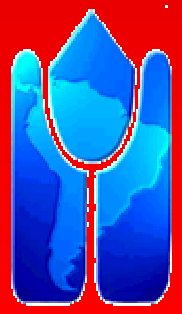
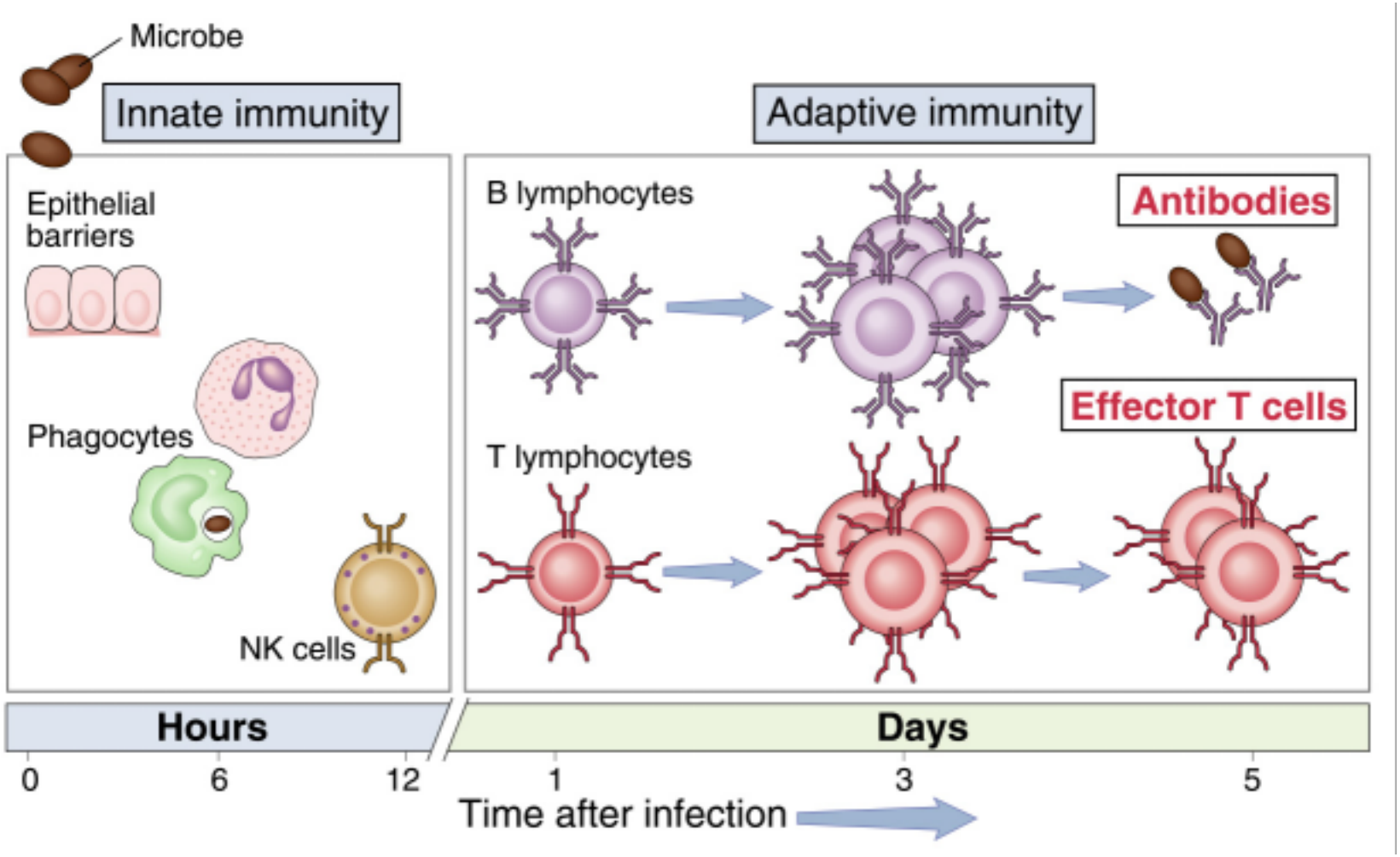
Receptores de los Linfocitos T (TCR)



FIDIC

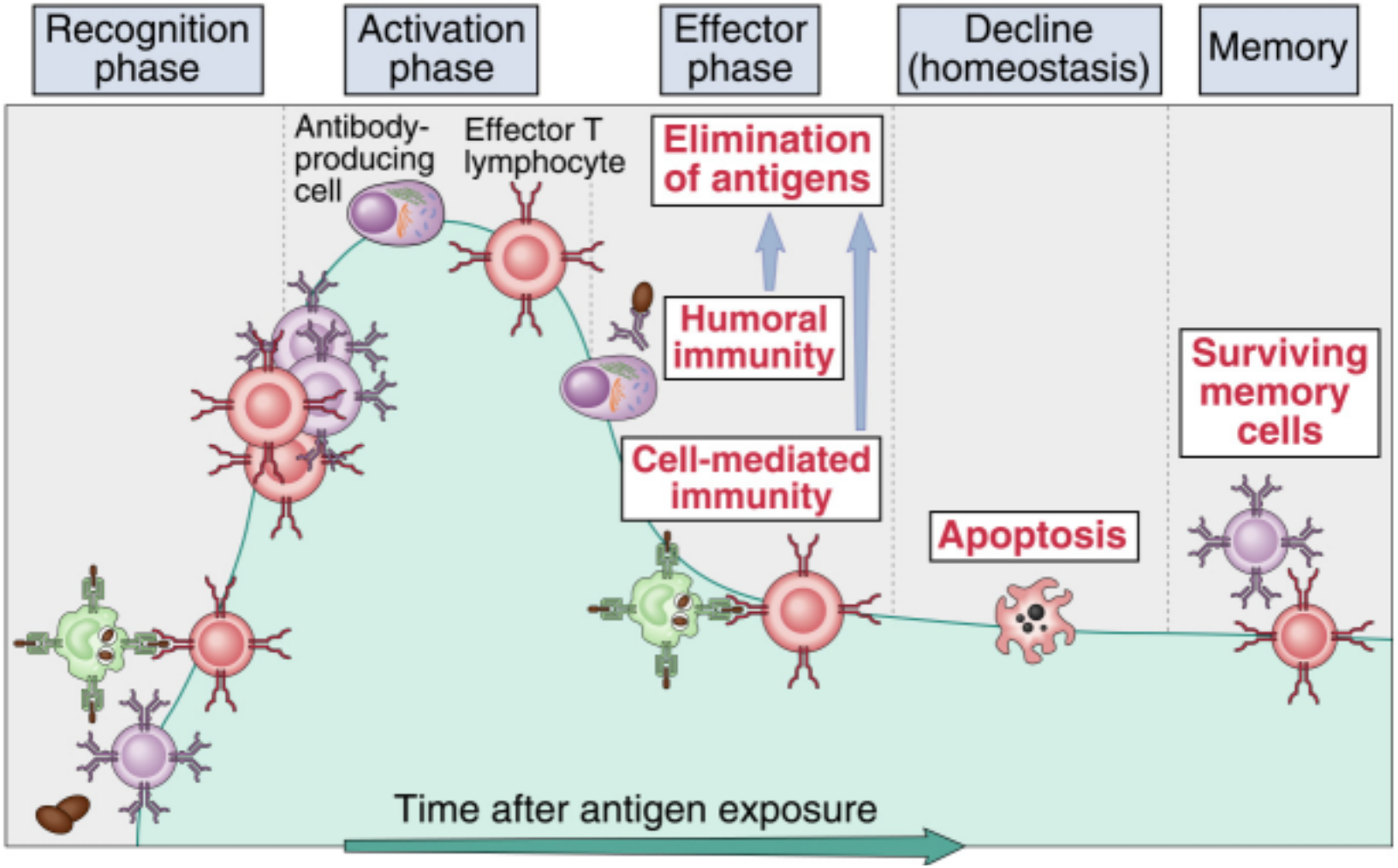
Generalidades de la Respuesta Inmune

INMUNIDAD INNATA Y ADQUIRIDA



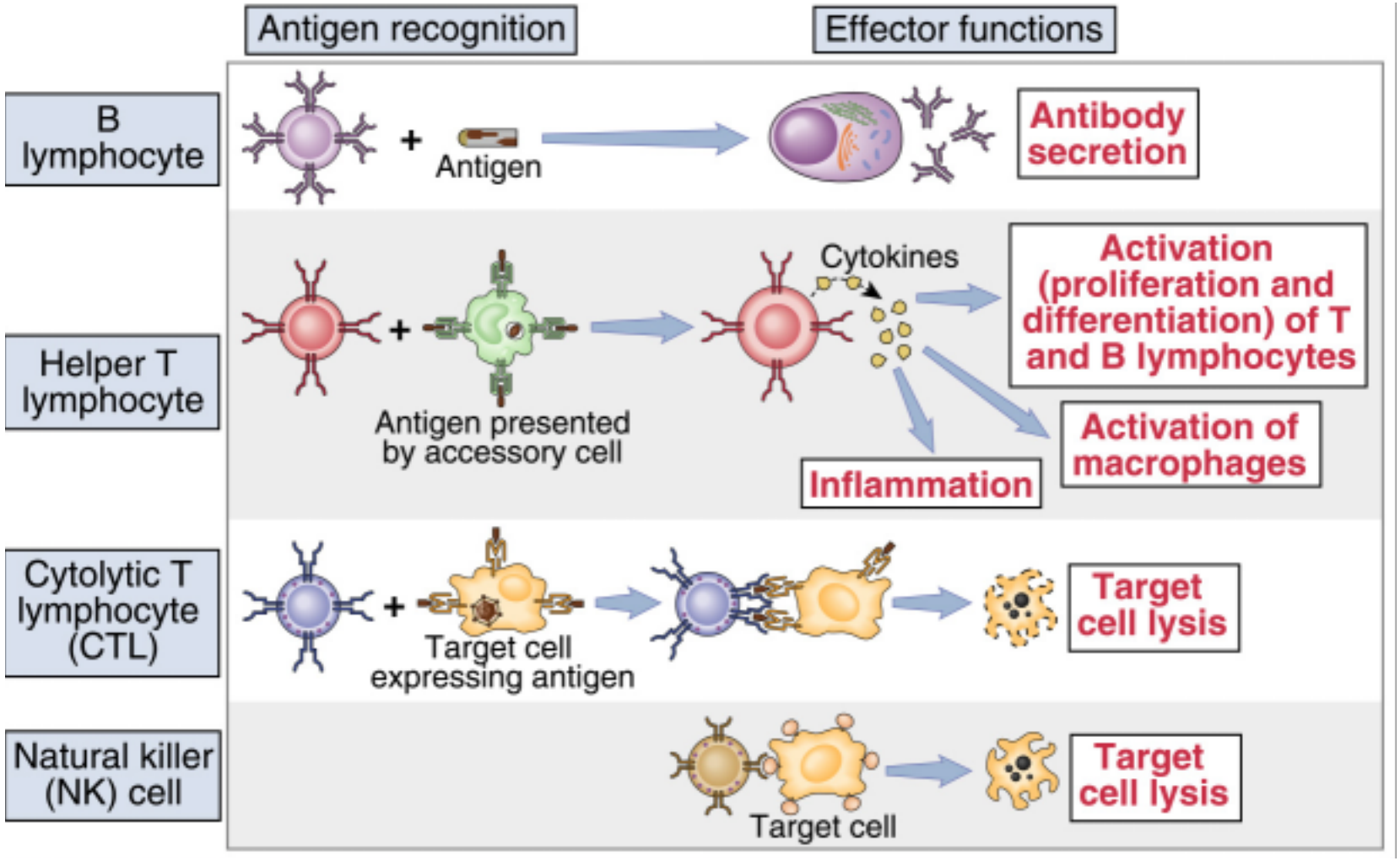
Abbas, et al Cellular and Molecular Immunology, 1999

FASES DE LA RESPUESTA INMUNE ADAPTATIVA



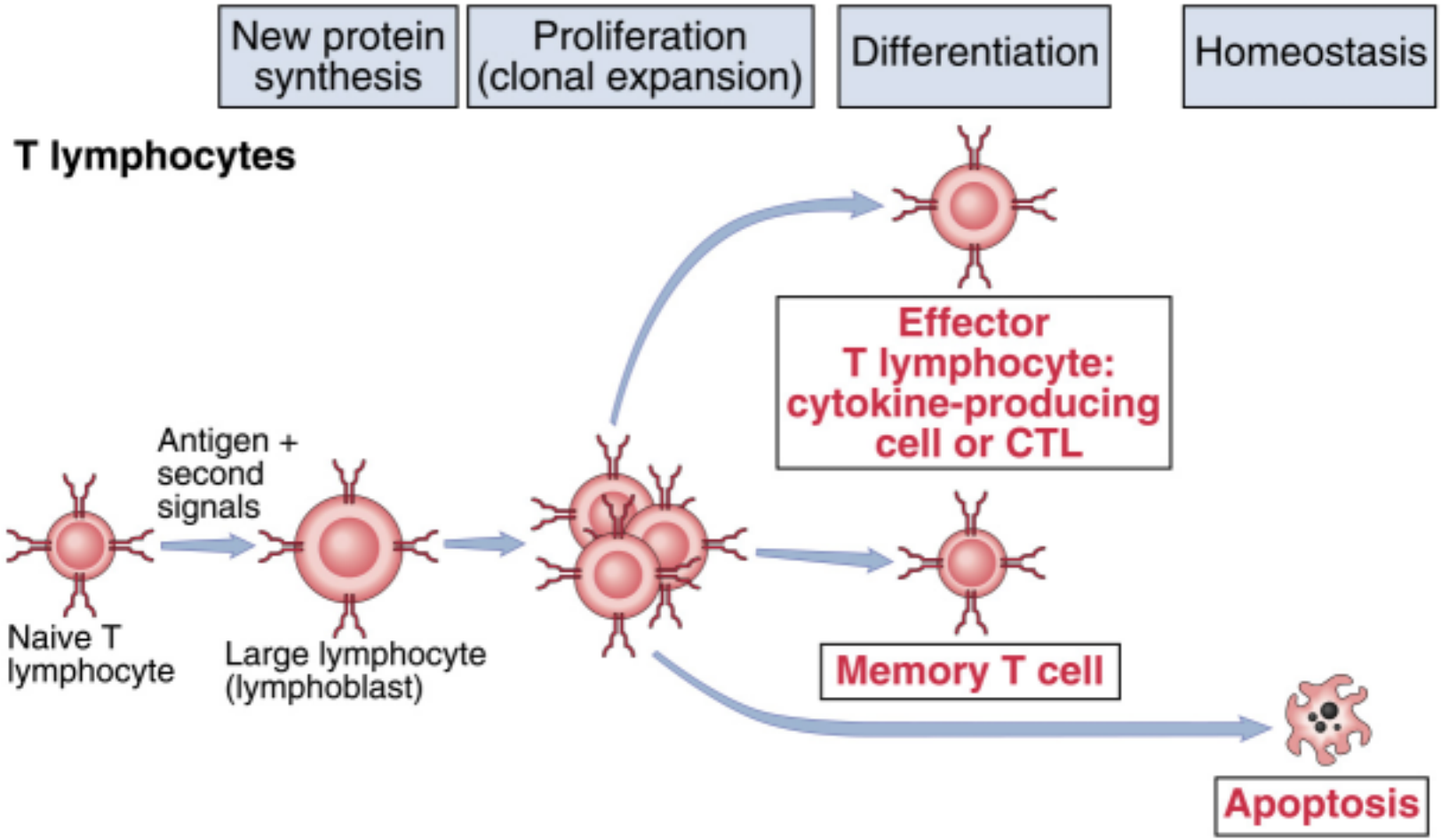
Abbas, et al Cellular and Molecular Immunology, 1999

CLASES DE LINFOCITOS



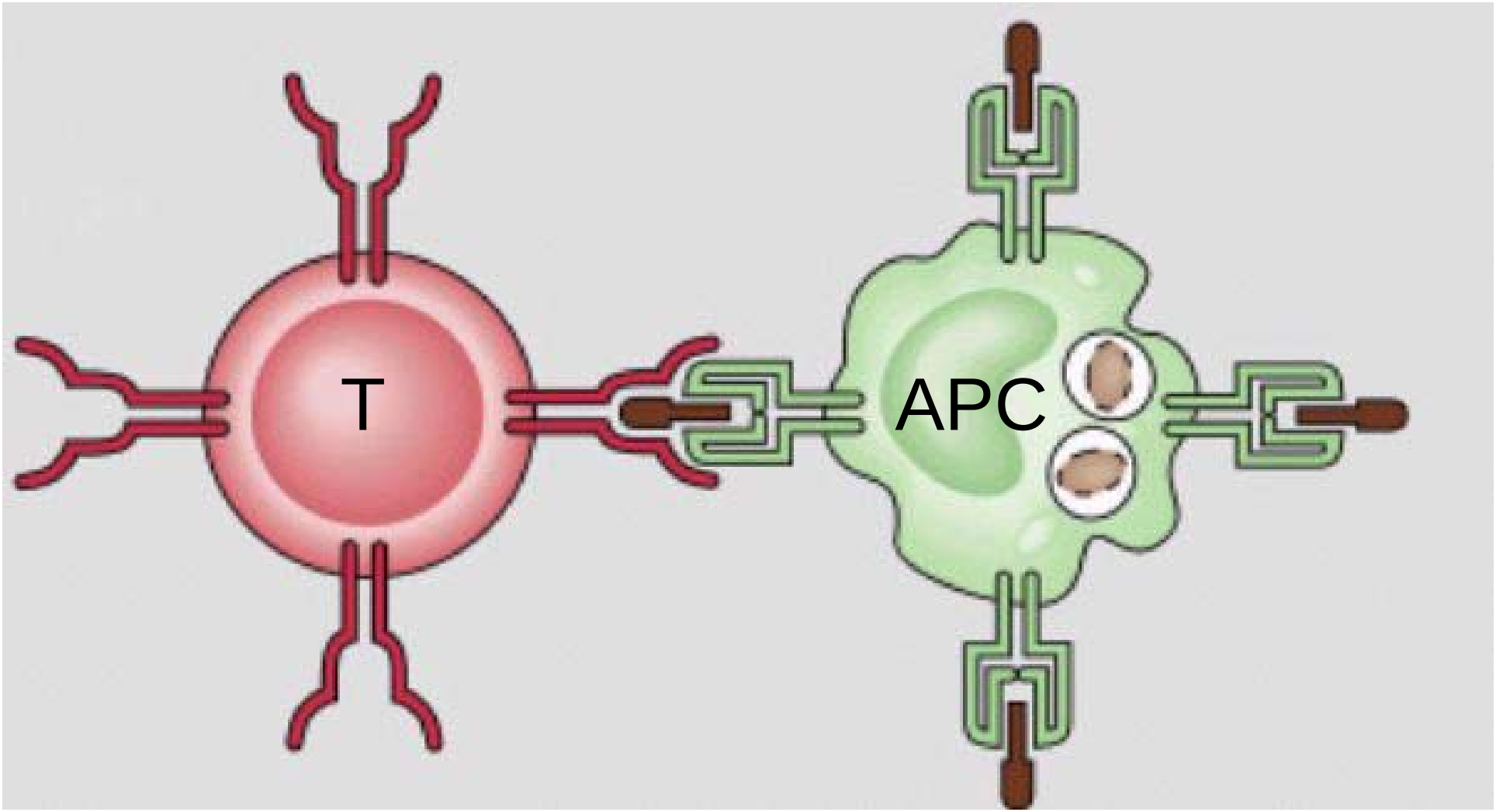
Abbas, et al Cellular and Molecular Immunology, 1999

FASES DEL PROCESO DE ACTIVACIÓN DE LOS LINFOCITOS

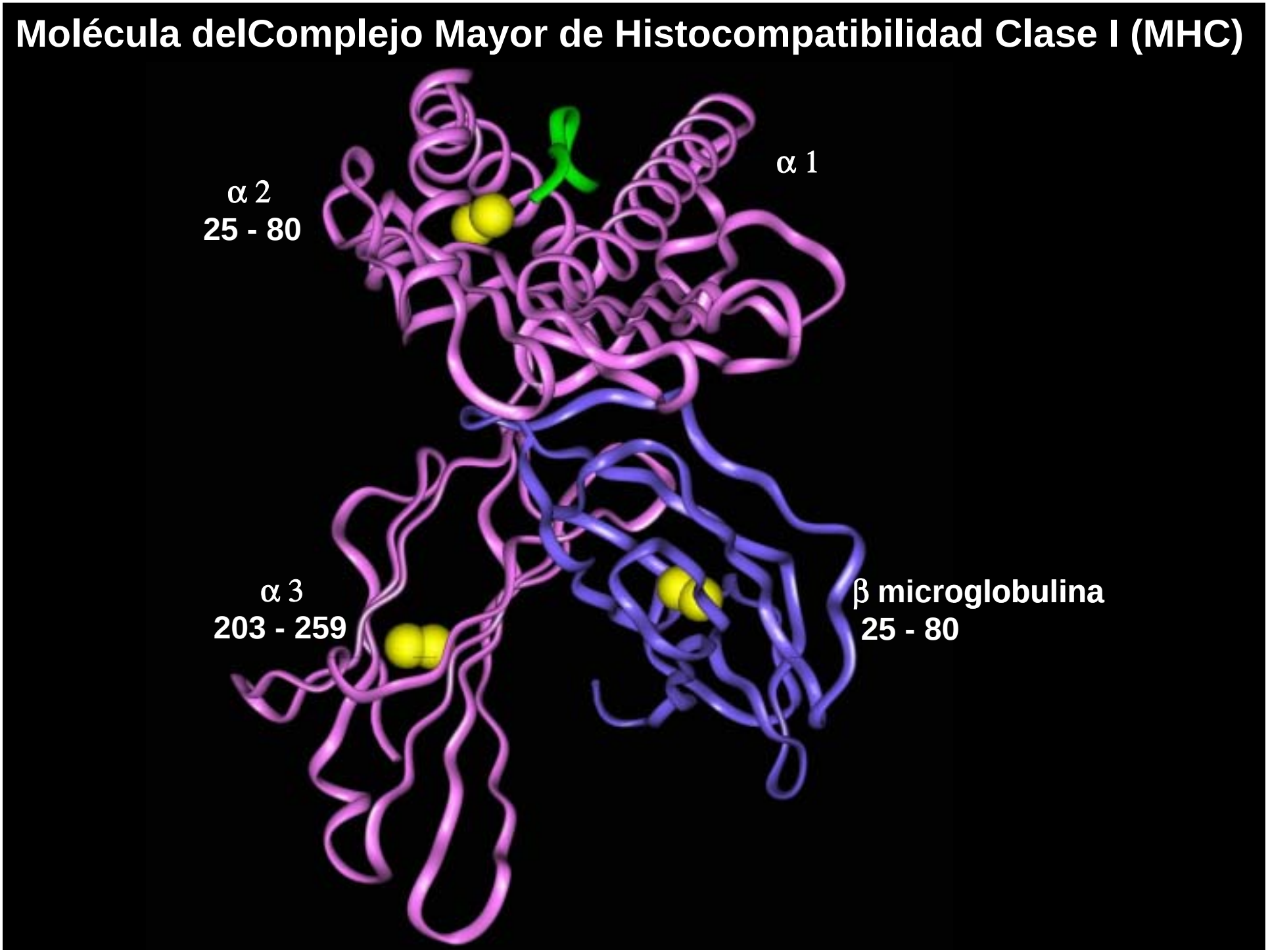


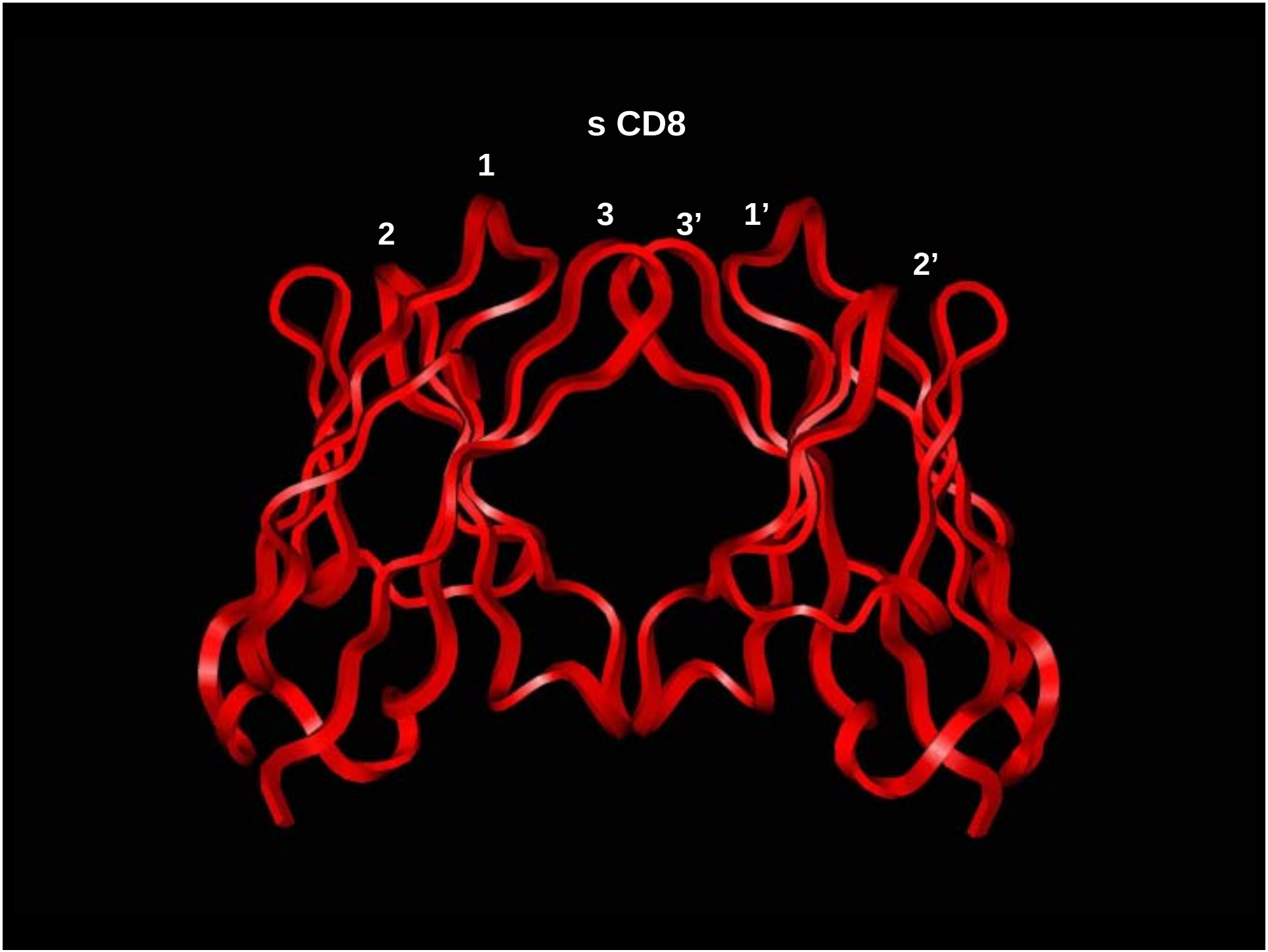
Abbas, et al Cellular and Molecular Immunology, 1999

Interacción Célula T vs APC

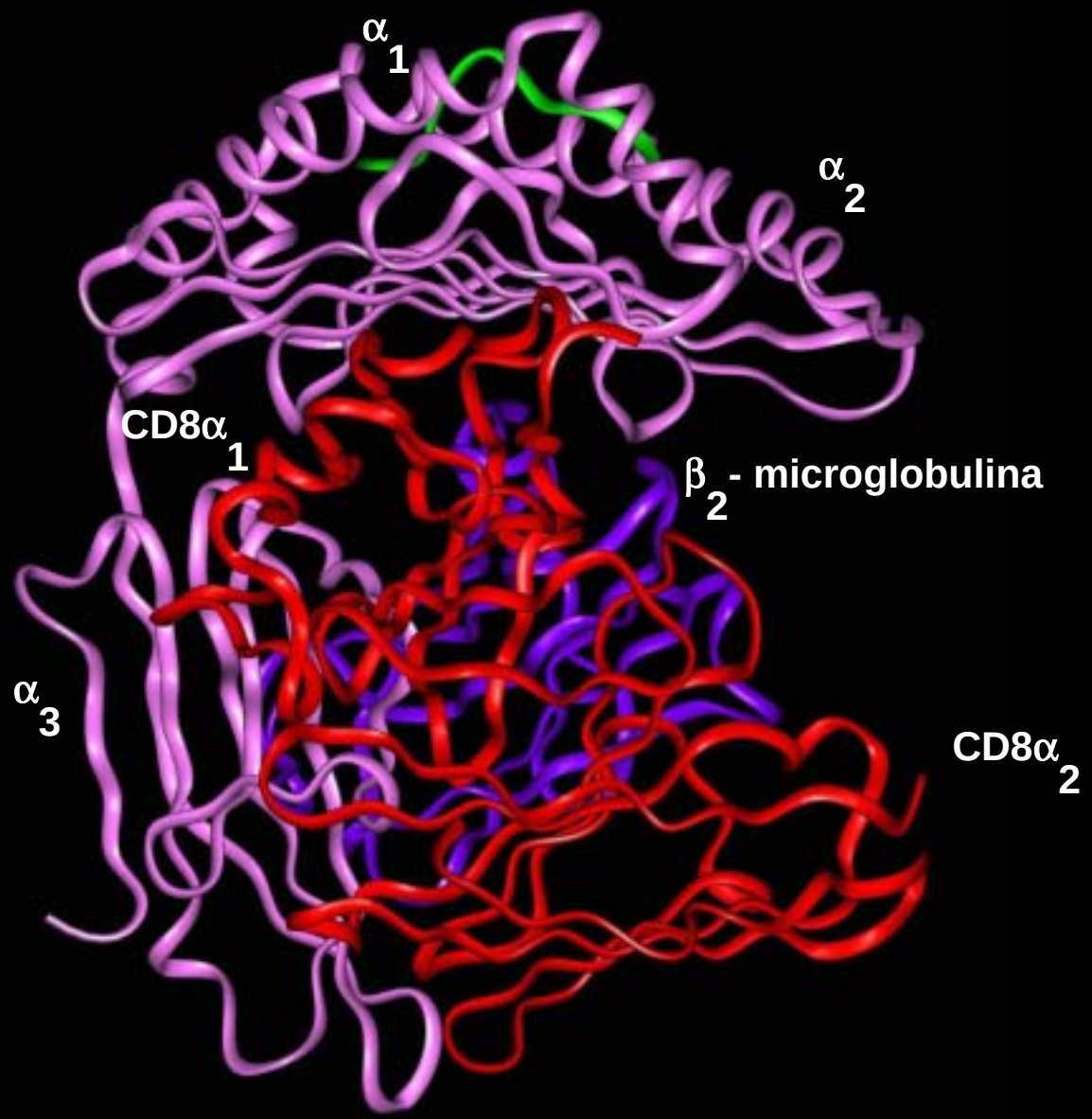


Molécula delComplejo Mayor de Histocompatibilidad Clase I (MHC)

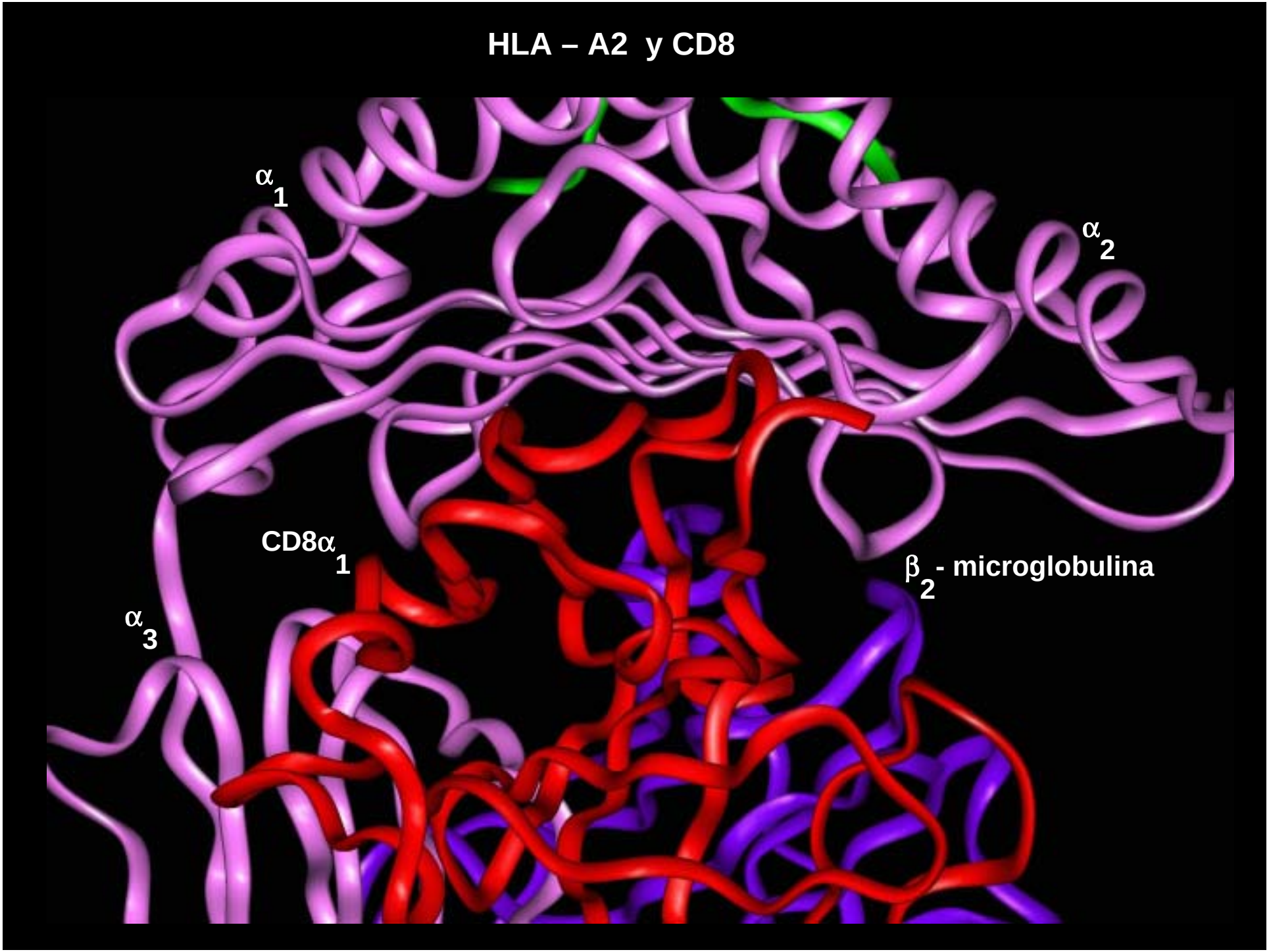



s CD8
1
2
3
3’
1’
2’

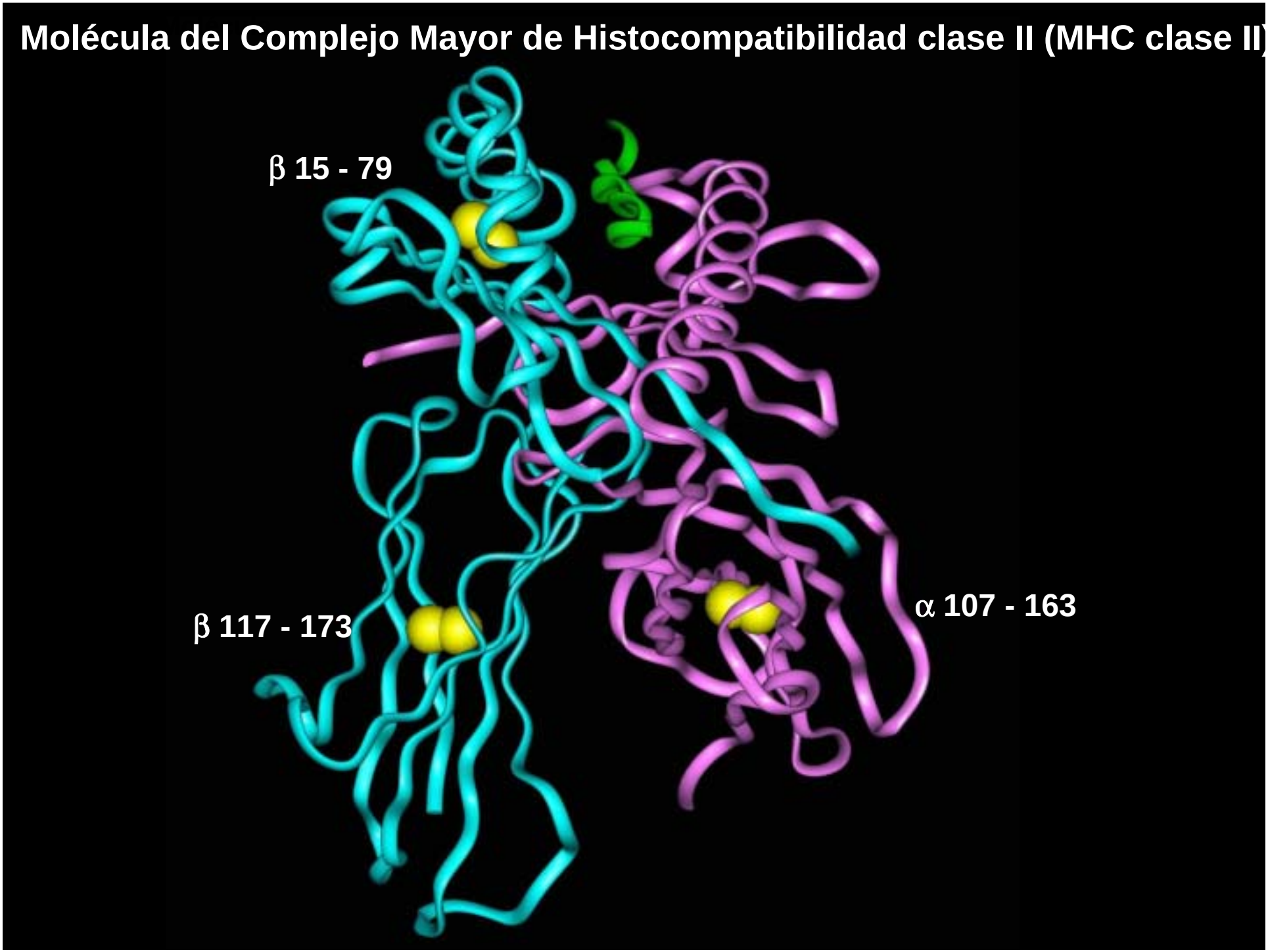
HLA – A2 y CD8



HLA – A2 y CD8

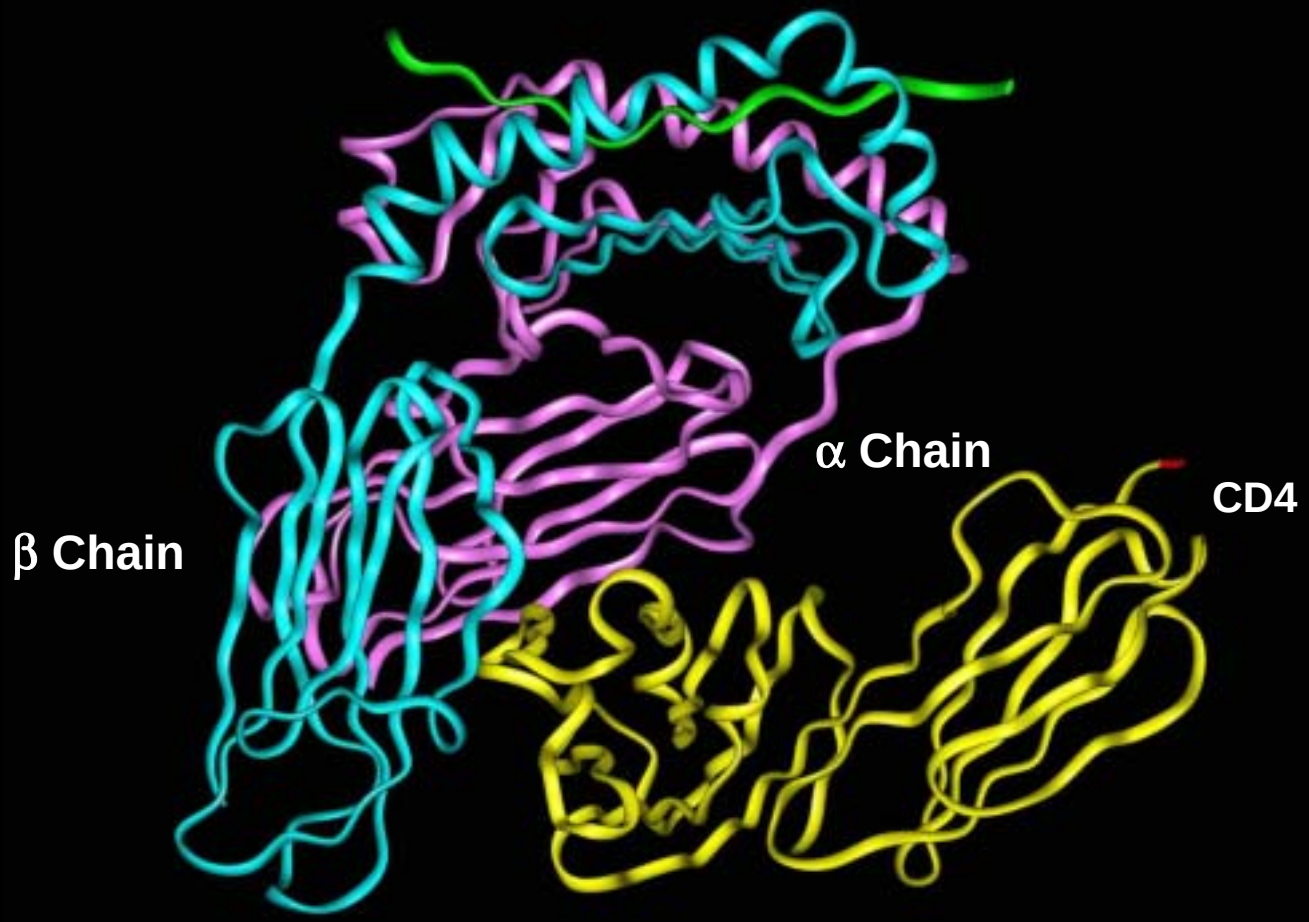


Molécula del Complejo Mayor de Histocompatibilidad clase II (MHC clase II)



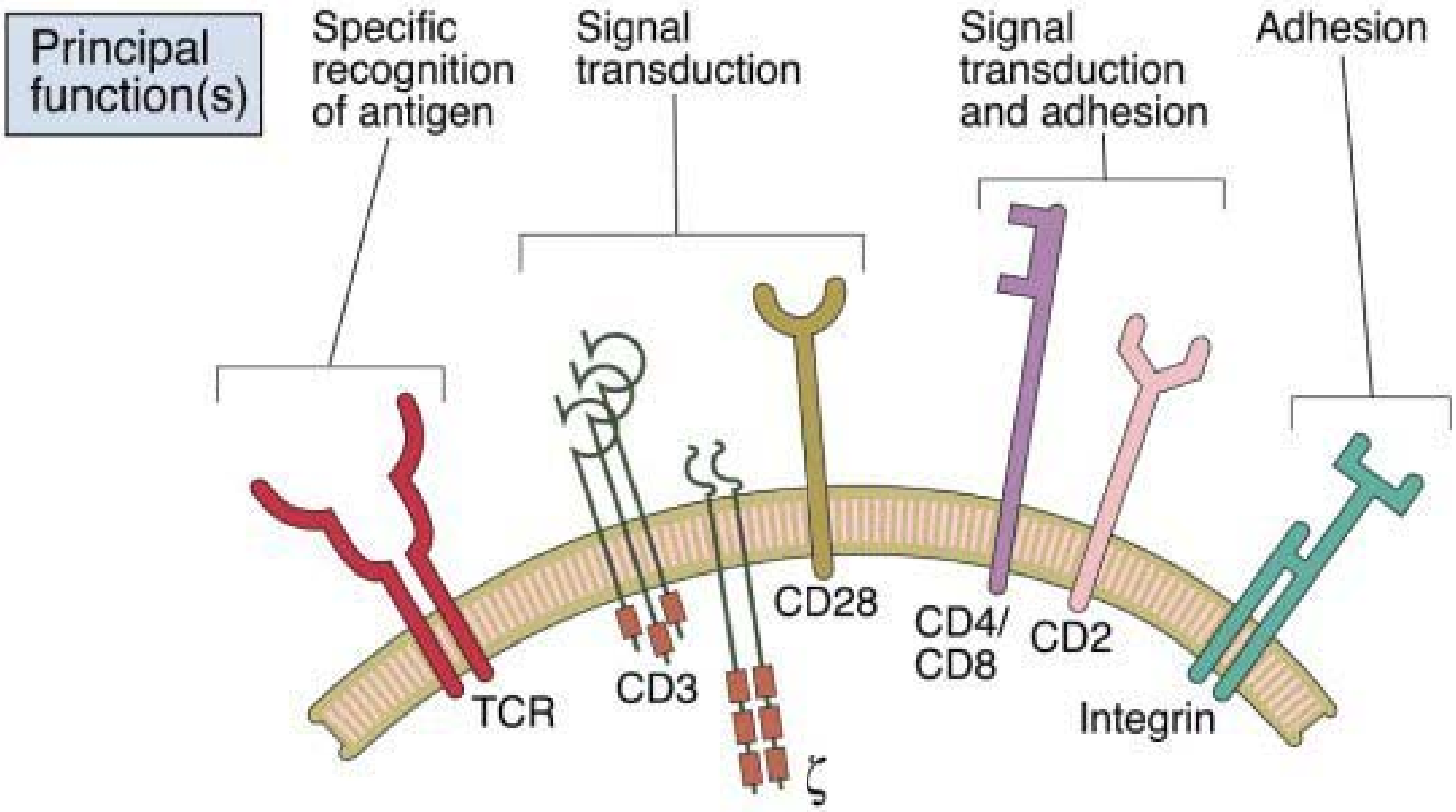

F
C
C’
C”
G
A
B
E
D
G
F
C’
C
E
B
CD4 dominios 1 y 2

MHC II - Peptide - CD4 N Terminus Complex



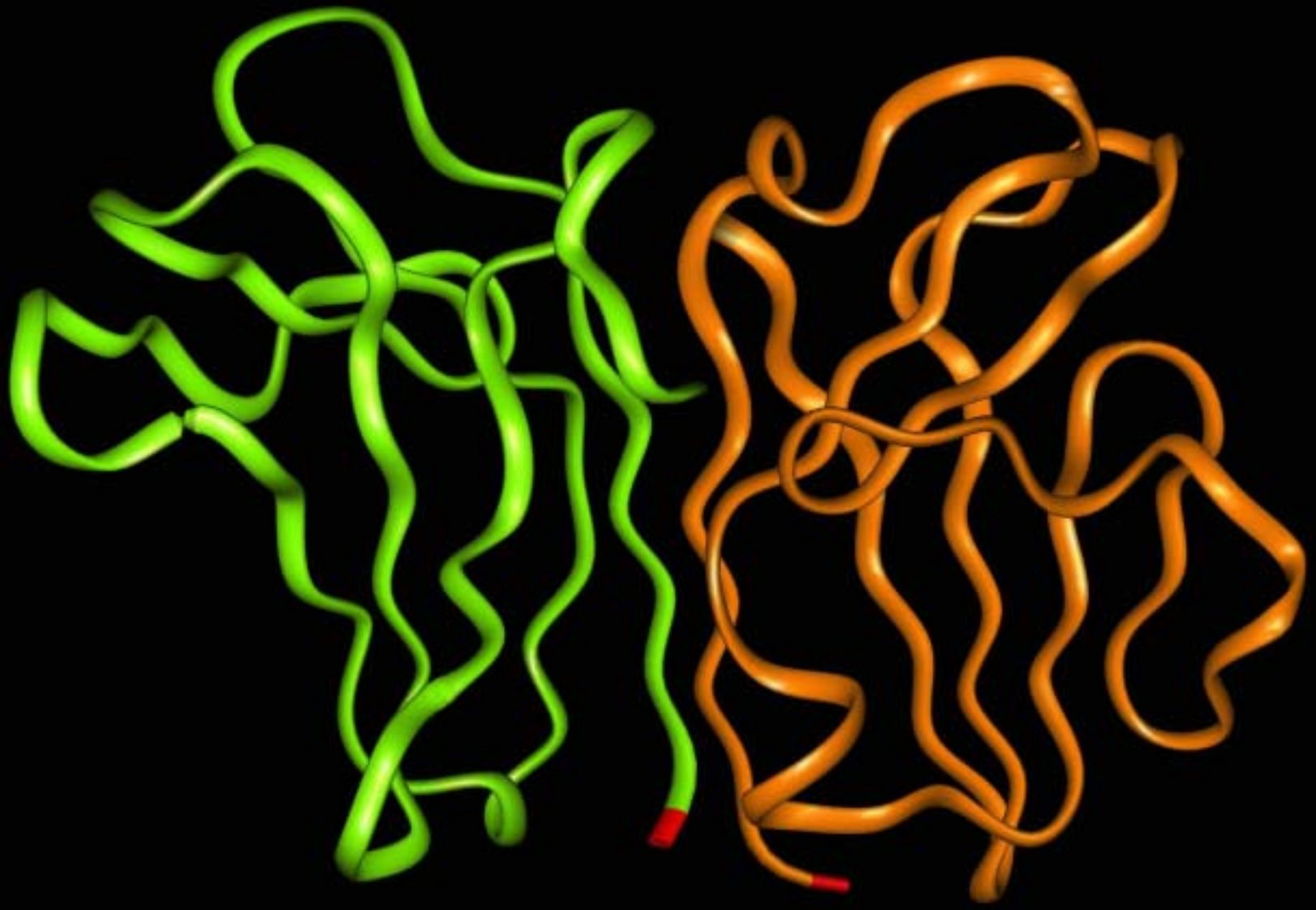
Wang, JH., et al. 2001, *Proc. Nat. Acad. Sci. USA*. 98:10799

Células T y Reconocimiento del Antígeno



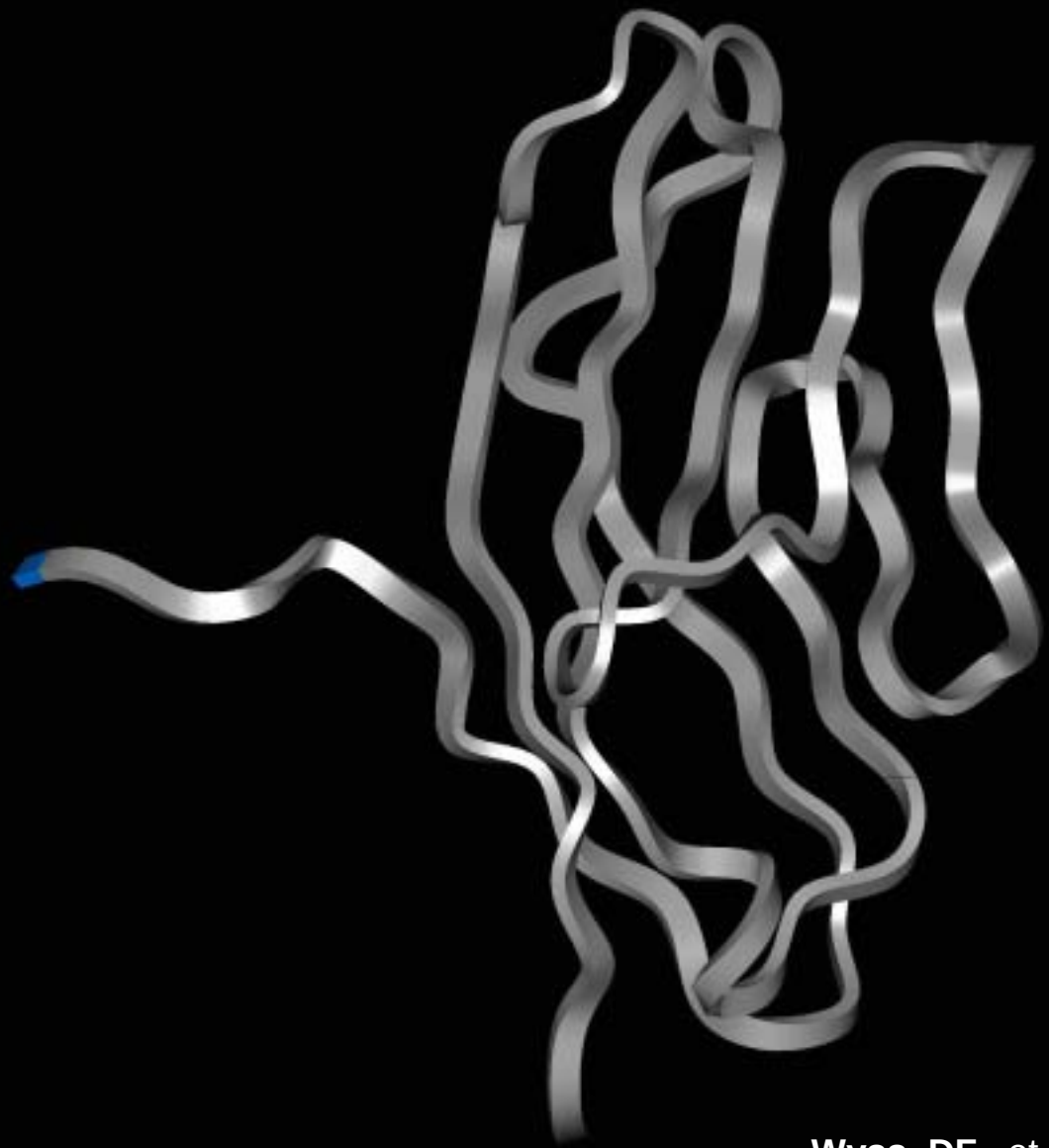
Abbas, et al Cellular and Molecular Immunology, 1999

Heterodimero CD3 γε



Kjer-Nielsen L., et al. 2004. *Proc. Nat. Acad. Sci. USA*. 101:7675

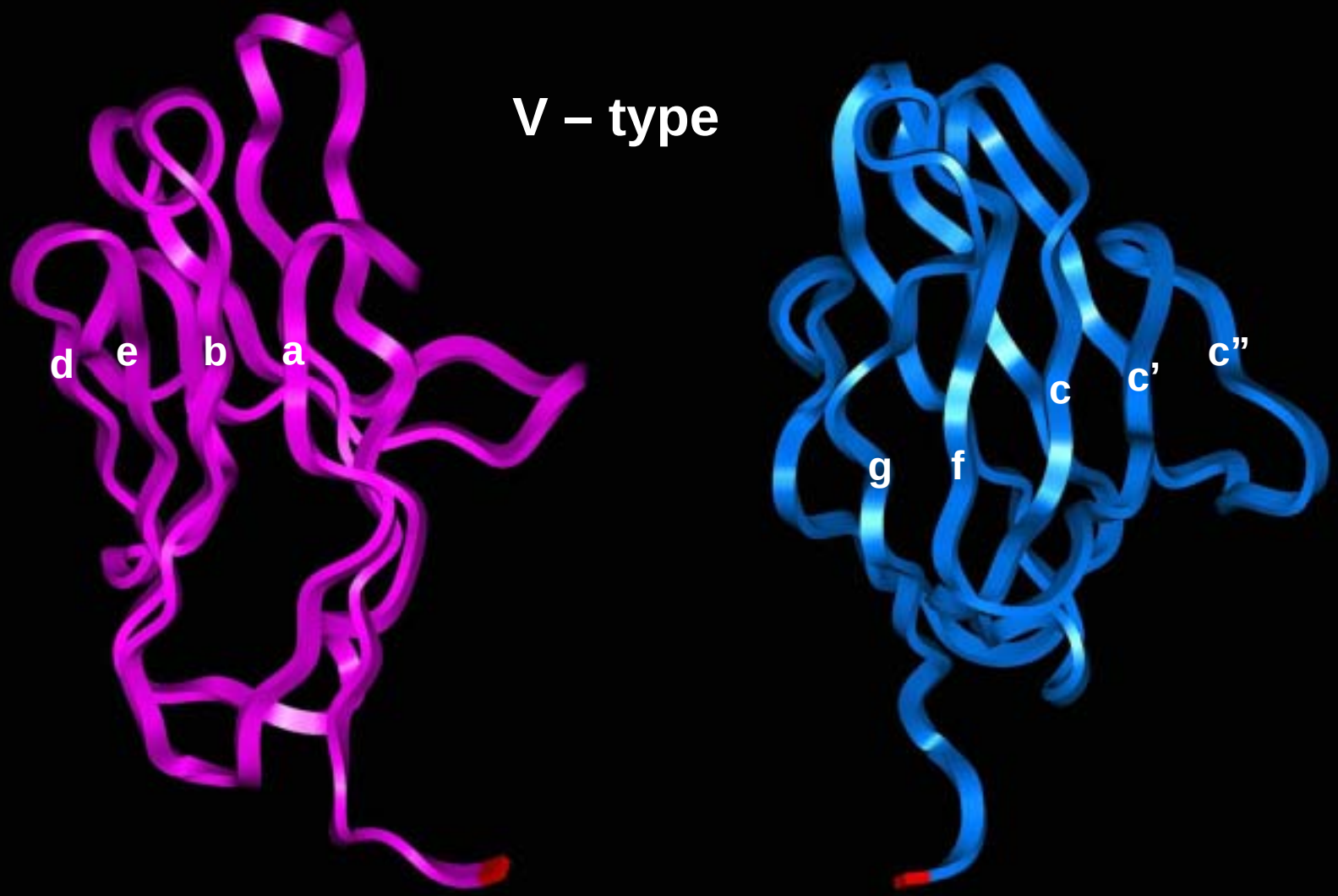
Human CD2



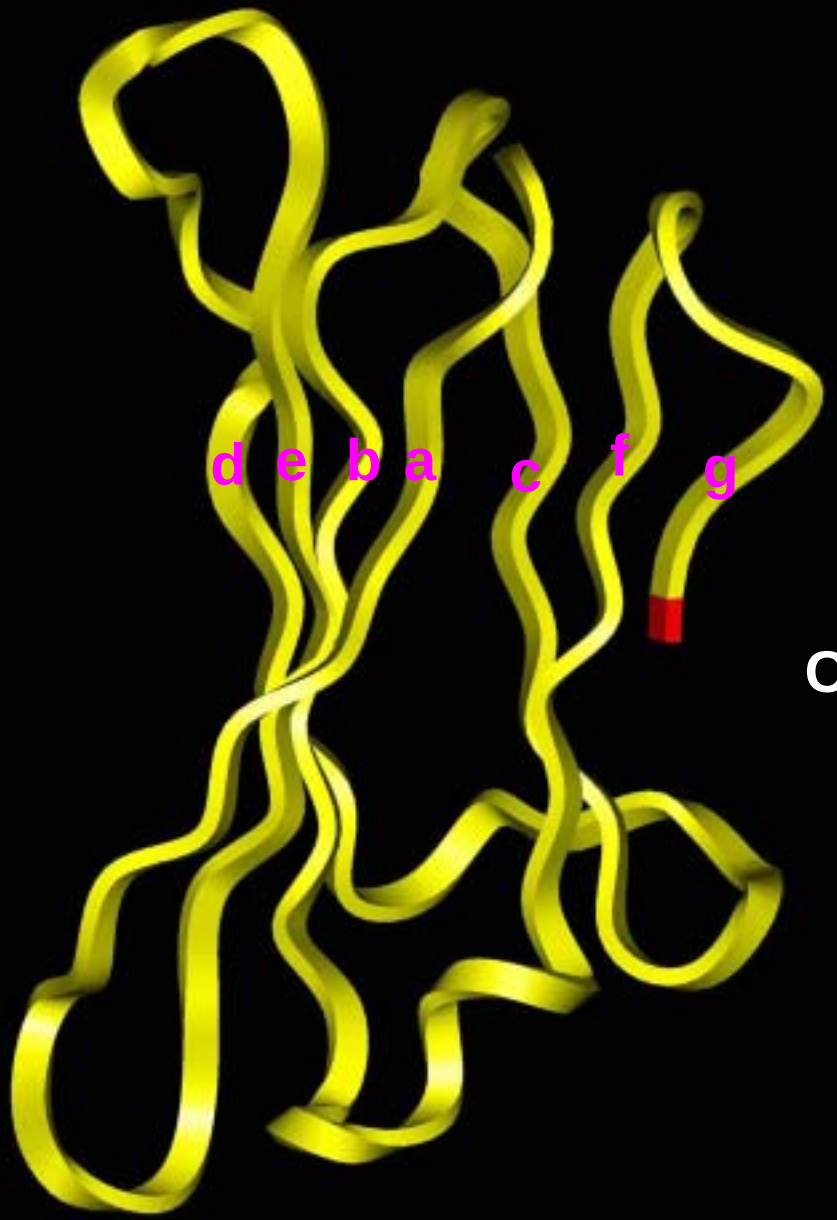
Wyss, DF., et al. 1995, *Science.* 269:1273

Patrones de enrollamiento de la superfamilia de las Igs

Ig Folds

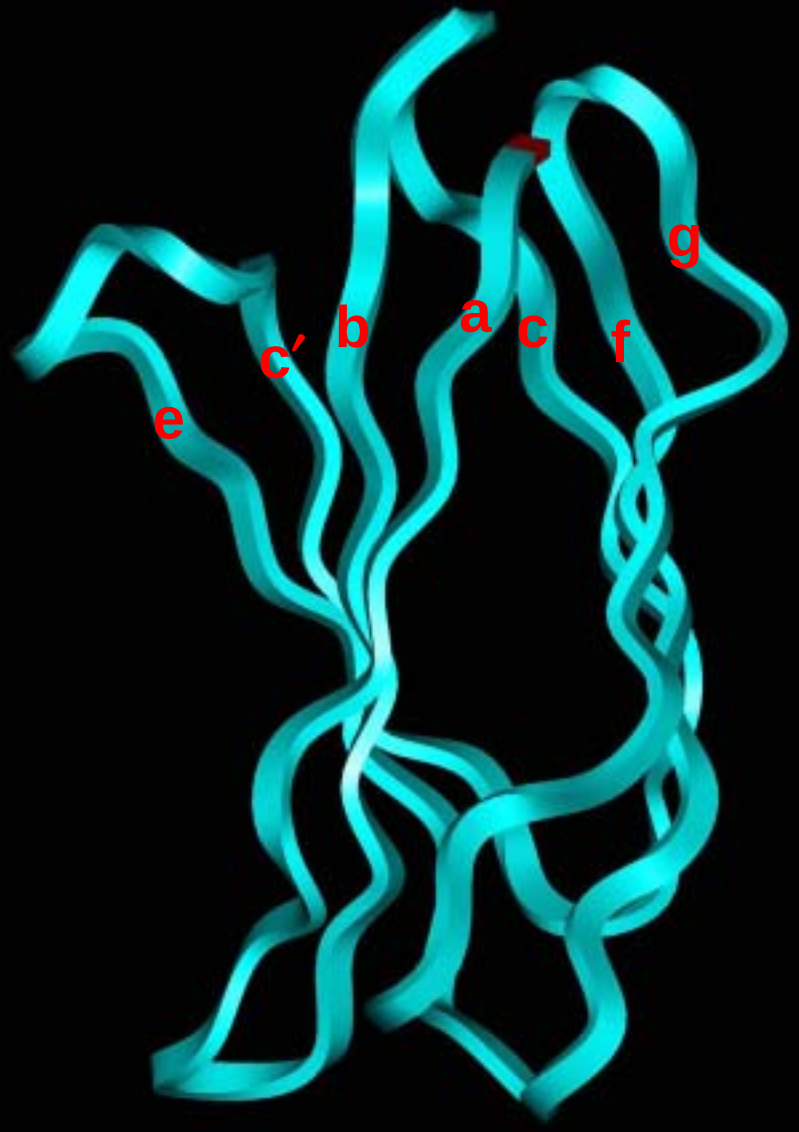


Ig Folds



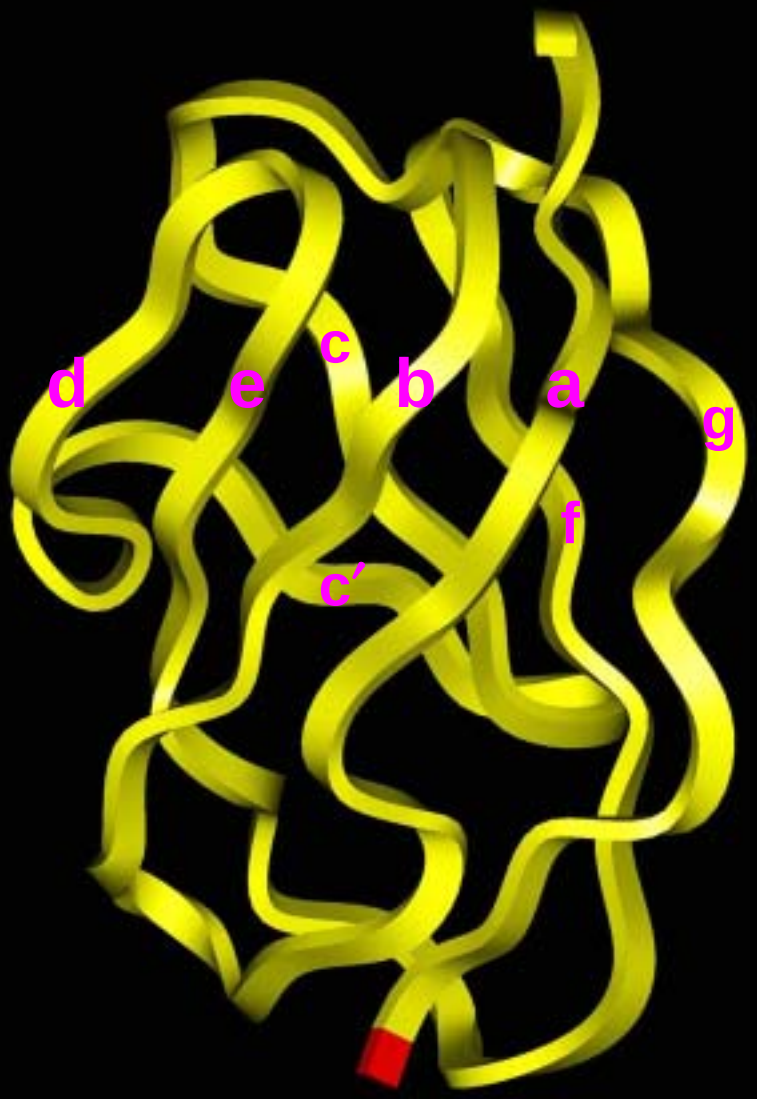
C - type

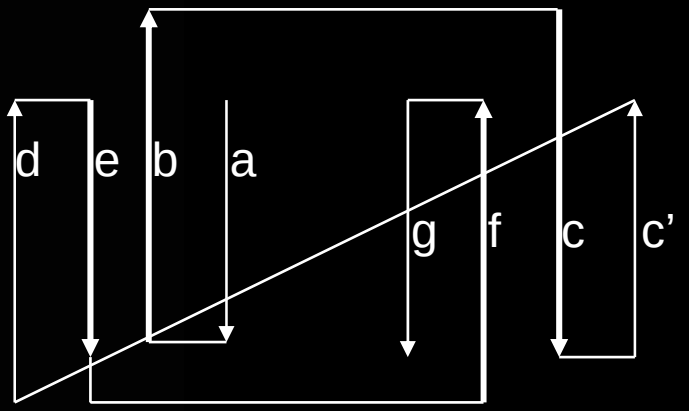
Ig Folds



S - type

Ig Folds

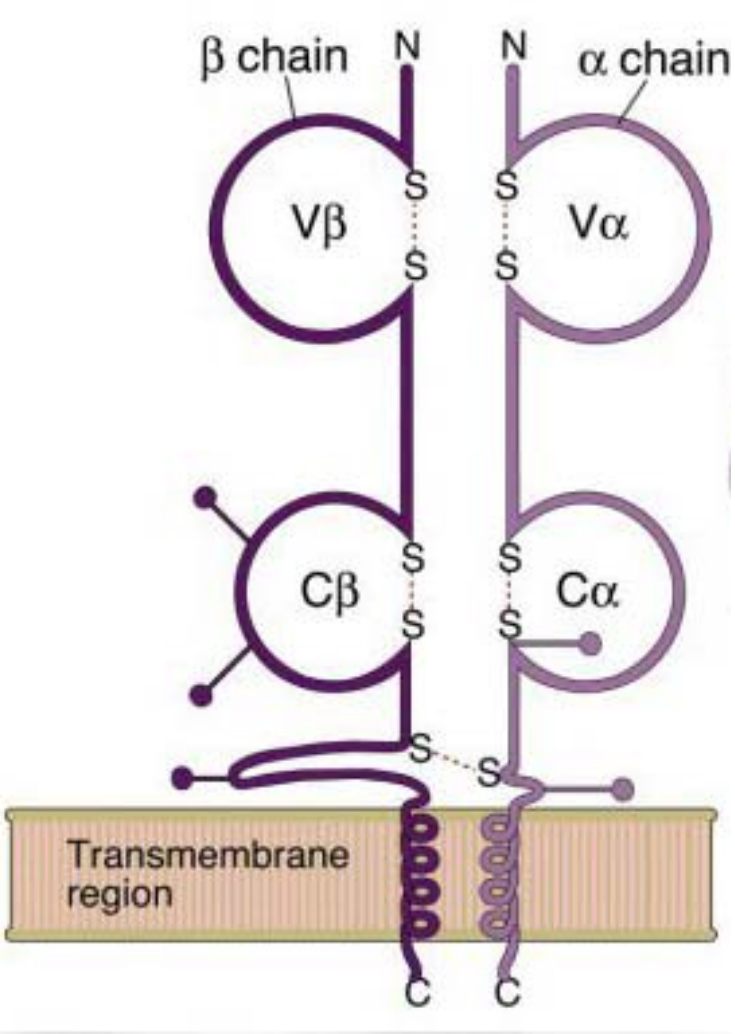


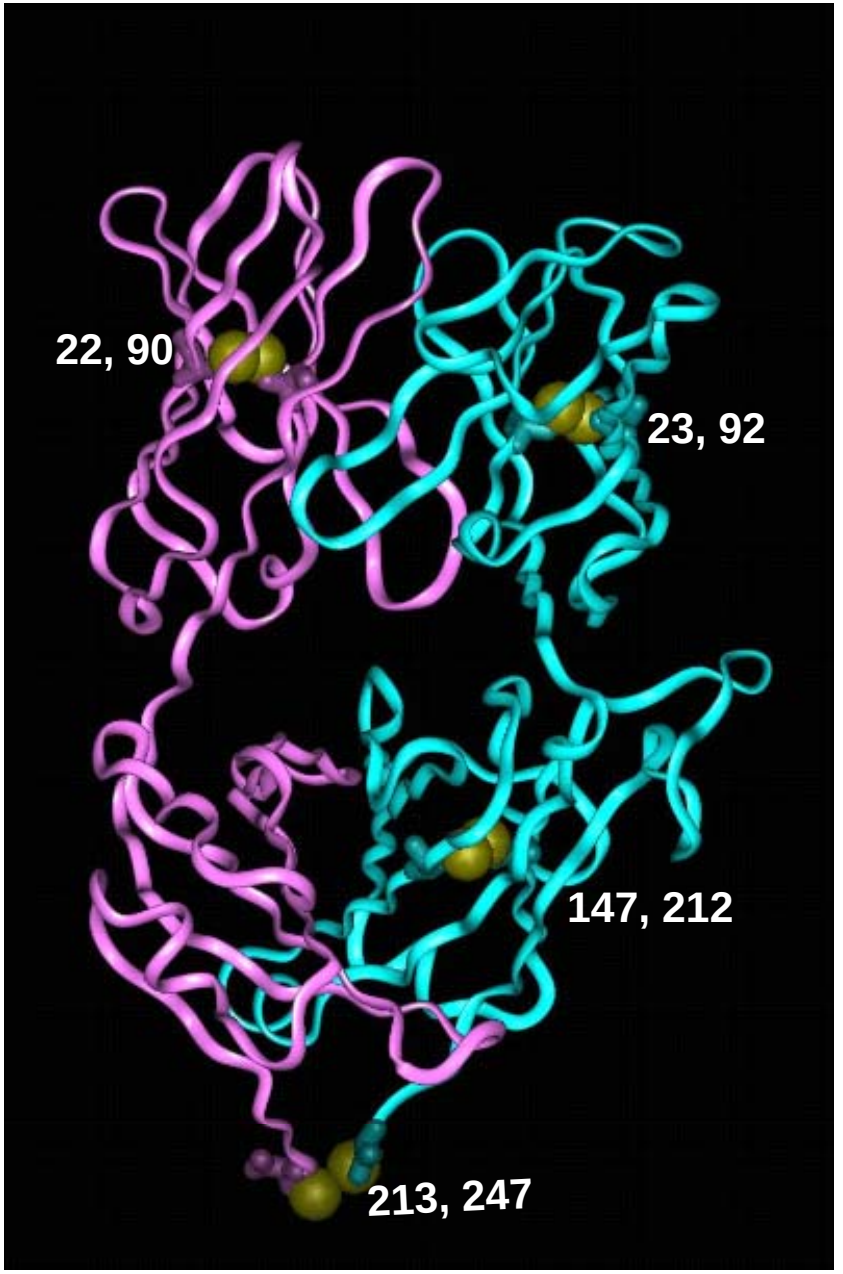


H - type

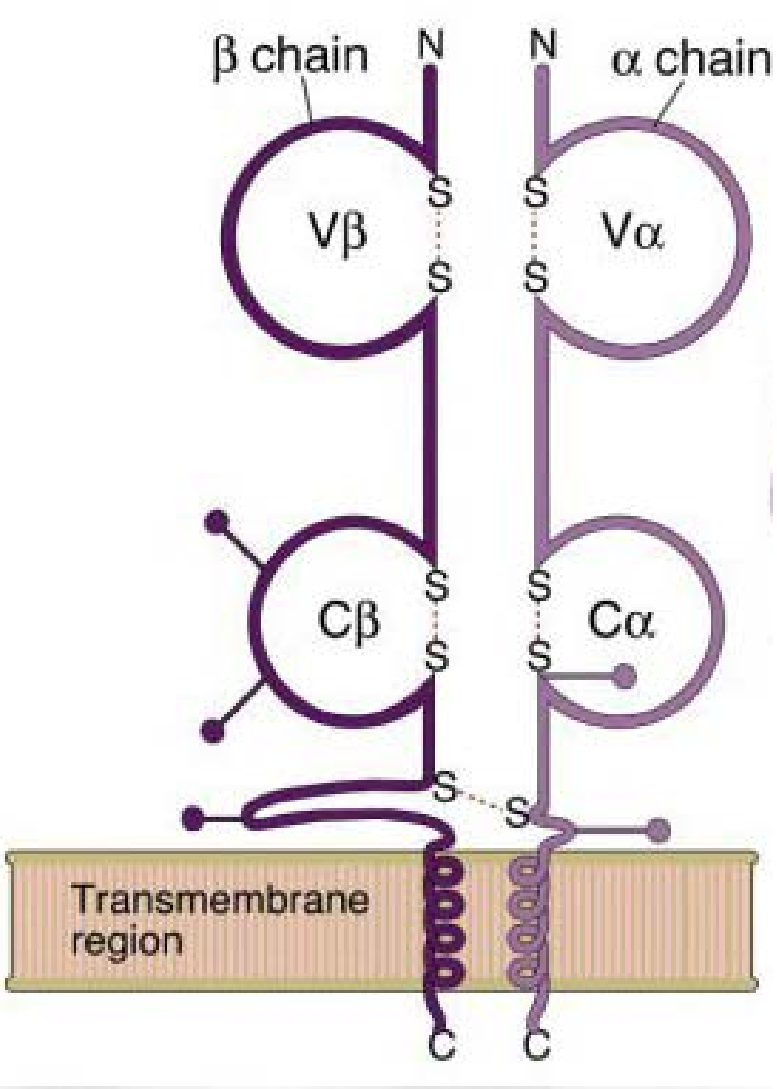
Estructura del TCR αβ

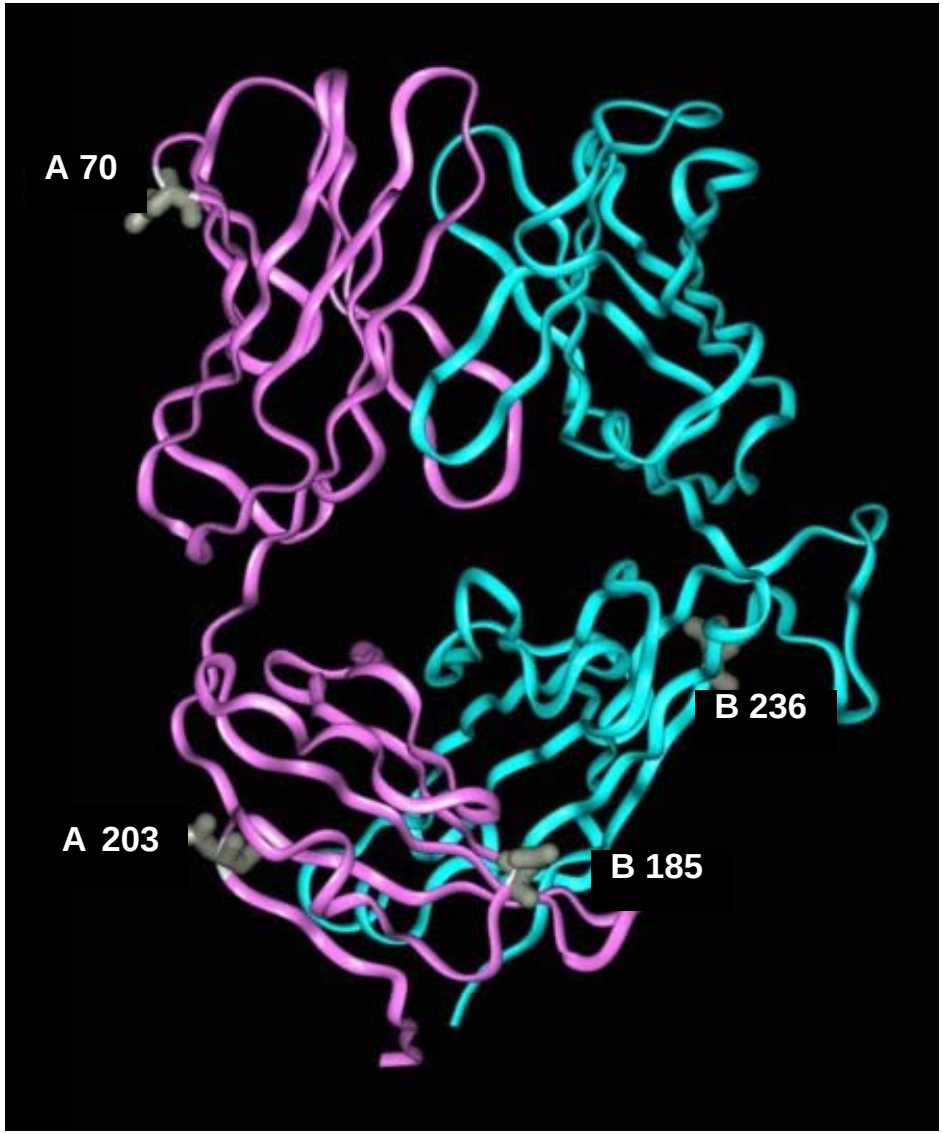
TCR Estructura (i)



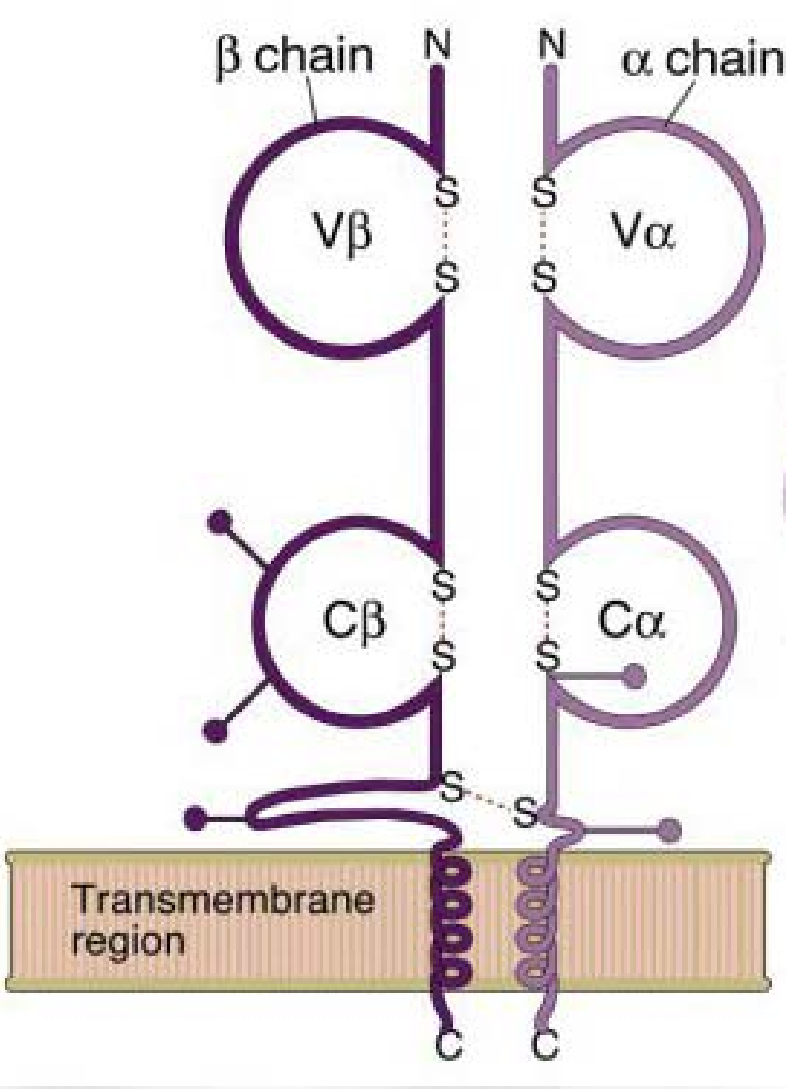


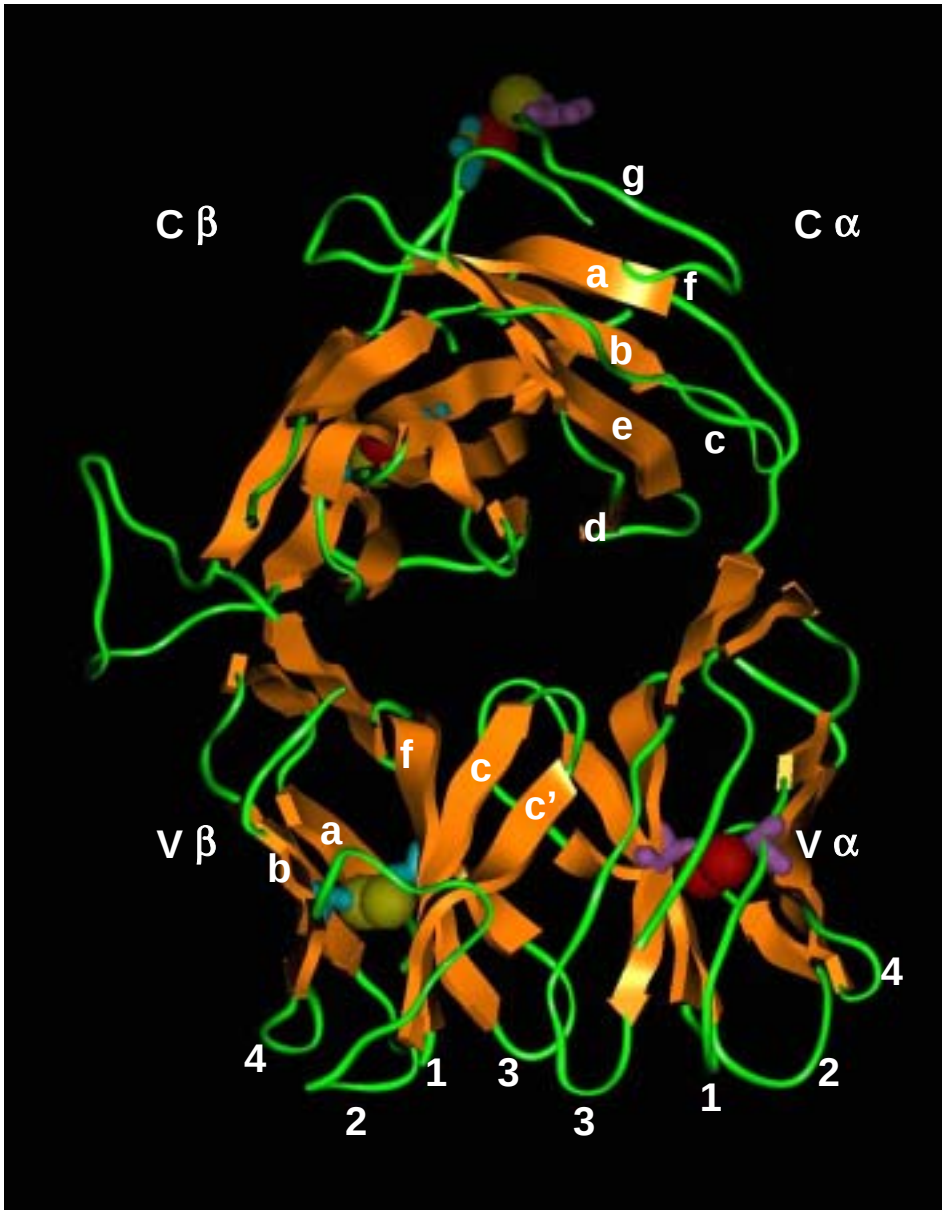
TCR Estructura (ii)





TCR Estructura (iii)





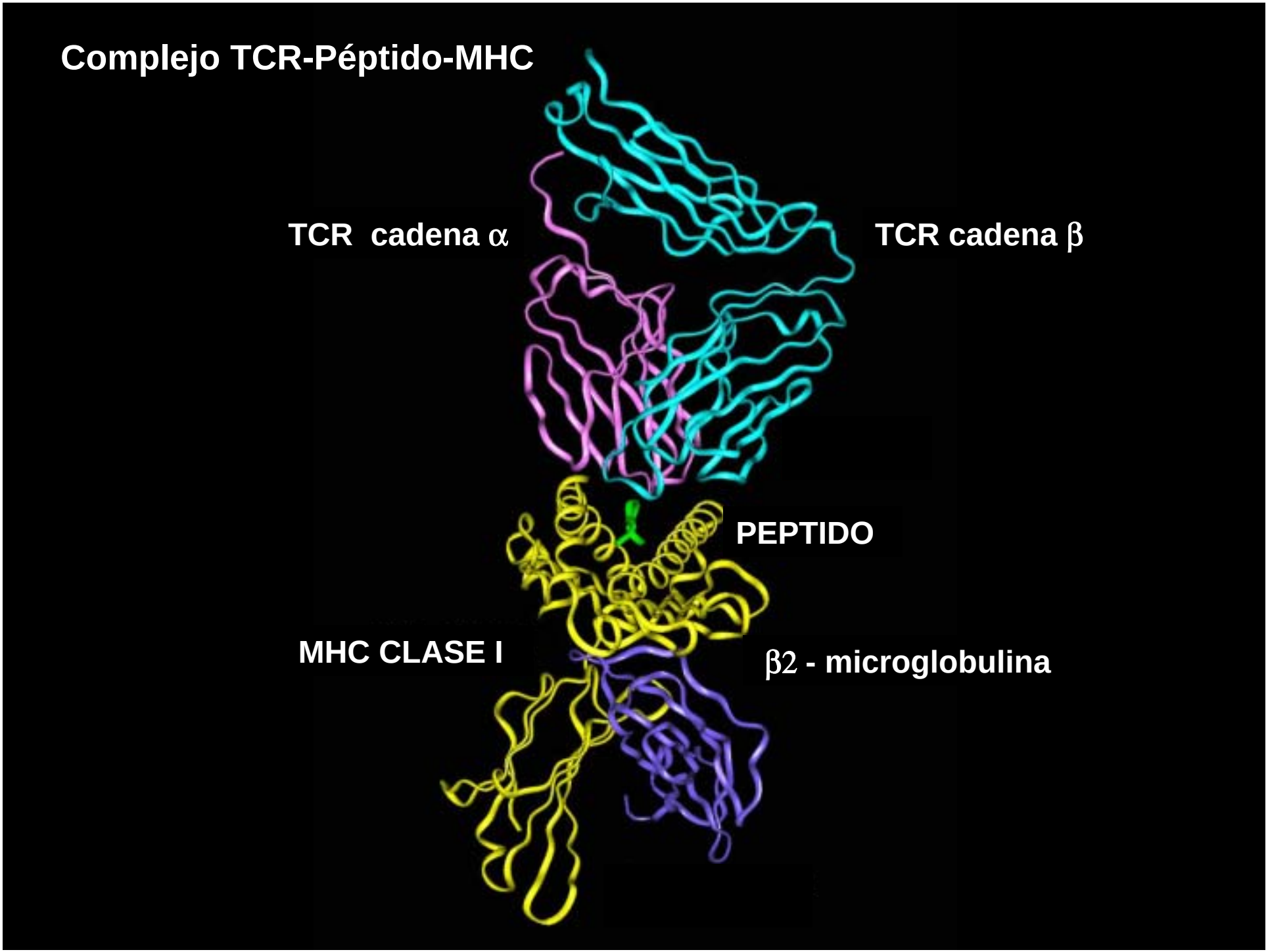
Complejo TCR-Péptido-MHC

TCR

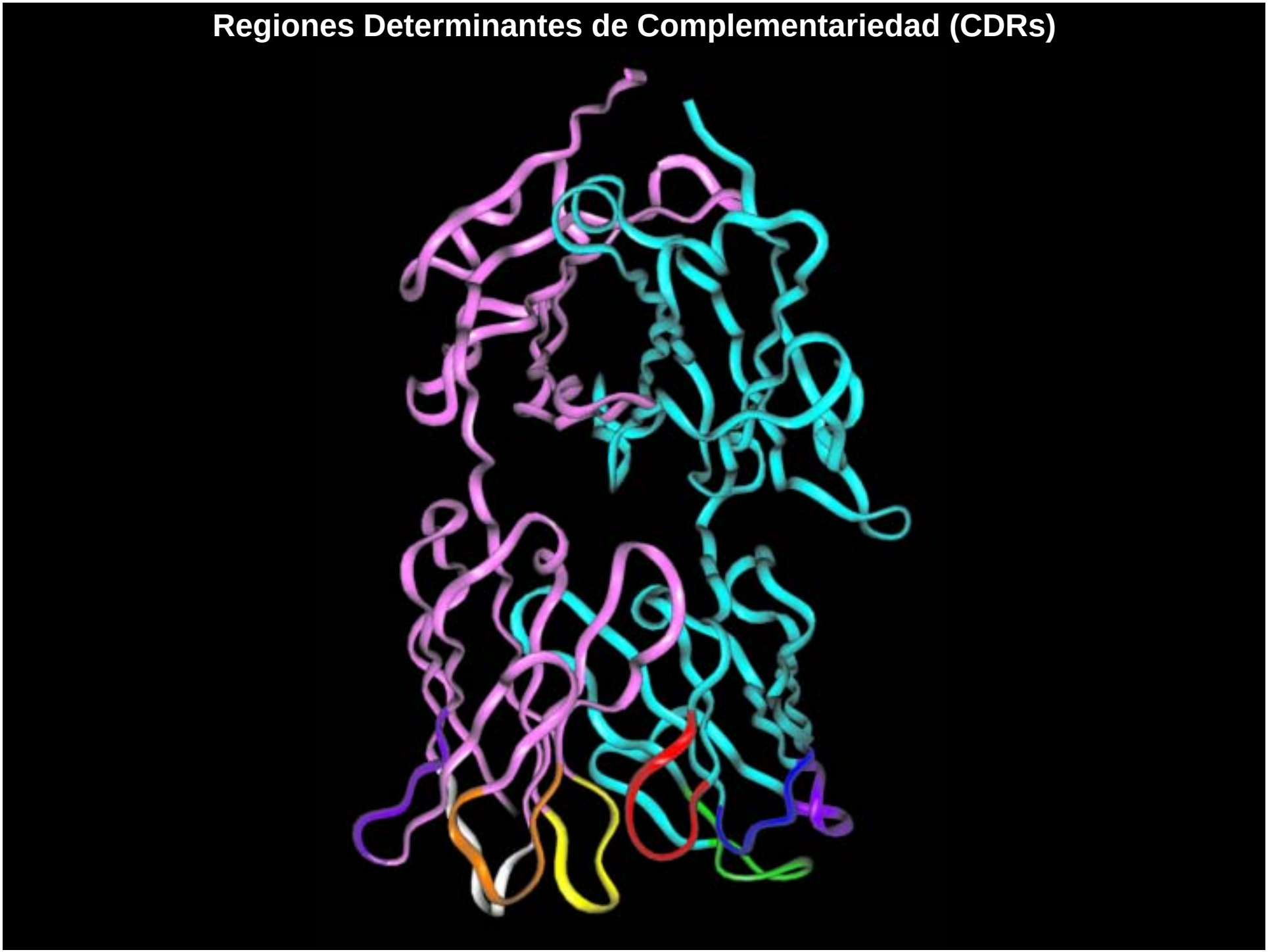
Péptido

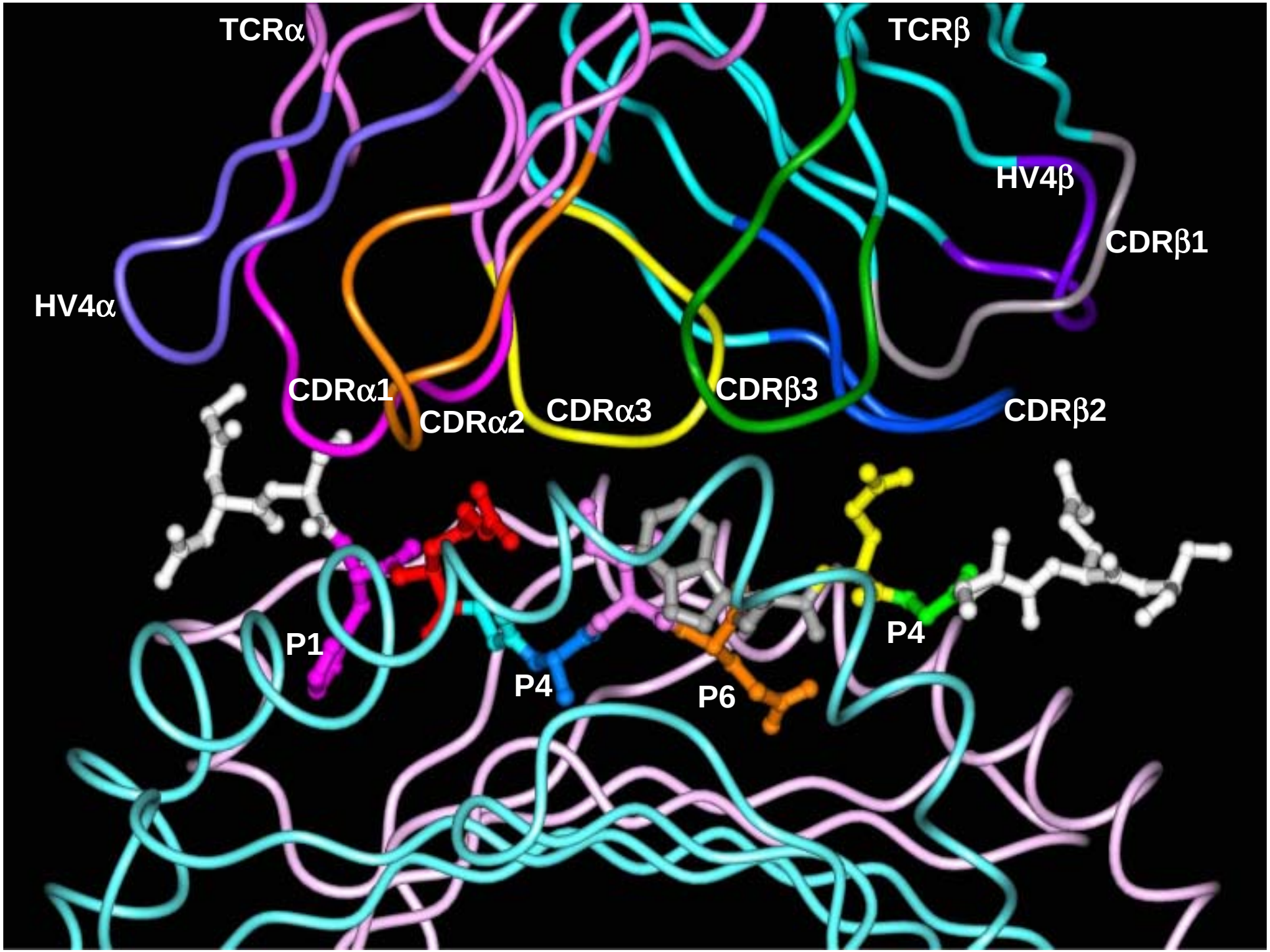
MHC

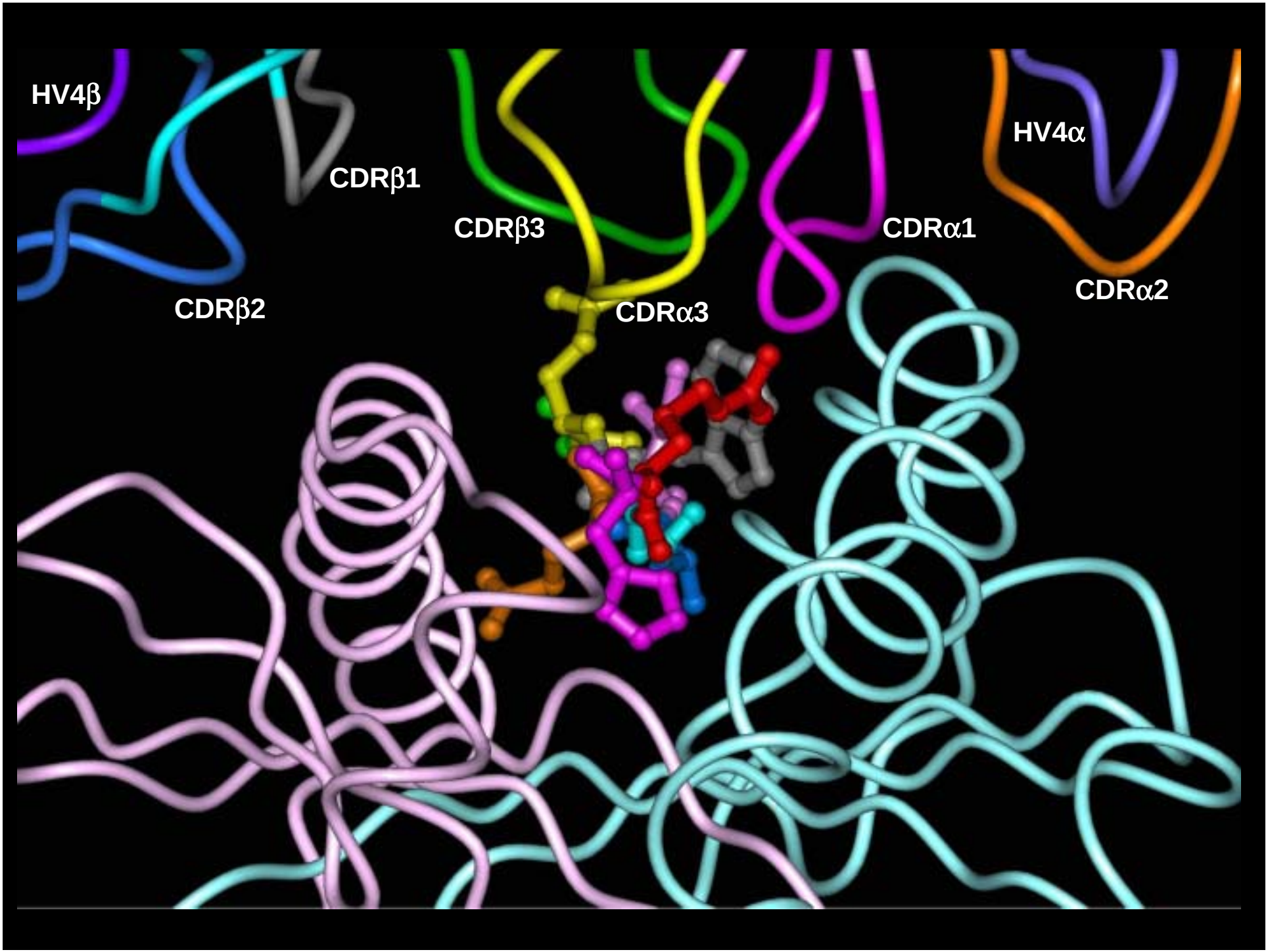
Complejo TCR-Péptido-MHC

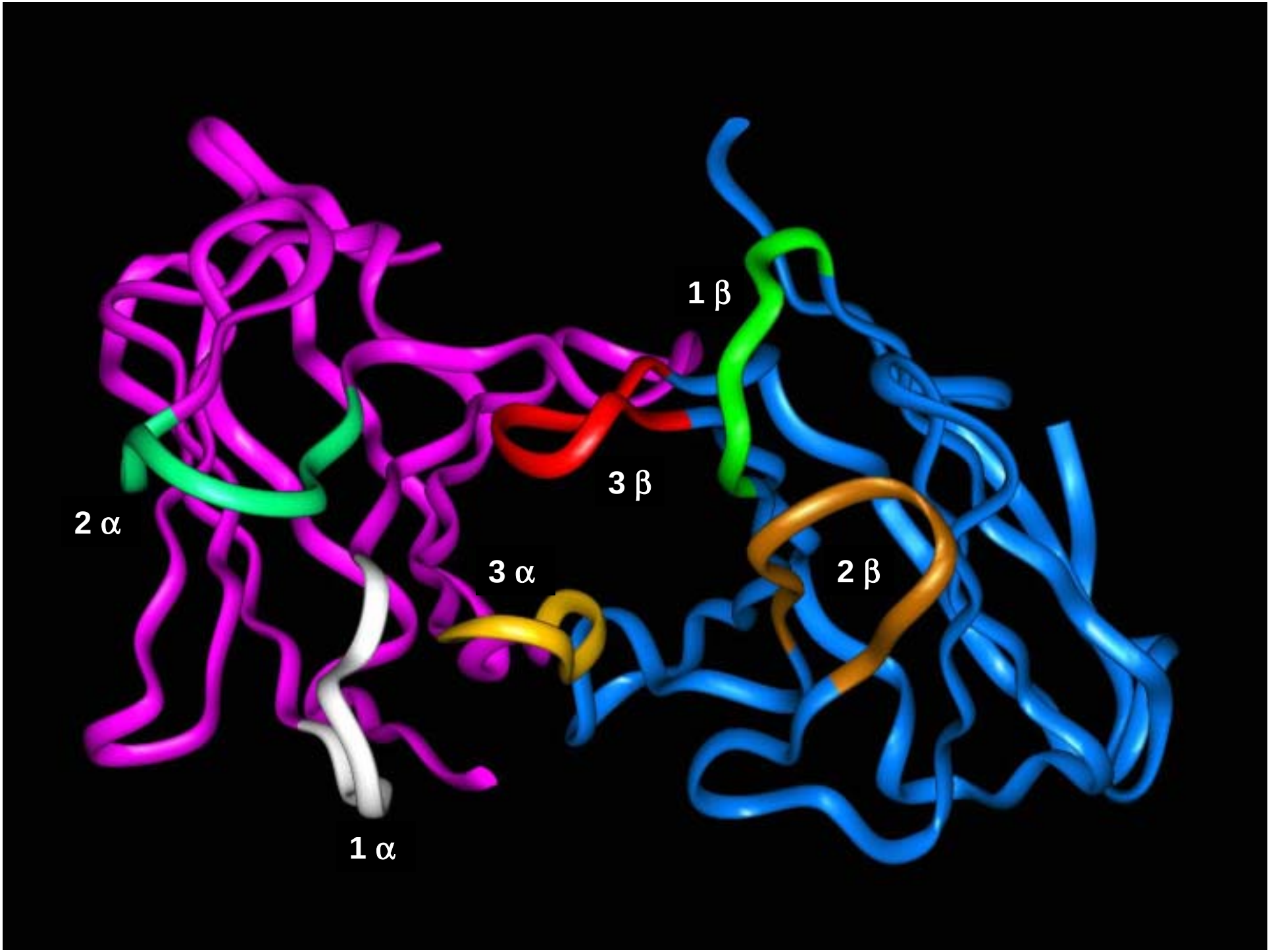


Regiones Determinantes de Complementariedad (CDRs)

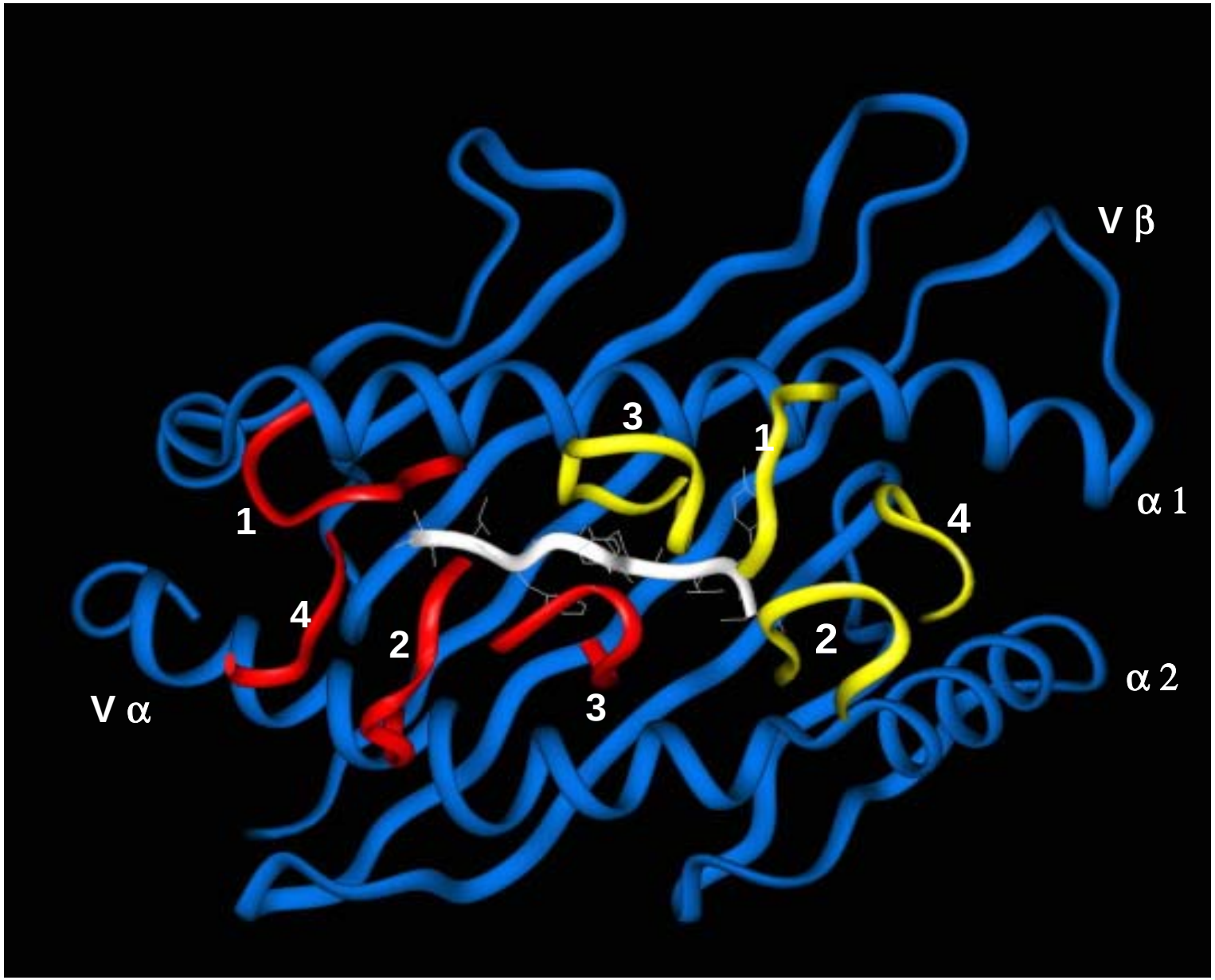



TCRα
TCRβ
HV4β
CDRβ1
HV4α
CDRα1
CDRα2
CDRα3
CDRβ3
CDRβ2
P1
P4
P6
P4


HV4β
CDRβ1
CDRβ3
CDRα1
HV4α
CDRβ2
CDRα3
CDRα2

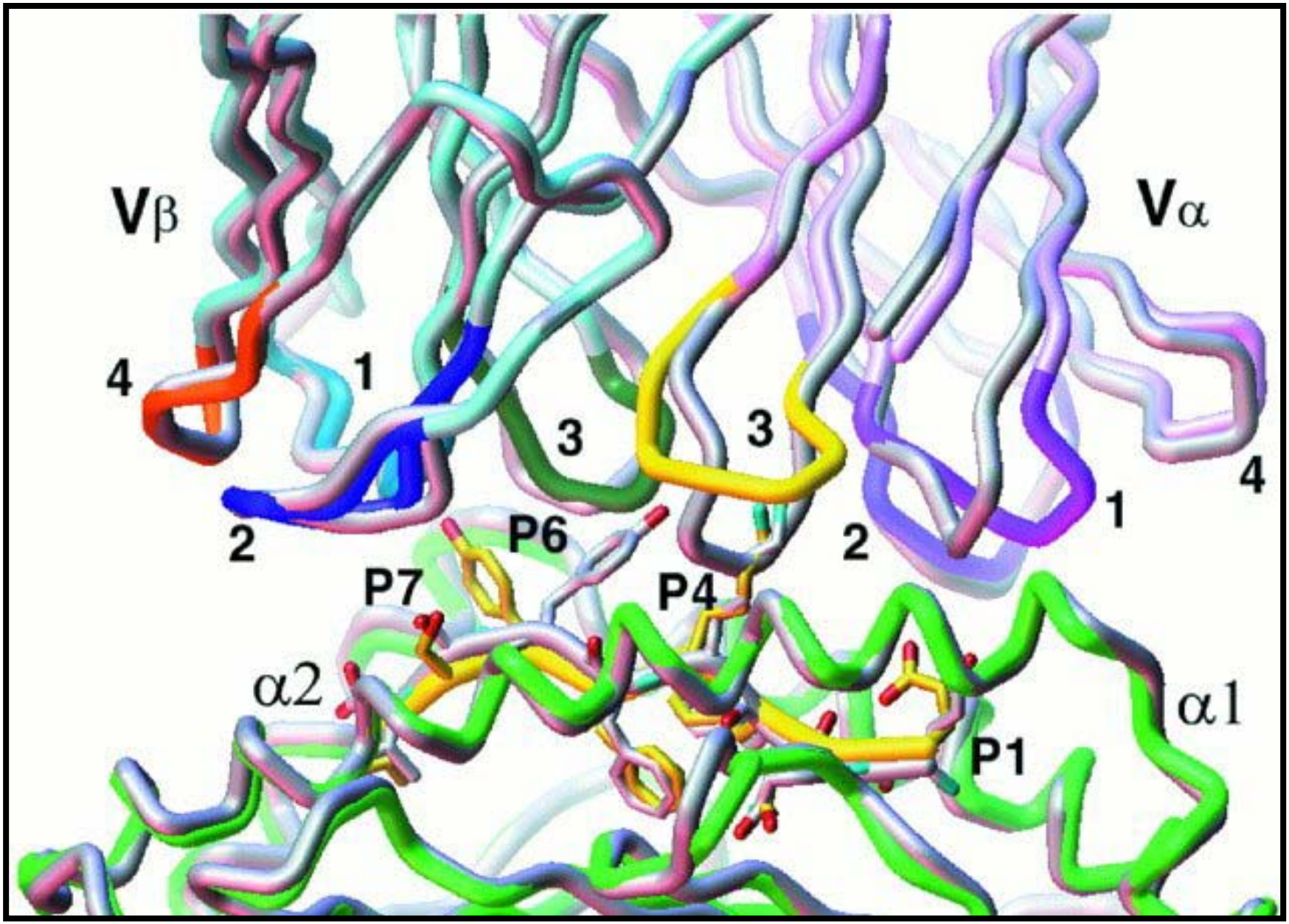

1 β
3 β
2 α
3 α
2 β
1 α

Complejo HLA – A2 – TAX – A.6



CLASE I: CAMBIOS PEPTIDO /DESOCUPADO
(2C / H-2Kb-dEV8)

Garcia KC *et al.* Science 1998.279:1166.



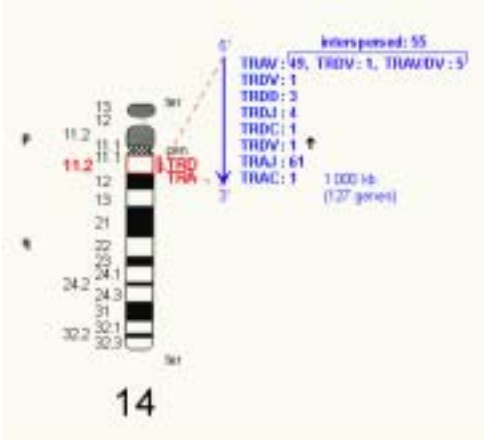
GENÉTICA DE LA DIVERSIDAD DEL TCR

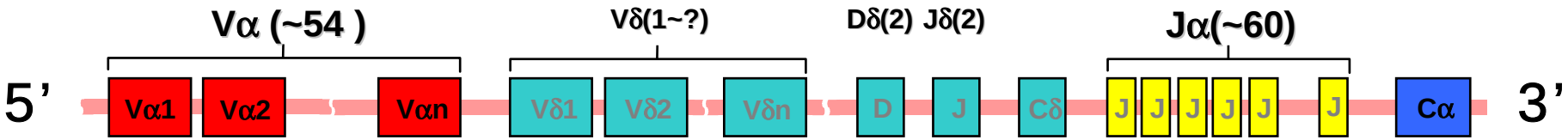
Mecanismos de Diversificación

1. Recombinación Somática
2. Diversidad de la unión
   - Diversidad en la Región N

El TCR está compuesto por dos cadenas, α y β que sufren recombinación somática

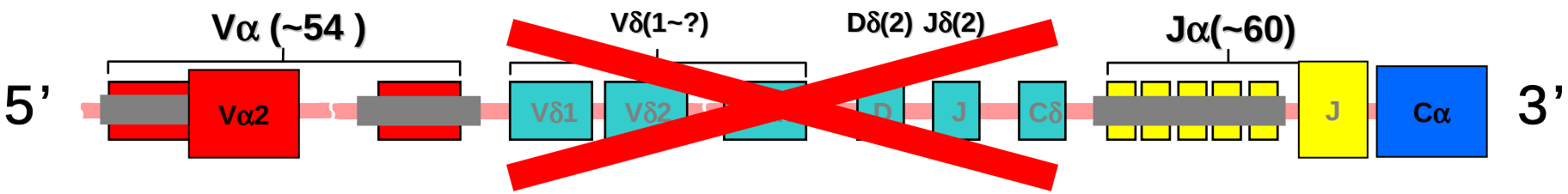
Cadena α: Rearreglo V-J

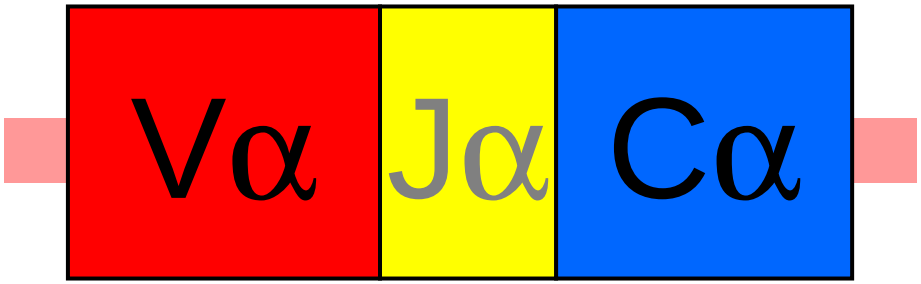




Janeway C.A. Immunobiology. Fifth edition, 2001

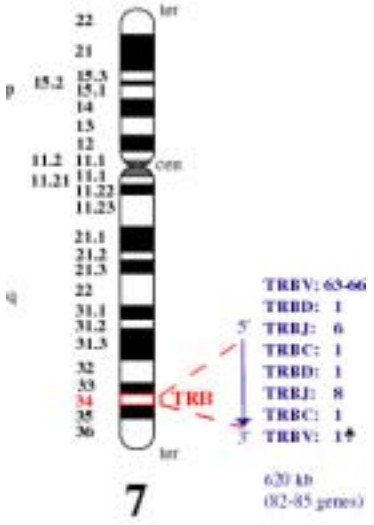
Cadena α: Rearreglo V-J

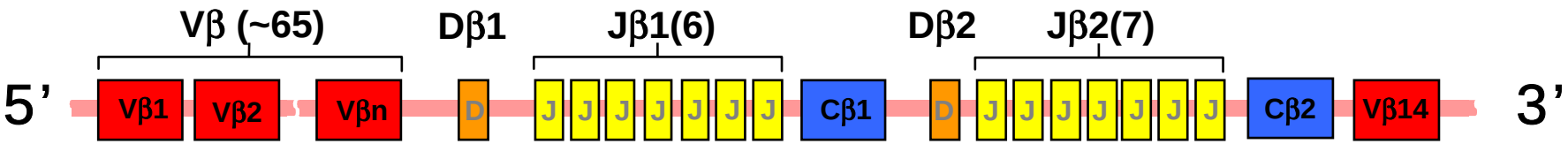




El TCR está compuesto por dos cadenas, α y β que sufren recombinación somática

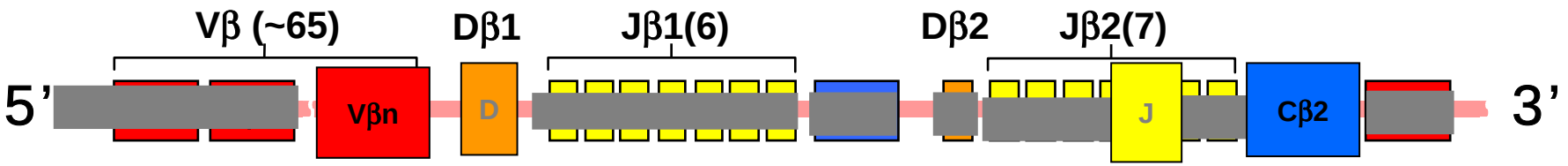
Cadena β: Rearreglo V-D-J

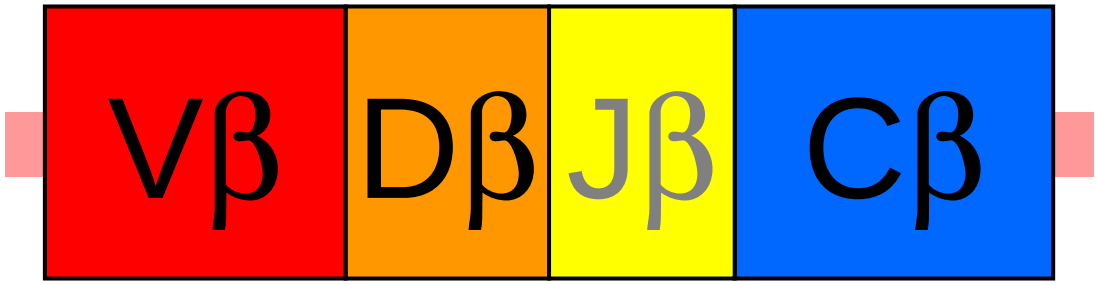




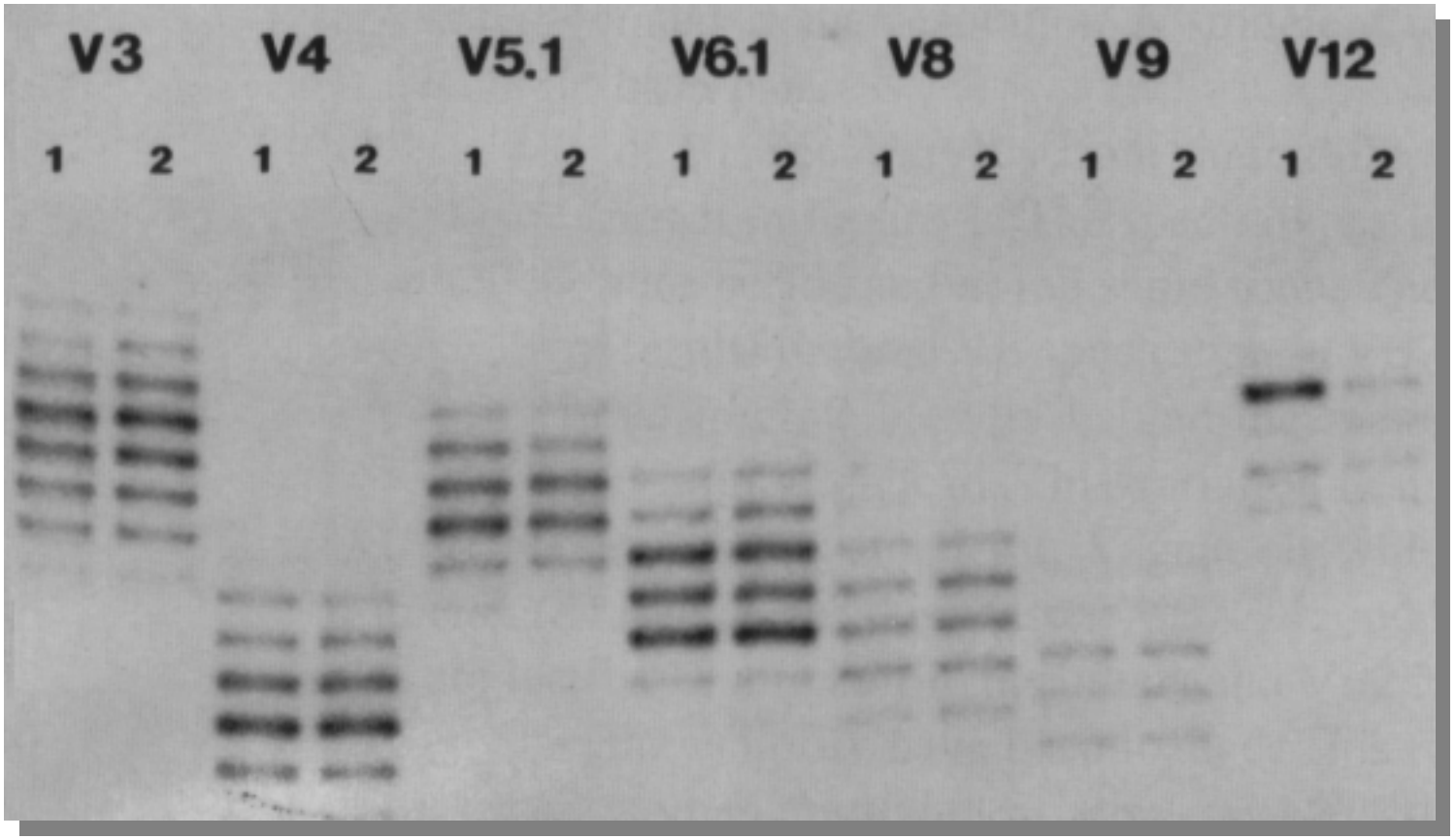
Janeway C.A. Immunobiology. Fifth edition, 2001

Cadena β: Rearreglo V-D-J



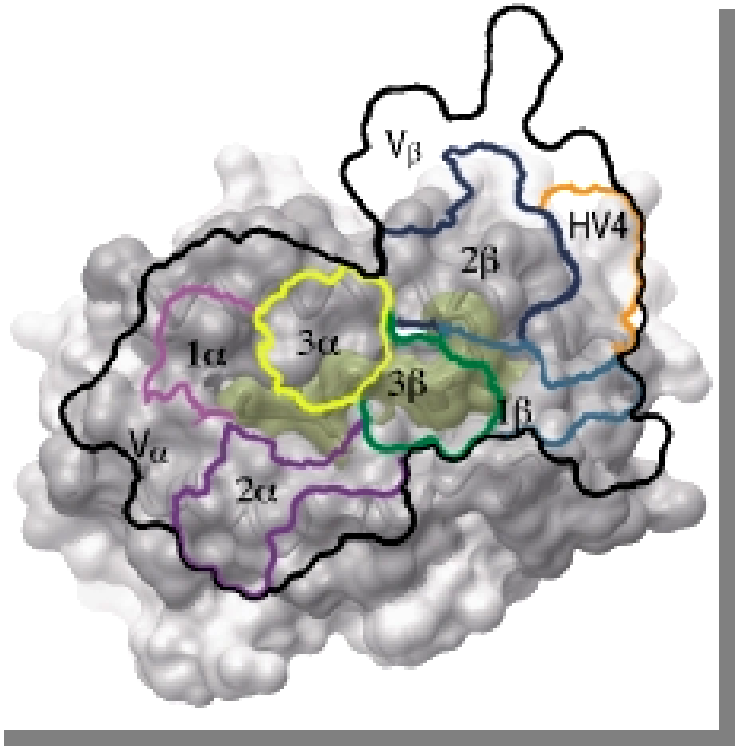


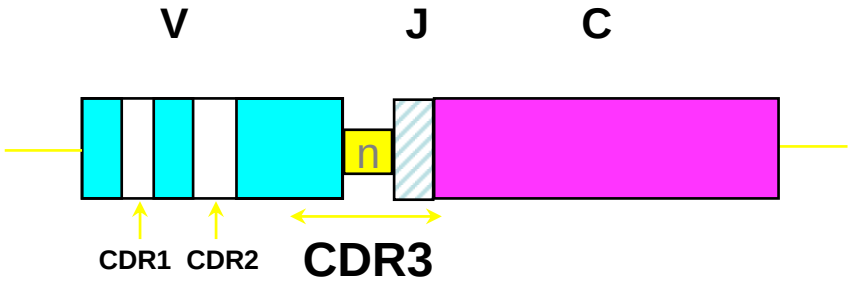
Los rearreglos VDJ funcionales siguen las variaciones del codón
- son múltiplos de 3 -



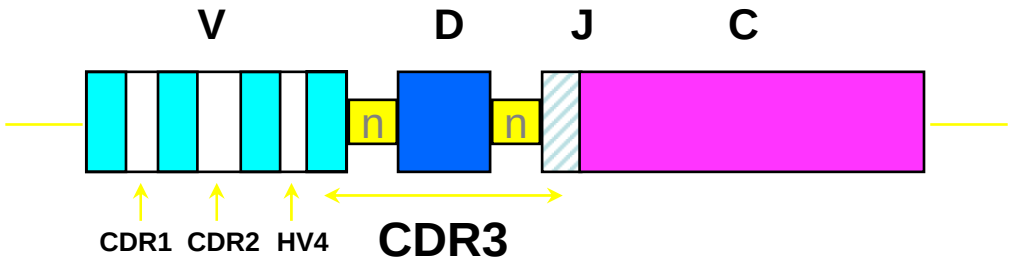
Los productos difieren en tamaño en 3 pares de bases

La Diversidad del TCR se centra principalmente en CDR3





Tamaño CDR3 α: 6 a 10 aminoácidos



Tamaño CDR3 β: 7 a 11 aminoácidos

Define un clon específico de linfocitos T

LaRoque *et al*. Human Immunol 1996; 48:3-11

Diversidad del TCR

Elemento	Cadena α	Cadena β
Segmentos V	~54	49
Segmentos D	0	2
Segmentos J	61	13
Uniones con N nucleótidos $\sum 4^n$	1 5461	2 5461^2
Pérdida de nucleótidos	7^2	7^4
Corrección por redundancia del codón(20aa/60 codones)	0.33	0.33
Combinación de cadenas individuales	$\sim 10^8$	$\sim 10^{12}$
Diversidad Total	$\sim 10^{20}$ (100.000.000.000.000.000.000)	

Lieber M.R. Site-Specific Recombination in the Immune System. FASEB J 1991; 5: 2934-2944.

LINFOCITOS T $\gamma\delta$

CARACTERÍSTICAS GENERALES

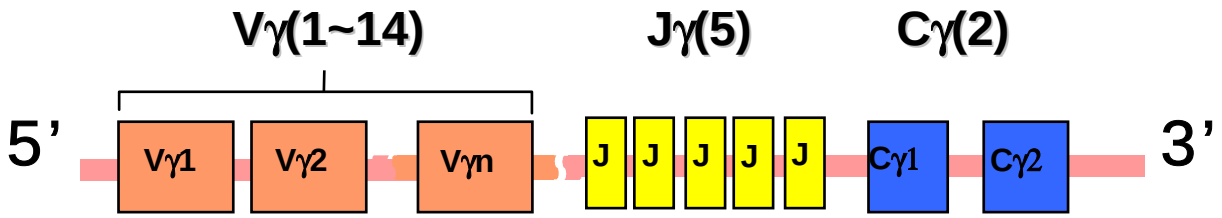
- Vinculados con respuesta inmune innata y adquirida.
- No necesitan presentación en contexto CMH.
- Reconocen moléculas no peptídicas como: moléculas hidrocarbonadas con residuos fosfato (IPP), HSP, MIC A y MIC B.

Comparación entre poblaciones de Linfocitos T

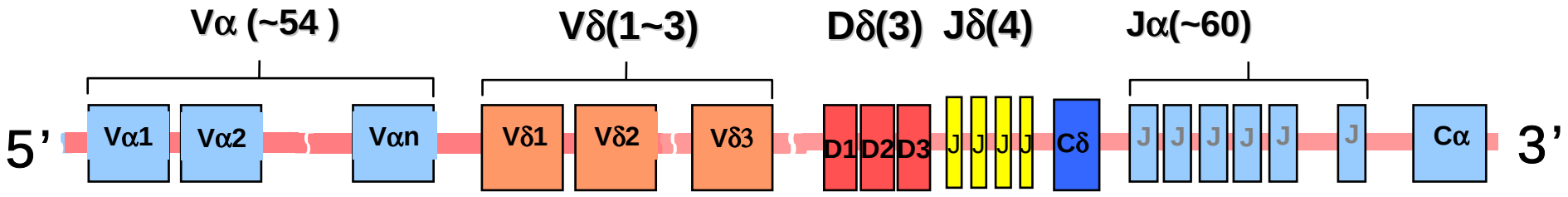
Receptor	**TCR αβ**	**TCR γδ**
Estructura	Heterodímero de cadena α y β	Heterodímero de cadena γ y δ
Antígeno que reconoce	Péptido corto presentado por MHC clase I y II	HSP, Fosfatos, aminas, MHC no clásicas
Moléculas accesorias	CD4 y CD8	Ninguna

Locus de las cadenas γ y δ del TCR Humano

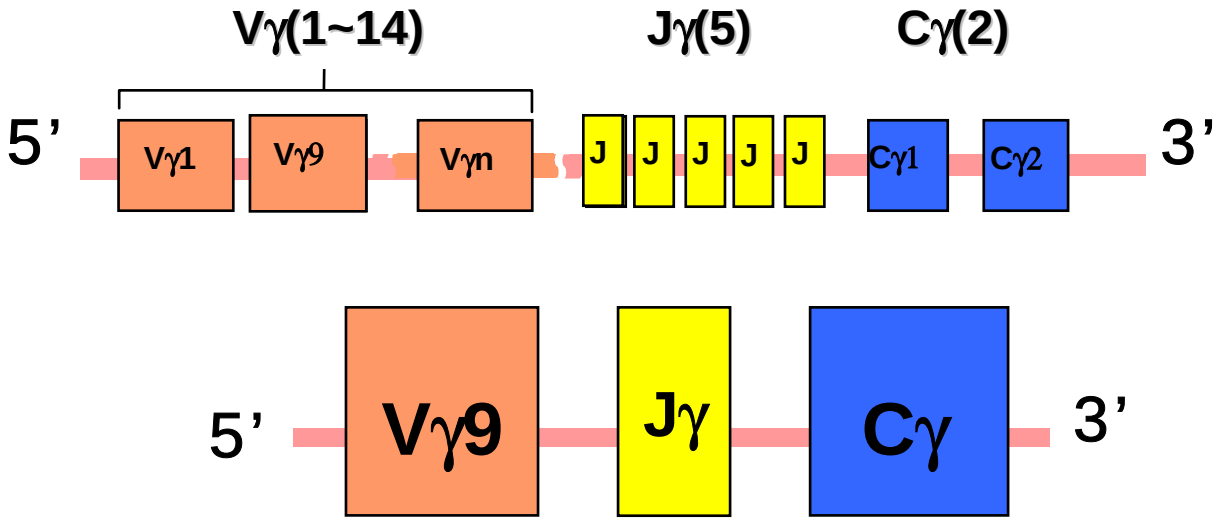
Locus TCR γ (Cromosoma 7)



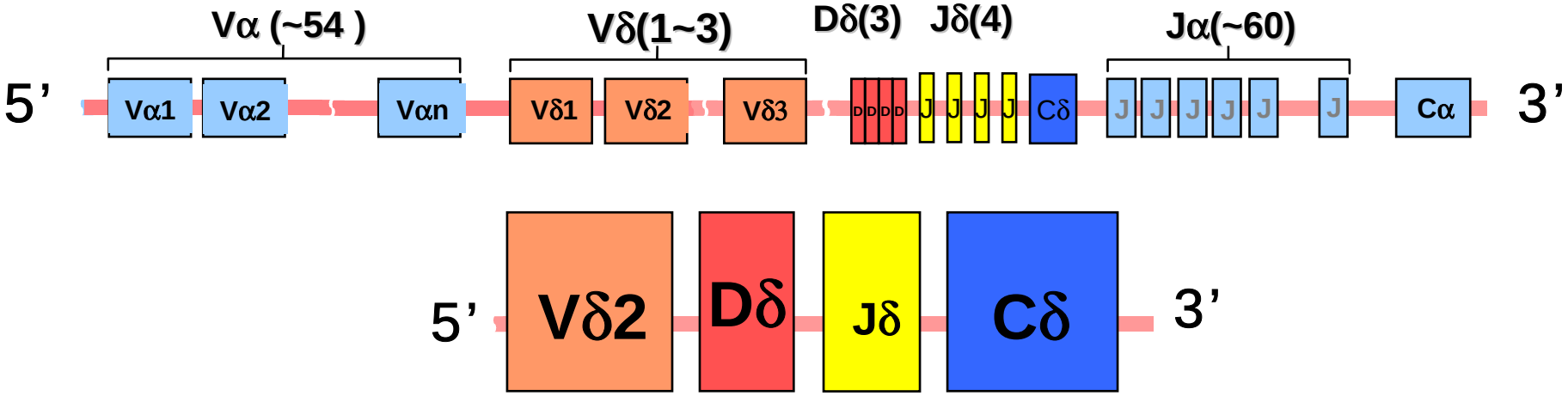
Locus TCR δ (Cromosoma 14)



Rearreglo de la cadena γ del TCR



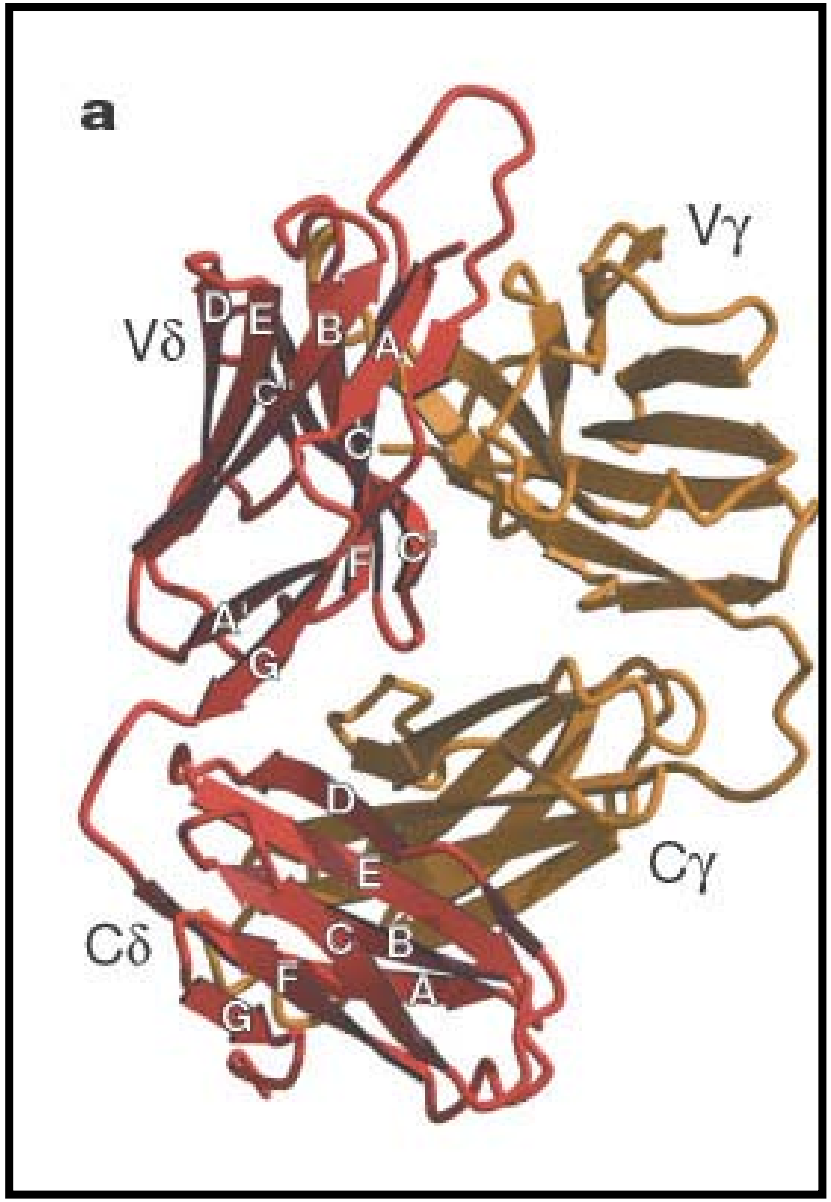
Rearreglo de la cadena δ del TCR



Subgrupos de los LT γ δ en el cuerpo humano

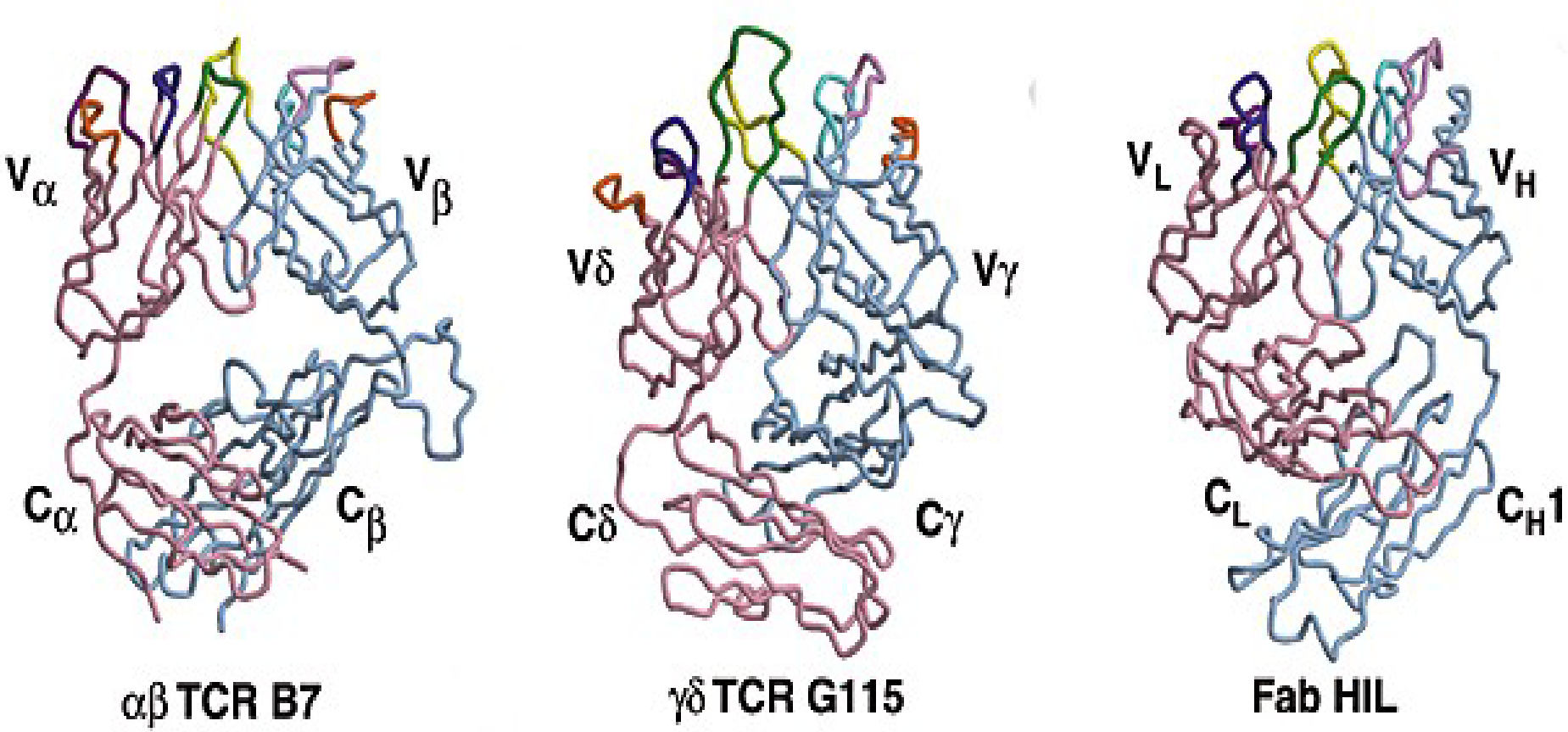
- **Subgrupo 1 comprende Vγ2, Vγ3, Vγ4, Vγ5 y Vγ8)**
- **Subgrupo 2 comprende Vγ9**
- **Subgrupo 3 comprende Vγ10**
- **Subgrupo 3 comprende Vγ11**

TCR γδ



Allison TJ *et al.*Nature 2001. 411:820.

Diferentes Receptores Antigénicos



Wilson, I. Stanfield, R. Nature Immunology 2001Vol 2 No 7: 579

Estudios de los Linfocitos T de los monos *Aotus*

Immunogenetics (1998) 48: 253–259

ORIGINAL PAPER

Nicolas Favre · Claudia Daubenberger · Jutta Marfurt
Alberto Moreno · Manuel P

Sequence and dive T-cell receptor alpha *V*, *J*, and *C* the owl monkey *Aotus nancymaae*

Abstract We cloned and sequenced TcR alpha chain cDNA of three healthy *Aotus nancymaae* monkeys. Fifteen different *TRAJ* segments and 9 different *TRAV* genes were identified in the 29 rearrangements analyzed. As expected from the greater phylogenetic distance, *A. nancymaae TRA* gene sequences diverged more from the human sequences than those of the chimpanzee or the rhesus macaque. However, no *Aotus TRAJ* segment or *TRAV* gene was found which lacked a human counterpart. These counterparts were *AJ02, AJ05, AJ09, AJ15, AJ22, AJ23, AJ28, AJ30, AJ32, AJ34, AJ37, AJ40, AJ42, AJ45, AJ52* and *AV2S1, AV2S3, AV3S1, AV8S1, AV12S1, AV15S1, ADV21S1, DV5, AV22S1S* cases the identity greater than responding *Aotu* 80%. This mark close structural relationship of *Ao-* R and demonstrates that the TcR re- es is remarkably stable. The results t of using *Aotus* monkeys, which are susceptible to infection with the human malaria parasite *Plasmodium falciparum*, as an animal model for the evaluation of molecularly defined malaria vaccine candidates.

80%.

Key words *Aotus nancymaae* · T-cell receptor alpha · *TRAV, TRAJ,* and *TRAC* genes

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank nucleotide sequence databases and have been assigned the accession numbers AF027541-AF027569 and AF 051312

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Immunogenetics (1999) 49:792–799

ORIGINAL PAPER

William Vecino · Claudia Daubenberger
Raul Rodriguez · Alberto Moreno
Manuel Patarroyo · Gerd Pluschke

Sequence and diversity of T-cell receptor β-chain *V* and *J* owl monkey *Aotus nancymaae*

Abstract The New World primate *Aotus nancymaae* is susceptible to infection with the human malaria parasite *Plasmodium falciparum* and has therefore been recommended by the World Health Organization as a model for the evaluation of malaria vaccine candidates. Recently, we have shown that *Aotus TCRVA* genes and *TCRJA* segments exhibit a high degree of similarity to human counterparts. In the present report we used reverse transcription polymerase chain reaction to analyze the sequences of *A. nancymaae TCR* β-chain gene rearrangements. Alignment with human sequences and phylogenetic comparison identified 18 distinct *Aotus TCRBV* genes homologous to the human *TCRBV* gene families *2, 4, 5, 6, 7, 9, 12, 15, 24,* and *28*. Multiple *Aotus* genes were found in the *TCRBV4, 5, 6,* and *7* families. Some of th
human gen
man homo
TCRBV ge
human cou
ments homologous to the human segments J1-1, J1-2, J1-4, J1-5, J1-6, J2-1, J2-2, J2-3, J2-4, J2-5 were found. In some cases the amino acid sequences of *Aotus* and human *TCRBJ* segments were completely identical. A comparison of the proportion of synonymous and nonsynonymous substitutions in *Aotus* vs human β-chain-encoding genes revealed a dominance of synonymous substitutions in *TCRBJ* segments and of nonsynonymous substitutions in *TCRBV* segments. Dominance of nonsynonymous substitutions was more pronounced in *TCRBV* CDR1 and CDR2 regions than in the framework regions. No evidence for the emergence of new *TCRBJ* segments or *TCRBV* families was found. These results confirm that the TCR repertoire in primates is remarkably stable and support the concept of using *Aotus* monkeys as an infection model for the evaluation of future subunit vaccine candidates.

· T-cell receptor
BJ genes

77 to 90% identical

Functional and Structural Similarities of Vγ9Vδ2 T Cells in Humans and *Aotus* Monkeys, a Primate Infection Model for *Plasmodium falciparum* Malaria[1,2]

Claudia A. Daubenberger,[3]* Maxence Salomon,* William Vecino,† Beatrice Hübner,* Heike Troll,‡ Raul Rodriques,† Manuel E. Patarroyo,† and Gerd Pluschke*

γδ T cells are implicated to play crucial roles during early immune responses to pathogens. A subset of human γδ T cells carrying the Vγ9Vδ2 TCR recognize small, phosphorylated nonpeptidic Ags. However, the precise role of these cells and the ligands recognized in human immune responses against pathogens remains unclear because of the lack of suitable animal models. We have analyzed the reactivity of spleen cells of the New World monkey *Aotus nancymaae* against isopentenyl pyrophosphate (IPP), a phosphorylated microbial metabolite selectively activating Vγ9Vδ2 T cells. Spleen cells were stimulated by IPP and the expanding cell population expressed the Vγ9 TCR. *TRGV-J* and *TRDV-D-J* rearrangements expressed by IPP-stimulated cells of *Aotus* were analyzed by RT-PCR and DNA sequencing. The *TRGV-J* and *TRDV-D-J* rearrangements expressed by IPP-stimulated *Aotus* and human γδ T cells were similar with respect to 1) TCR gene segment usage, 2) a high degree of germline sequence homology of the TCR gene segments used, and 3) the diversity of the CDR3 regions. Phylogenetic analysis of human, *Pan troglodytes*, and *A. nancymaae TRGV* gene segments showed that the interspecies differences are smaller than the intraspecies differences with *TRGV9* gene segments located on a distinct clade of the phylogenetic tree. The structural and functional conservation of Vγ9Vδ2 T cells in *A. nancymaae* and humans implicates a functionally important and evolutionary conserved mechanism of recognition of phosphorylated microbial metabolites. *The Journal of Immunology*, 2001, 167: 6421–6430.

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Chara T-cell receptor γ-variable gene in *Aotus nancymaae* owl monkey peripheral blood

Key words:
Aotus; evolution; γδ T lymphocytes; Platyrrhini; T-cell receptor

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We are greatly indebted to P. Cárdenas, Y.P. de Coaña, F. Carrillo, M. Mancera, and F. Guzmán for their valuable help in preparing this manuscript, to R. Rodríguez for his help in obtaining the monkey blood samples, and to J. Garry for patiently revising the manuscript. This work was financed by the Colombian Ministry of Public Health.

Abstract: γδ T lymphocytes have a heterodimeric complex formed by the association of γ and δ chains as receptor. Proliferation of this lymphocyte population has been observed, when infection by several pathogens such as *Mycobacterium tuberculosis* and *Plasmodium* spp. occurs. The New World Monkey *Aotus nancymaae* has become a very good experimental model for the immunological and physiopathological study of these infectious agents. The *A. nancymaae* γ-variable region was characterized from peripheral blood samples by using cDNA and genomic DNA polymerase chain reaction amplification, DNA sequencing, and dot-blot hybridization techniques. Seventeen different T-cell receptor γ-variable (TCRGV) sequences were obtained. These sequences were distributed among TCRGV subsets 1, 2, or 3, according to human subset classification. Although no subset 4 amplification was obtained, this subset was detected by dot-blot hybridization. The presence of these 4 subsets resembles the behavior displayed by 'γδ-low sp
c
h
1 and 3 highlights *Aotus* as a promising model for studying these lymphocyte functions.

Homologies greater than 70%

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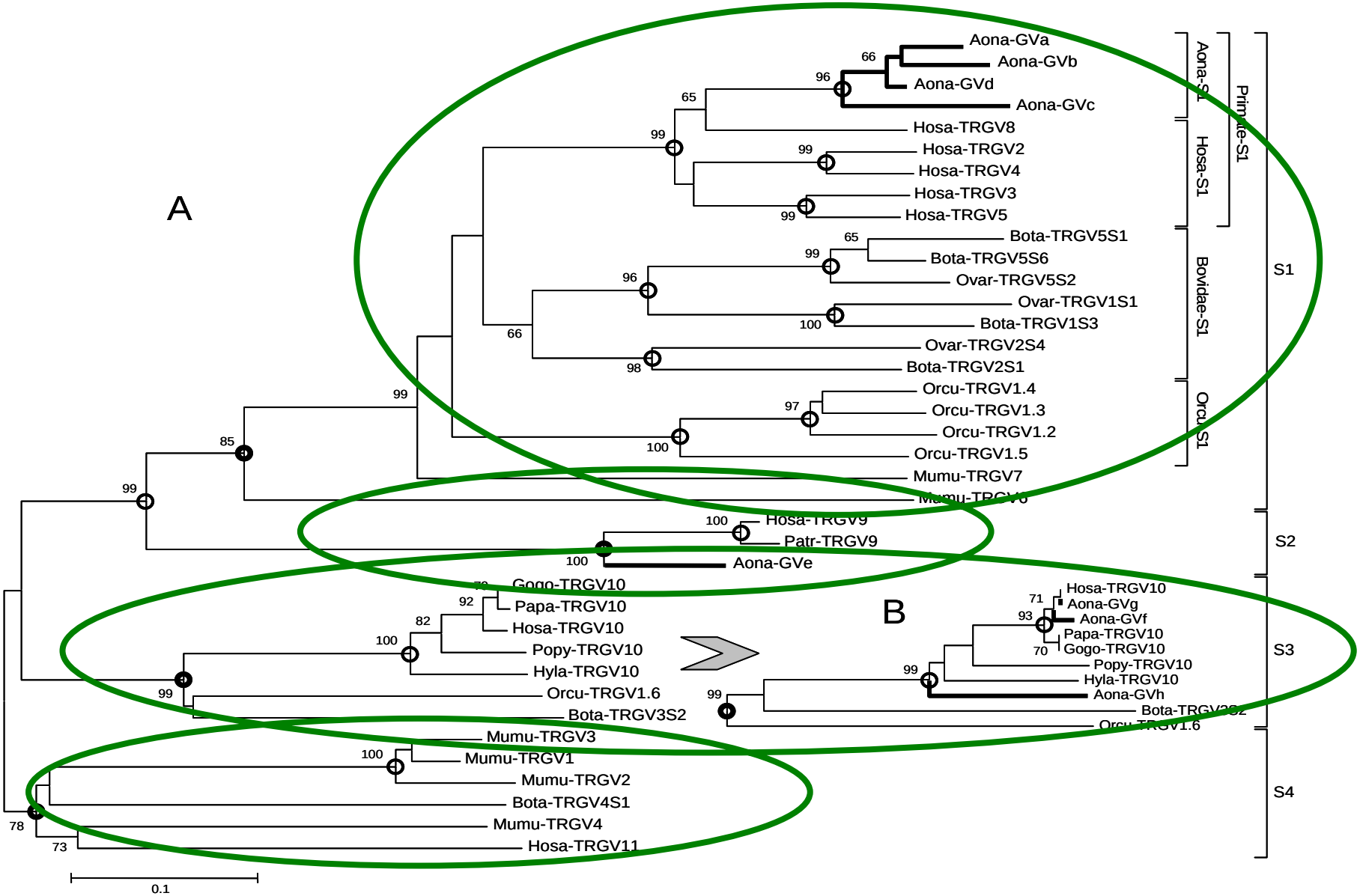
ANÁLISIS EVOLUTIVO



HOMOLOGÍAS

subgrupos	Porcentaje homología *Aotus* y humano (%)	
	Nucleótidos	Aminoácidos
2	>90	>90
3	> 90	>90
1	76-83	72-84

T cell receptor in primates: Identifying and sequencing new owl monkey *TRBV* subgroups

Camilo A. Moncada [1,§], Eduar Guerrero [1], Paula Cardenas [1], Carlos F. Suarez [2,§], Manuel E. Patarroyo [1,3], Manuel A. Patarroyo [1,3,*]

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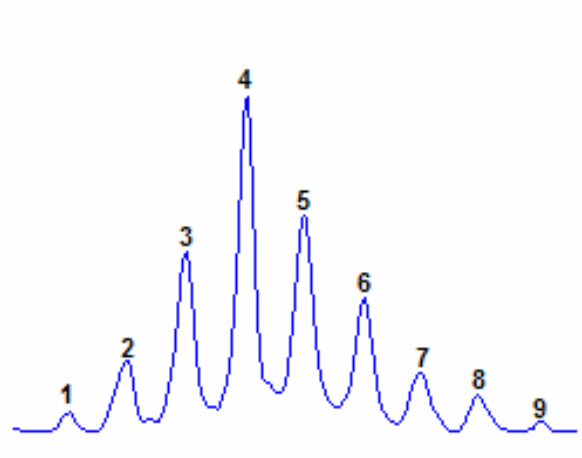
Enviado a Immunogenetics

Familias del TCRVB identificadas en *Aotus*

Aotus TRBV	Human TRBV	Nucleotide		Amino acid	
		Length	Homology	Length	Homology
AoBV2	TRBV2-1*01	255	82	85	84
AoBV3	TRBV3-1*01	255	86	85	87
AoBV4-1*	TRBV4-1*01	255	83	85	86
AoBV5-1*	TRBV5-1*01	252	80	83	78
AoBV6-1*	TRBV6-5*01	258	84	86	82
AoBV7-1*	TRBV7-6*01	255	81	85	80
AoBV9*	TRBV9-1*01	255	84	85	81
AoBV10	TRBV10-2*01	255	80	85	76
AoBV11*	TRBV11-2*01	237	78	79	78
AoBV12	TRBV12-5*01	252	83	83	85
AoBV14	TRBV14*01	258	82	85	80
AoBV15	TRBV15-1*01	252	82	83	83
AoBV18	TRBV18*01	270	88	90	92
AoBV19	TRBV19*01	252	86	84	87
AoBV20	TRBV20-1*02	258	81	86	79
AoBV24	TRBV24*01	255	78	85	79
AoBV25	TRBV25-1*01	252	83	83	81
AoBV27	TRBV27*01	255	87	85	89
AoBV28	TRBV28*01	255	83	85	83
AoBV29*	TRBV29-1*01	258	82	86	81
AoBV30	TRBV30*04	252	84	84	83
AoJB2-7	TRBJ 2-7*01	47	94	15	87

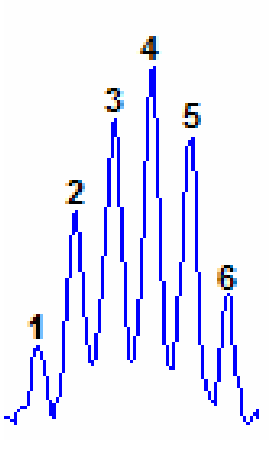
Estudio del repertorio de los Linfocitos T αβ por espectratipificación

Humano Vβ7



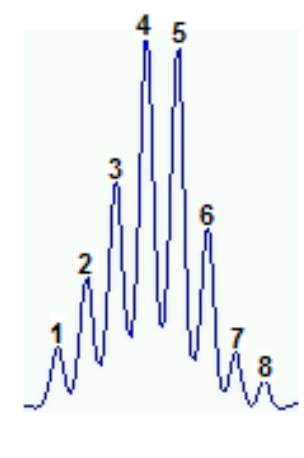
Num.	Size
1	183.87
2	186.73
3	189.59
4	192.54
5	195.26
6	198.2
7	200.92
8	203.7
9	206.83

Humano Vβ15



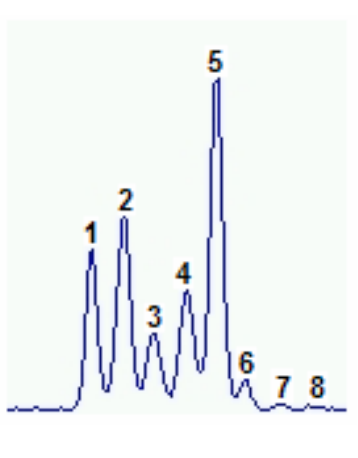
Num.	Size
1	353.18
2	356.04
3	359.03
4	361.81
5	364.86
6	367.68

Aotus Vβ6



Num.	Size
1	349.12
2	351.76
3	354.47
4	357.32
5	360.31
6	363.09
7	365.77
8	368.59

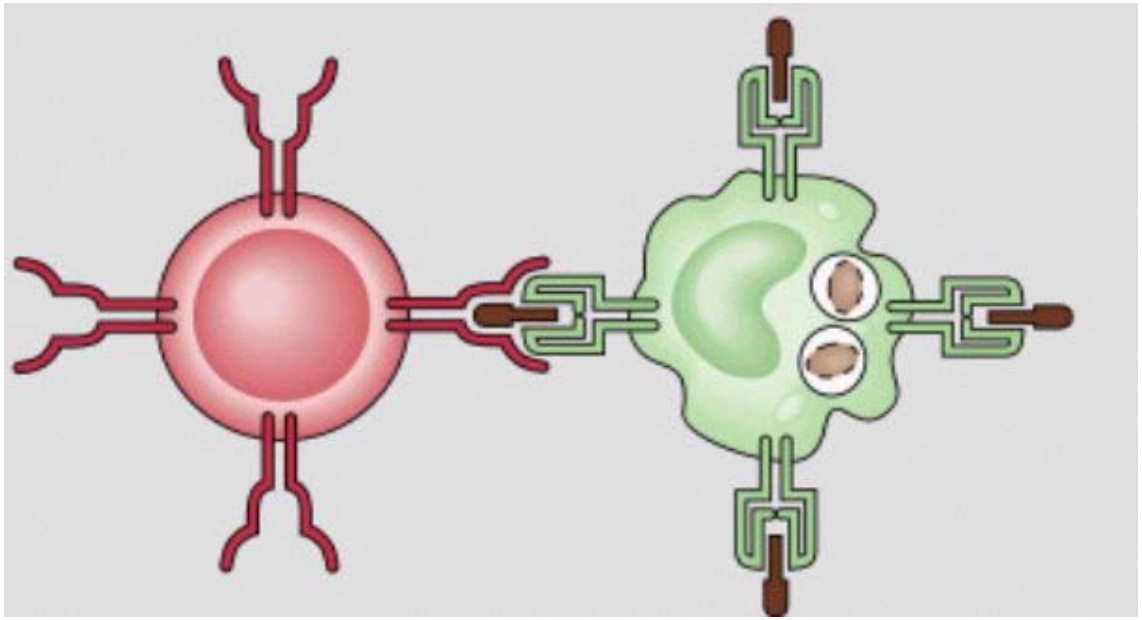
Aotus Vβ5



Num.	Size
1	350.35
2	353.19
3	355.93
4	358.92
5	361.7
6	364.38
7	367.45
8	370.05

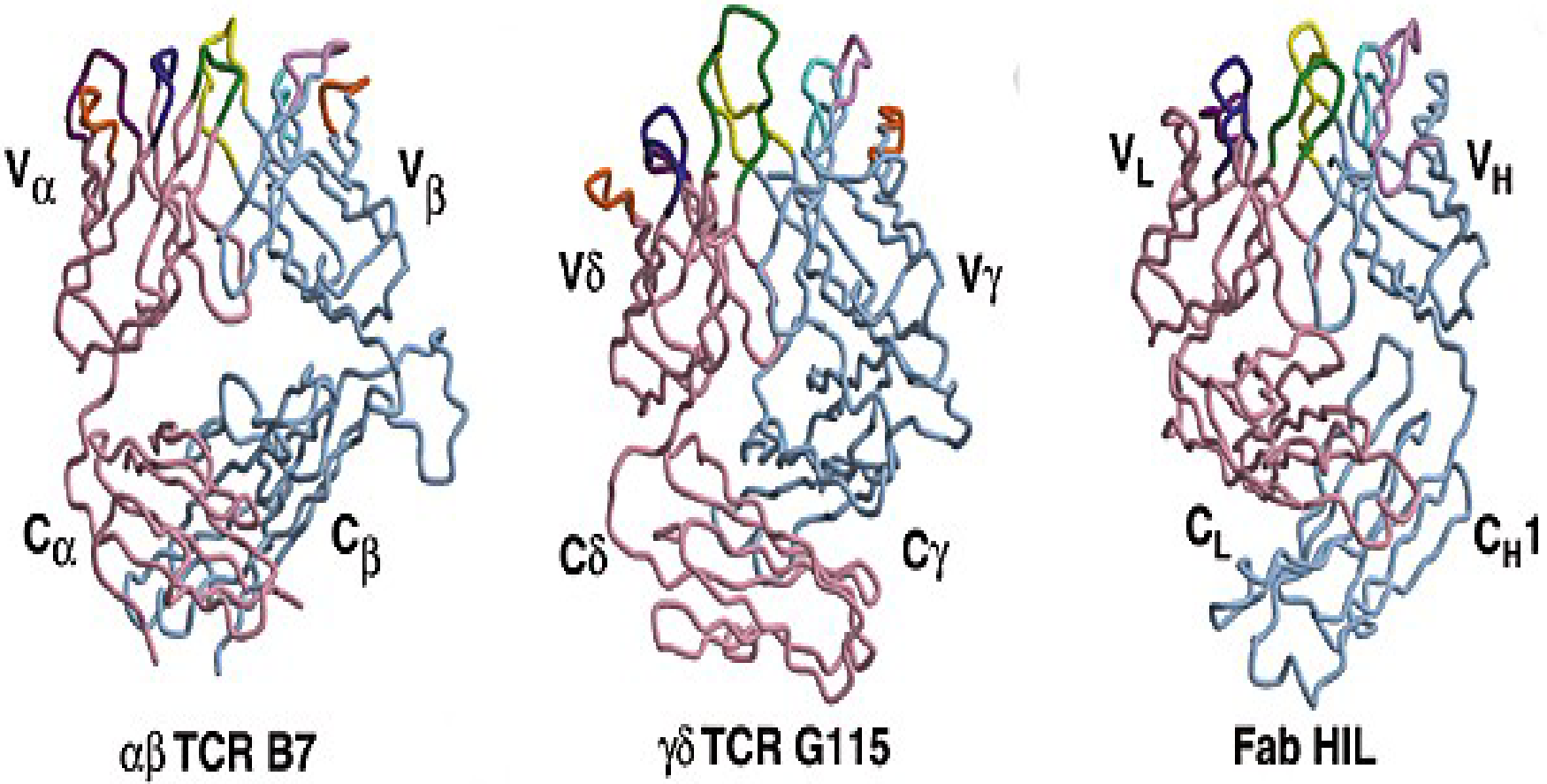
Resumen

Un linfocito T reconoce antígenos foráneos a través de su TCR en un contexto de presentación MHC clase I para LTC y clase II para LT ayudadores.

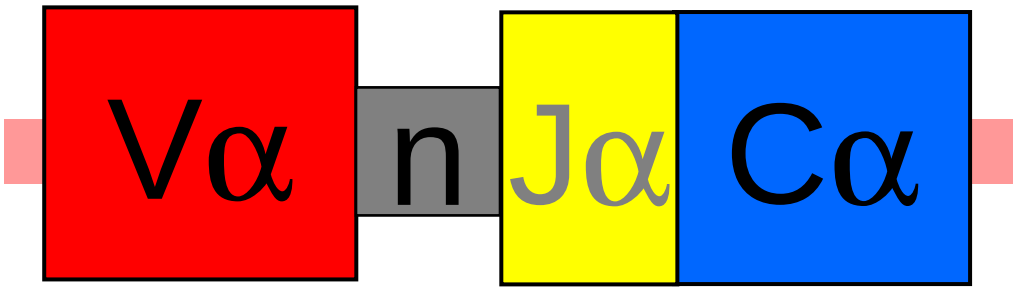


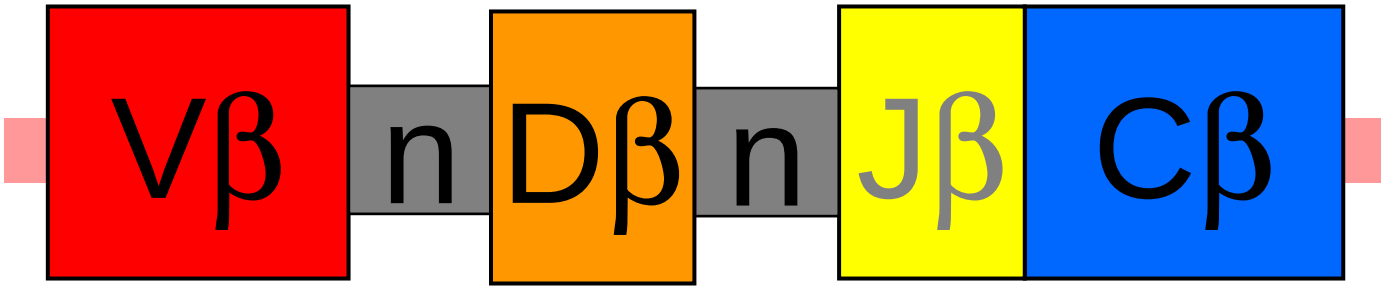
La Información es transmitida al núcleo mediante interacciones moleculares para proporcionar una eficiente respuesta (señalización)

- La organización génica de las inmunoglobulinas y el TCR es muy semejante

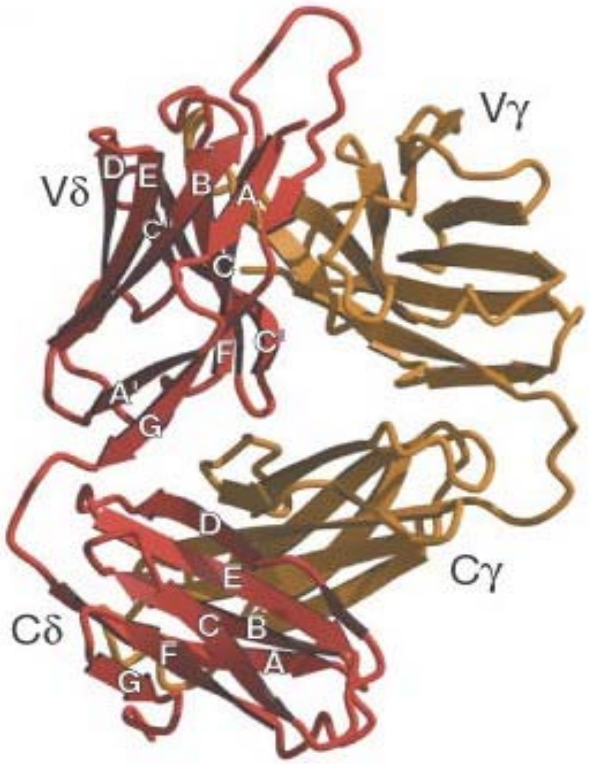


- La diversidad del TCR es generada por recombinación somática





Los LT γδ son una población minoritaria de Linfocitos T que comparte mecanismos efectores con los LT αβ.



Desempeñan una función complementaria y reguladora de otras células del sistema inmune. Además vigilan la integridad celular ya que reconocen moléculas inducidas por estrés celular.

Existe un alto grado de homología entre los genes codificantes para las moléculas del TCR de humano y los reportados hasta la fecha en *Aotus*