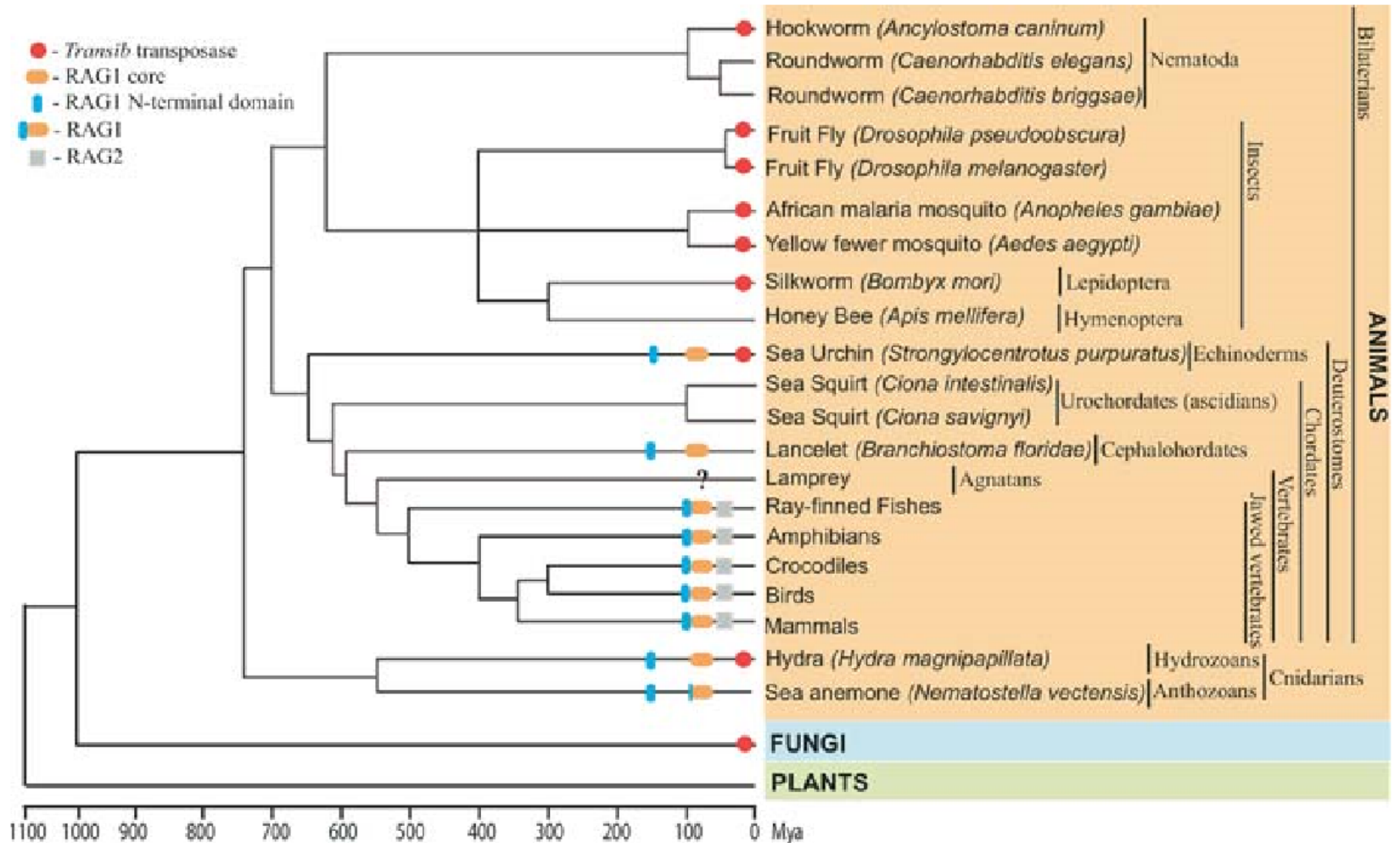
RAG和Transib转座酶在不同物种中的表达



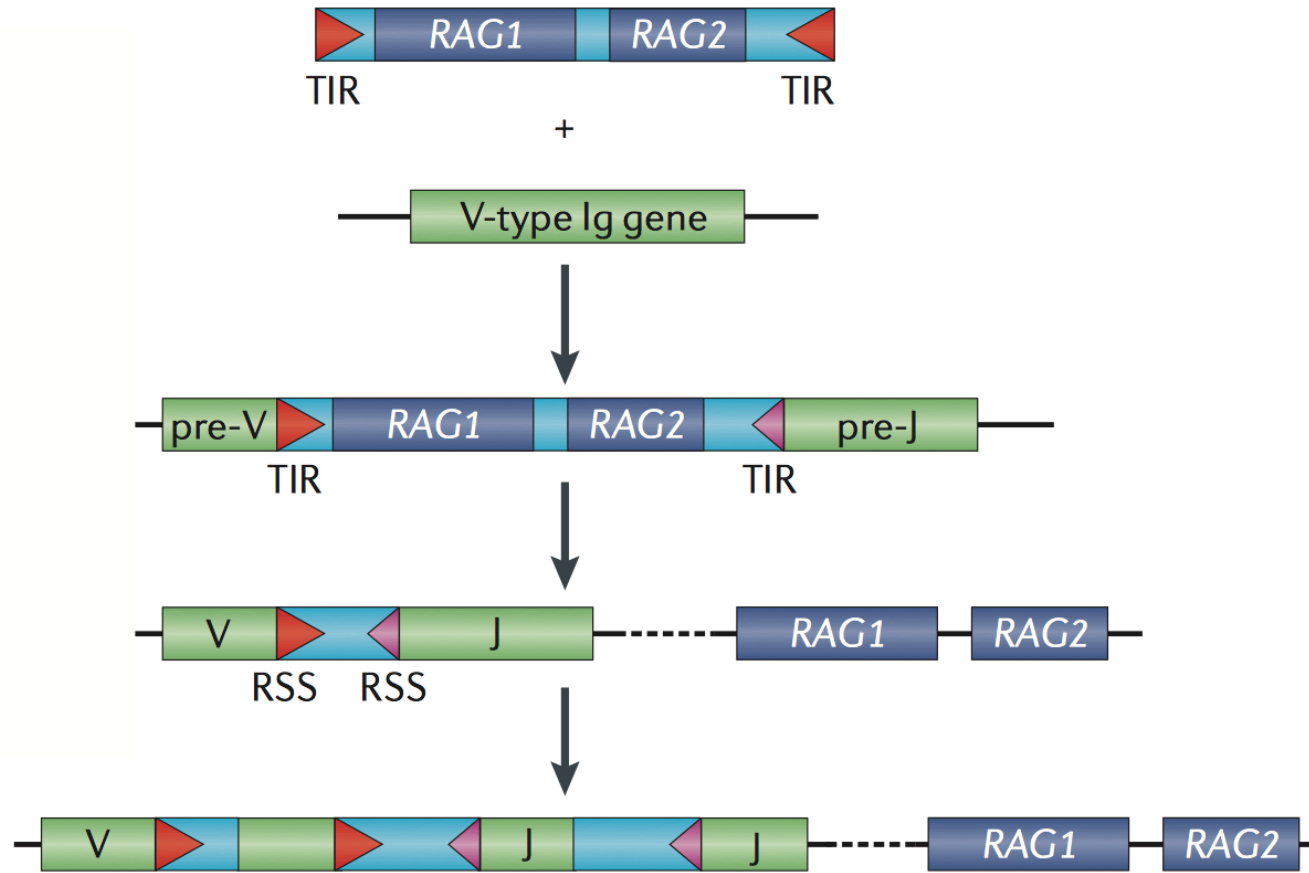
海胆的RAG1-Like序列



RAG和Transib转座酶



从转座子到V-D-J重排



总结

- 结构与功能：

- RAG1/2以四聚体形式结合两条DNA链行使功能
- RAG1-NBD的N端与DNA小沟结合，H1螺旋与DNA大沟结合

- 演化分析：

- RAG1最早出现于软骨鱼中
- RAG1的ring-zf区主要参与泛素化作用，可能来自其他泛素化相关的基因
- RAG1核心区由Transib转座酶演化而来，从软骨鱼开始稳定于特异性免疫中，之后保持缓慢的演化。

致谢

- 感谢罗老师一学期的教导
- 感谢柯岚师姐的帮助
- 感谢我们小组的小伙伴以及ABC课上的所有同学