Microbiology 204

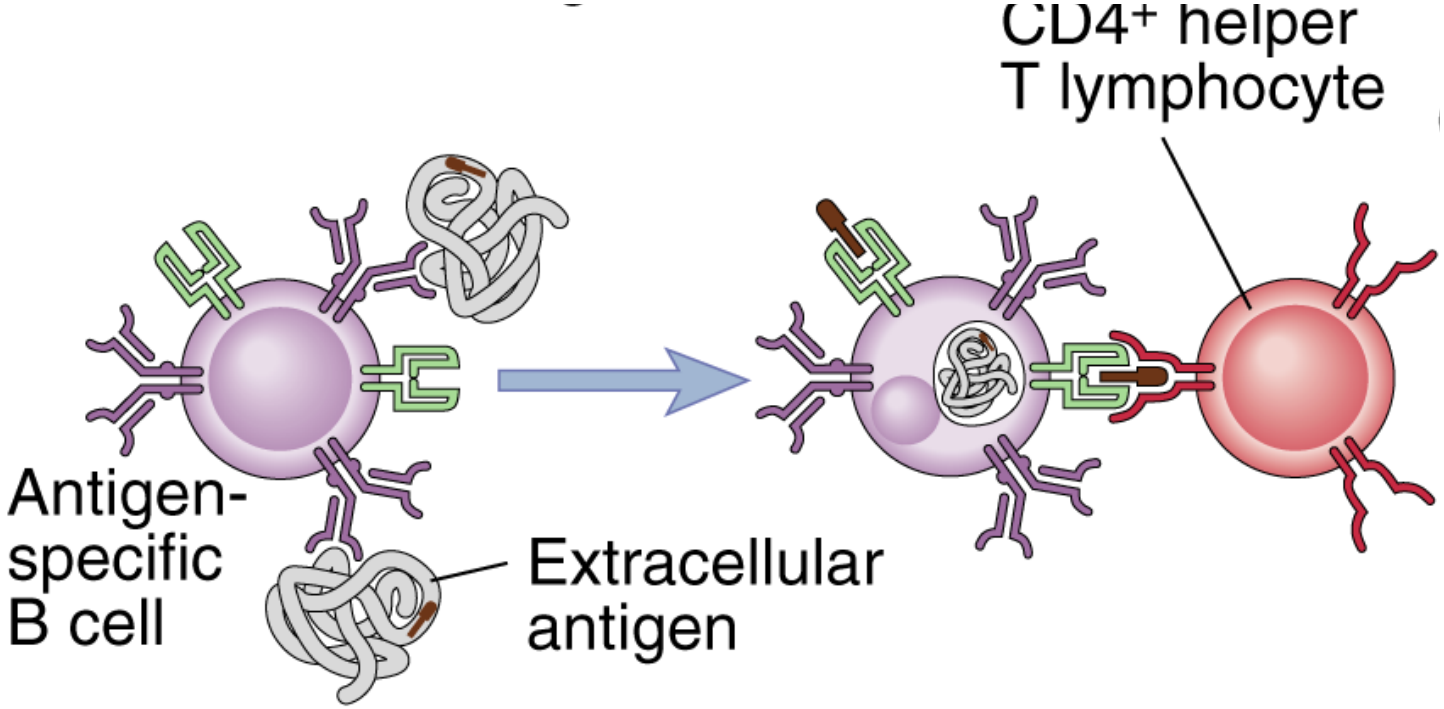
TCR Structure

Art Weiss

October 10, 2018

What do T cells see with their antigen receptors?

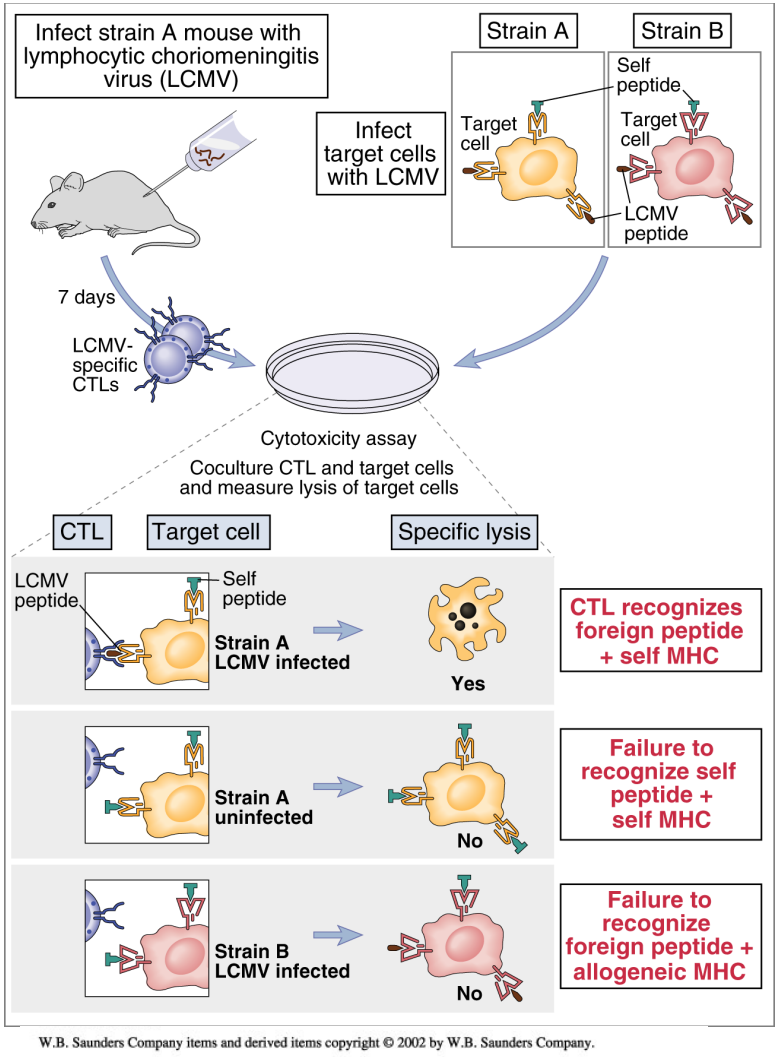
T cells and B cells use distinct antigen receptors to recognize fundamentally different forms of antigen



B cells can recognize either ***linear or conformational epitopes*** of proteins, of carbohydrates or of lipids. The B cell antigen receptor is a form of membrane Ig.

T cells generally recognize "only" ***linear peptide fragments*** that are bound to MHC class I or class II molecules.

MHC Restricted Recognition of Antigen



Zinkernagel and Dougherty

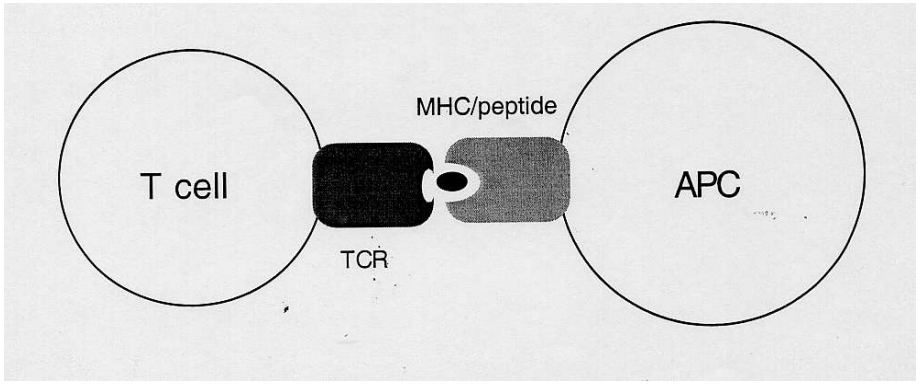
Bevan

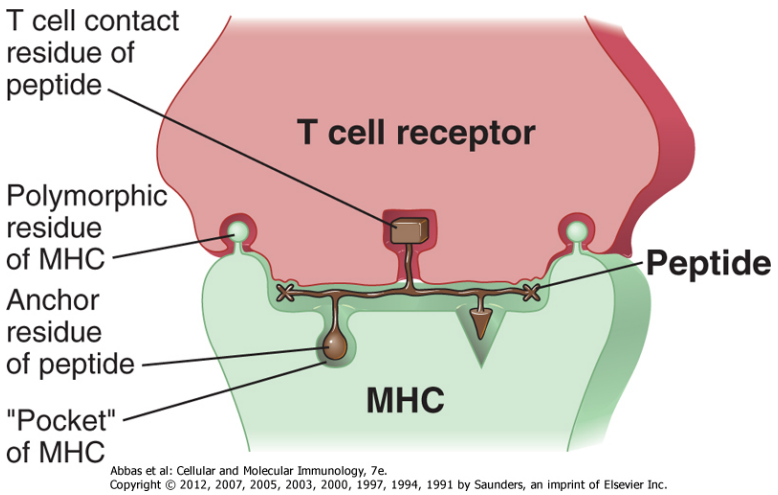
Mid -1970's

T cells only recognize specific peptide antigen <u>in the context of self</u>: MHC restriction. Specificity for self recognition is encoded in the MHC (Major Histocompatibility Complex).

MHC Restriction: How does the TCR simultaneously recognize MHC specificity and antigen specificity?

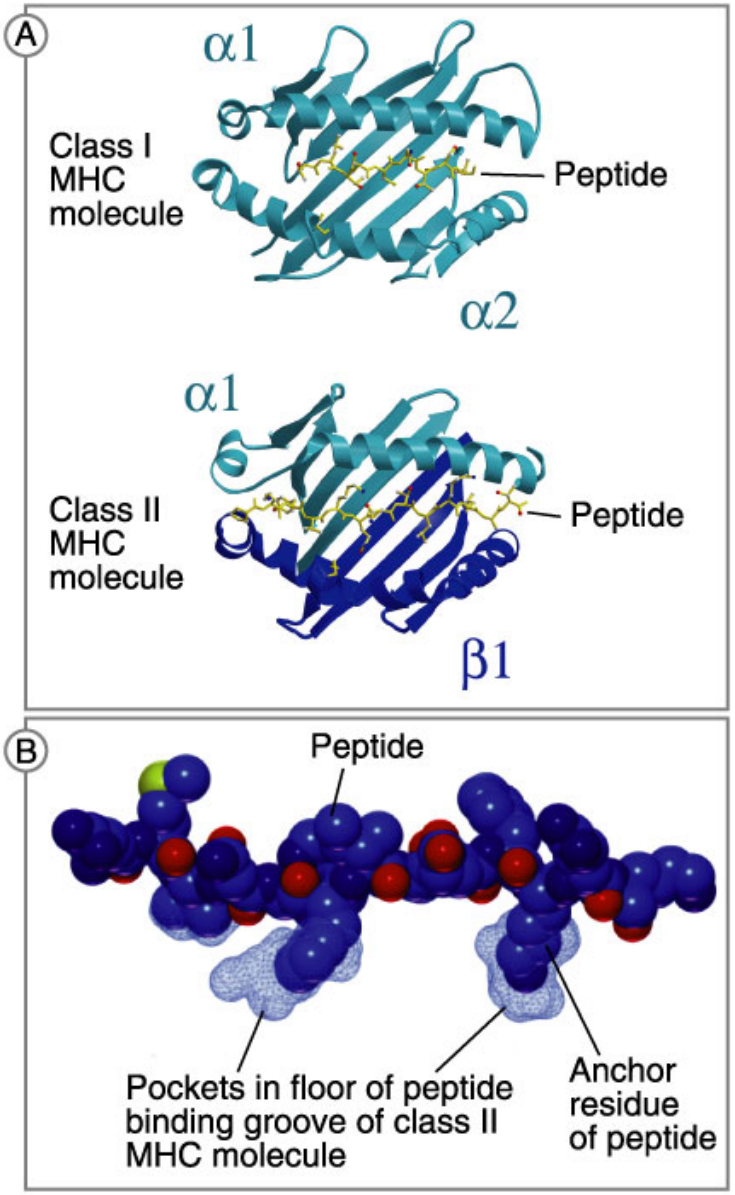
- One receptor or two receptors?
- Structure of the MHC provided the insight
- MHC molecules are designed to present peptides.

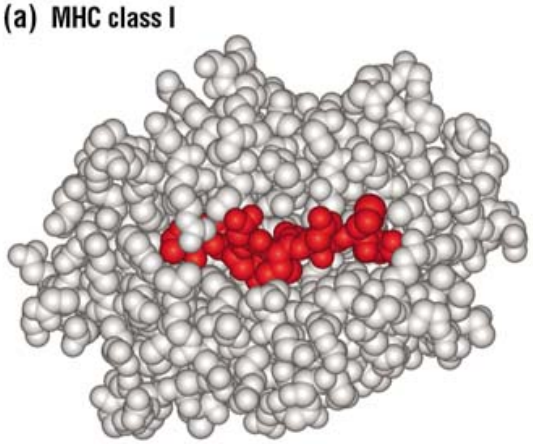


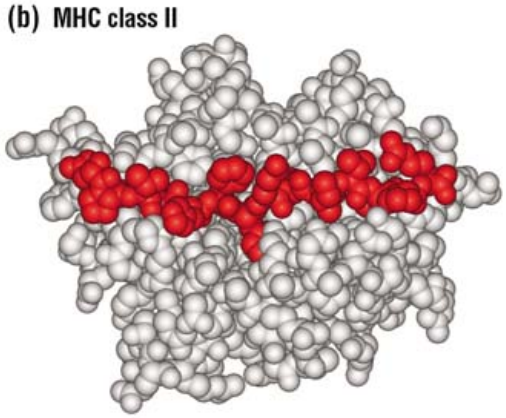


So, T cells simultaneously recognize a single peptide and MHC molecular complex!

Binding of Class I and Class II MHC Molecules to Peptide Ags





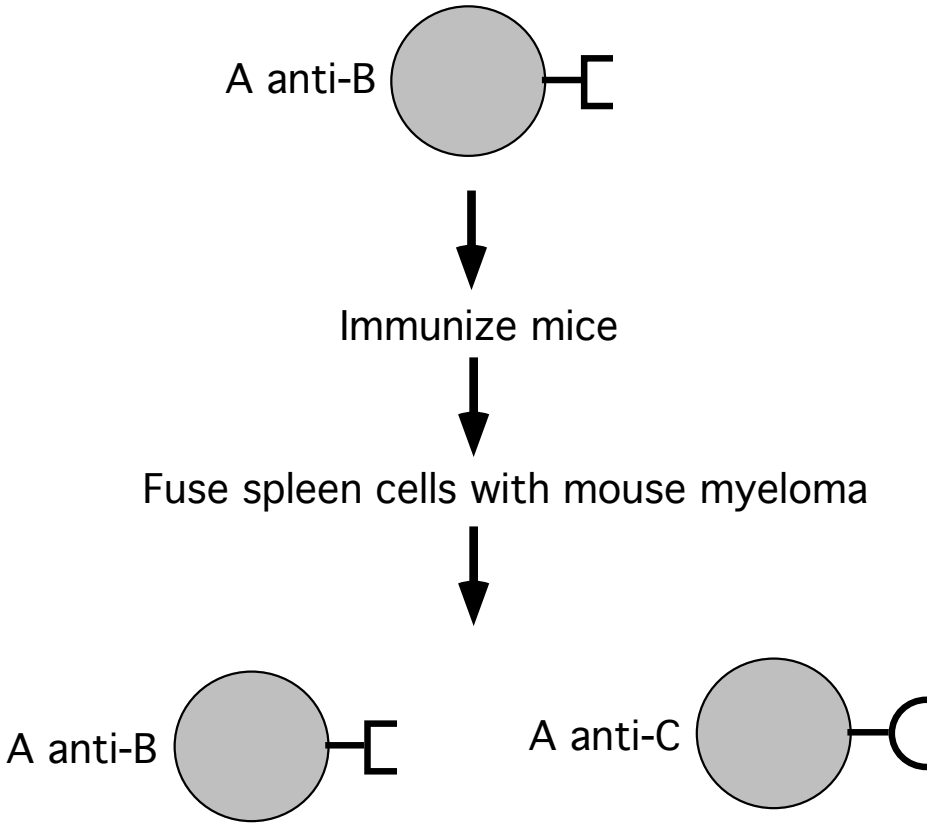


From **Immunity: The Immune Response in Infectious and Inflammatory Disease** by DeFranco, Locksley and Robertson

Discovery and characterization of the TCR protein and genes

Identification of the TCR Protein

Generation of T cell *clone-specific* monoclonal antibodies (Allison, Reinherz, Kappler and Marrack, '82-' 83)

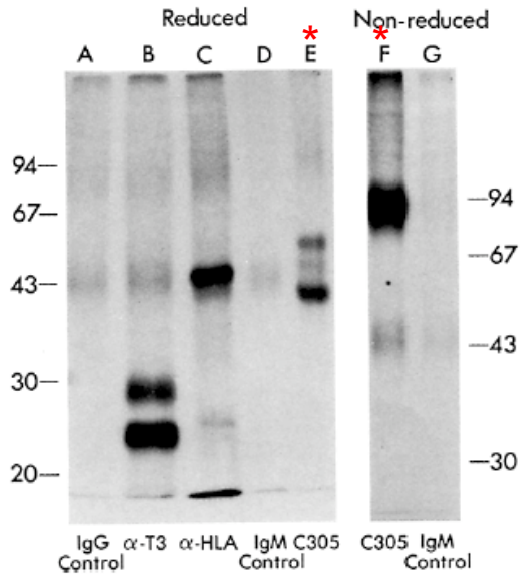


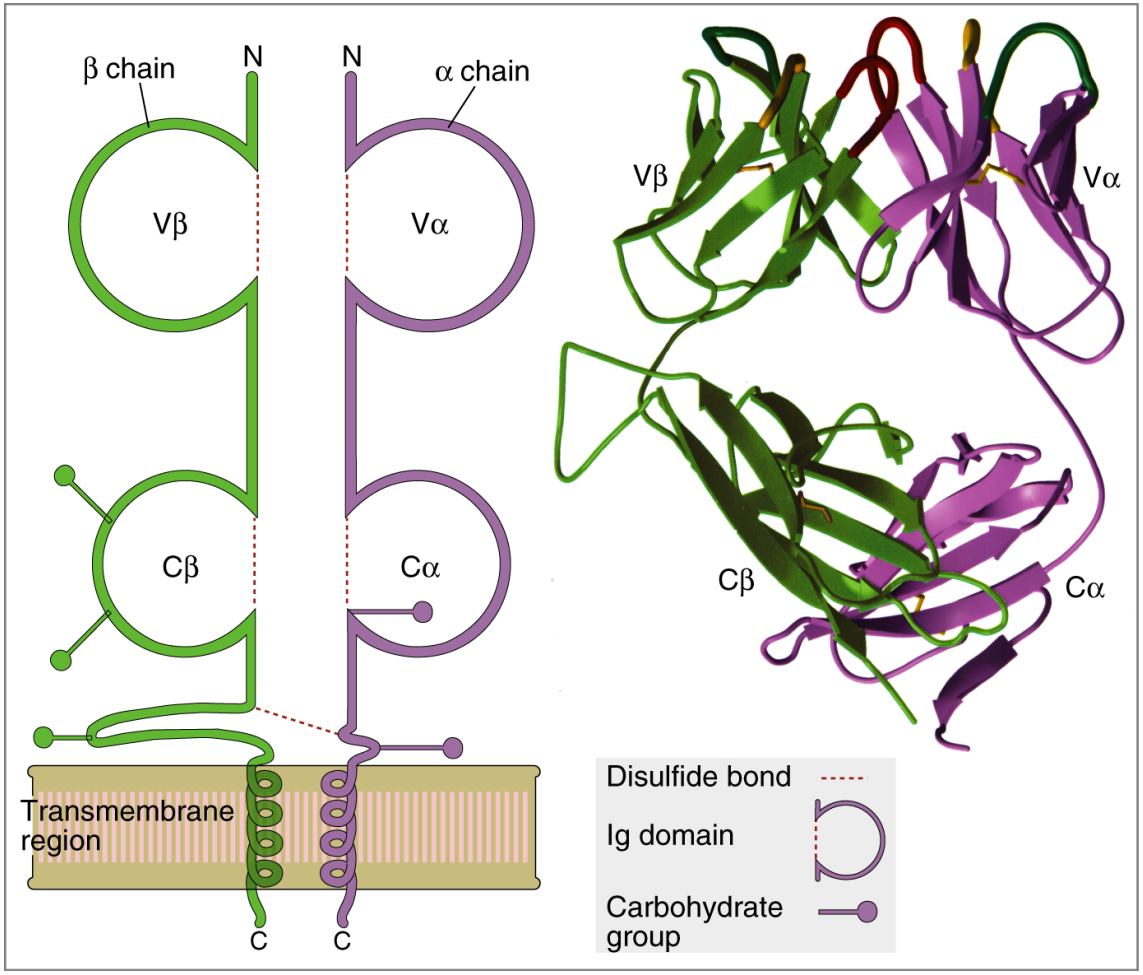
Screen for mAbs that react only with clone A anti - B

Biochemical characterization of the TCR

Biochemical Characterization:

1. Disulfide-linked heterodimer
2. Transmembrane protein
3. Constant and variable regions
4. Both chains are glycoproteins





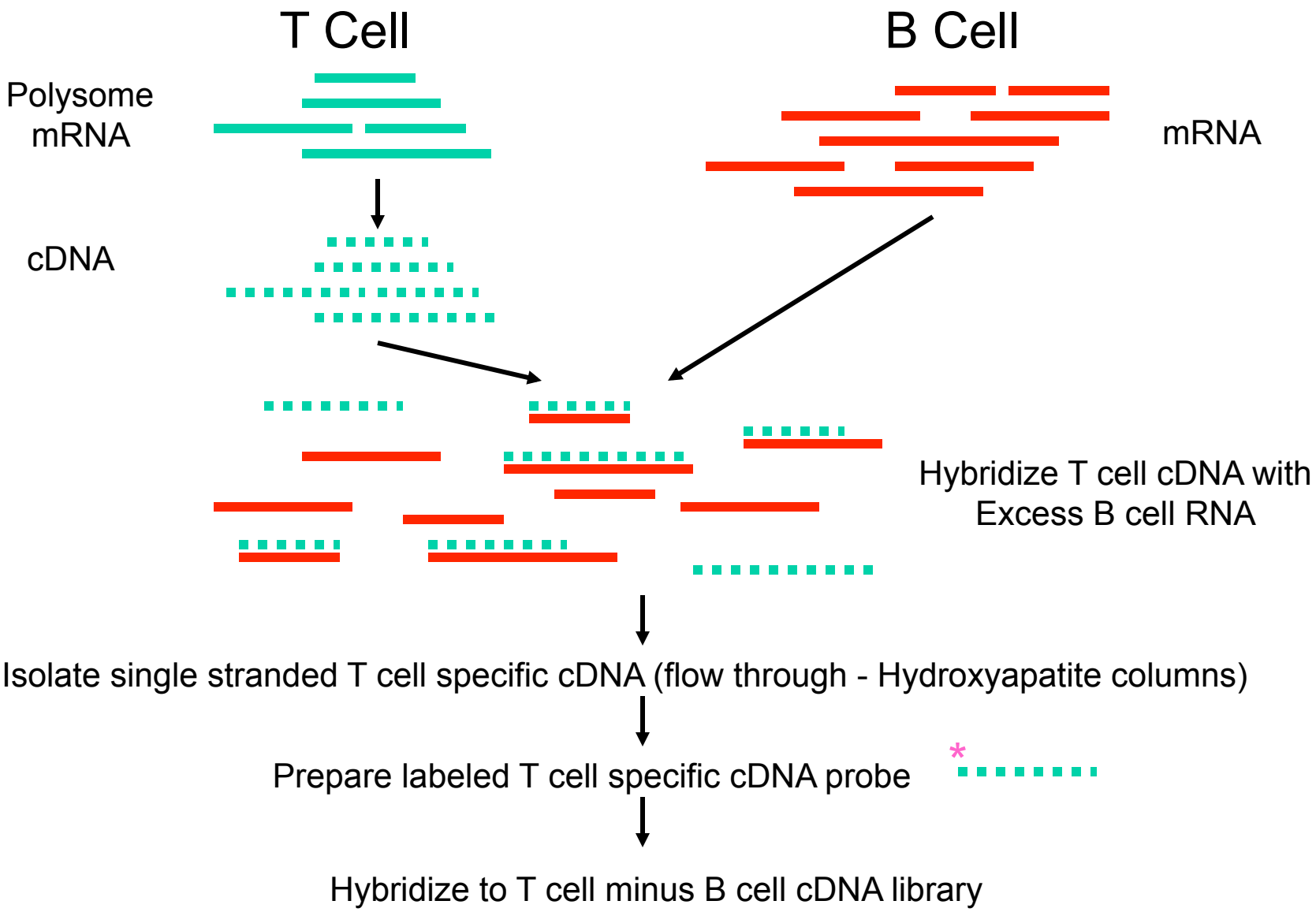
Cloning the TCR β-chain cDNA

Hedrick and Davis, 1984
Yanagi and Mak, 1984

Predictions:

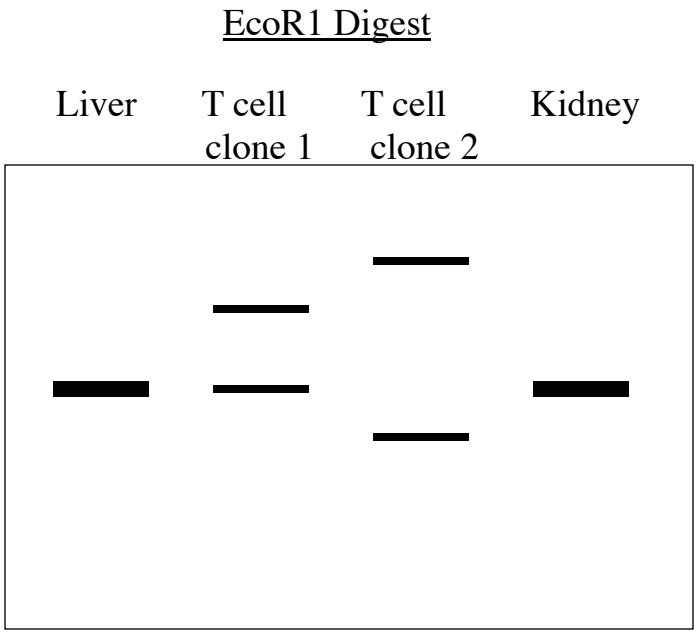
1. T cell specific
2. Transmembrane protein
3. Genes should be rearranged in T cell but not in non-T cells
4. cDNA should encode **C**onstant and **V**ariable domains

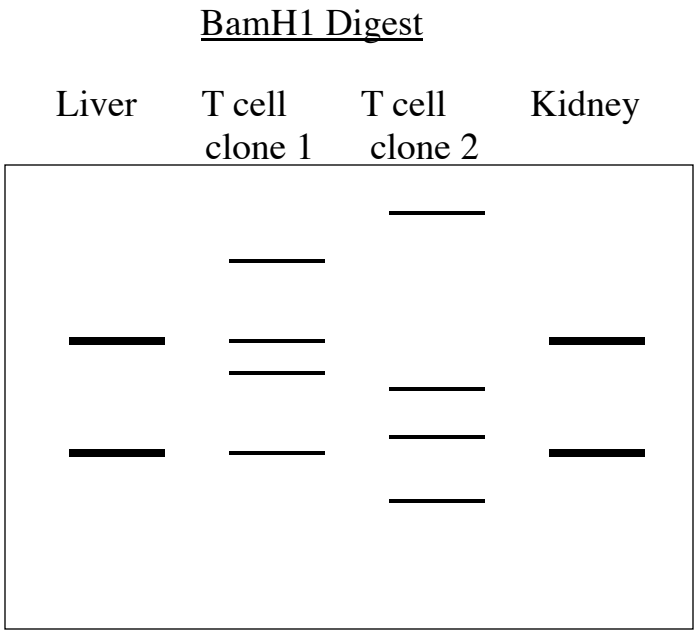
Isolation of TCR β-chain cDNA
(Hedrick and Davis, 1984)



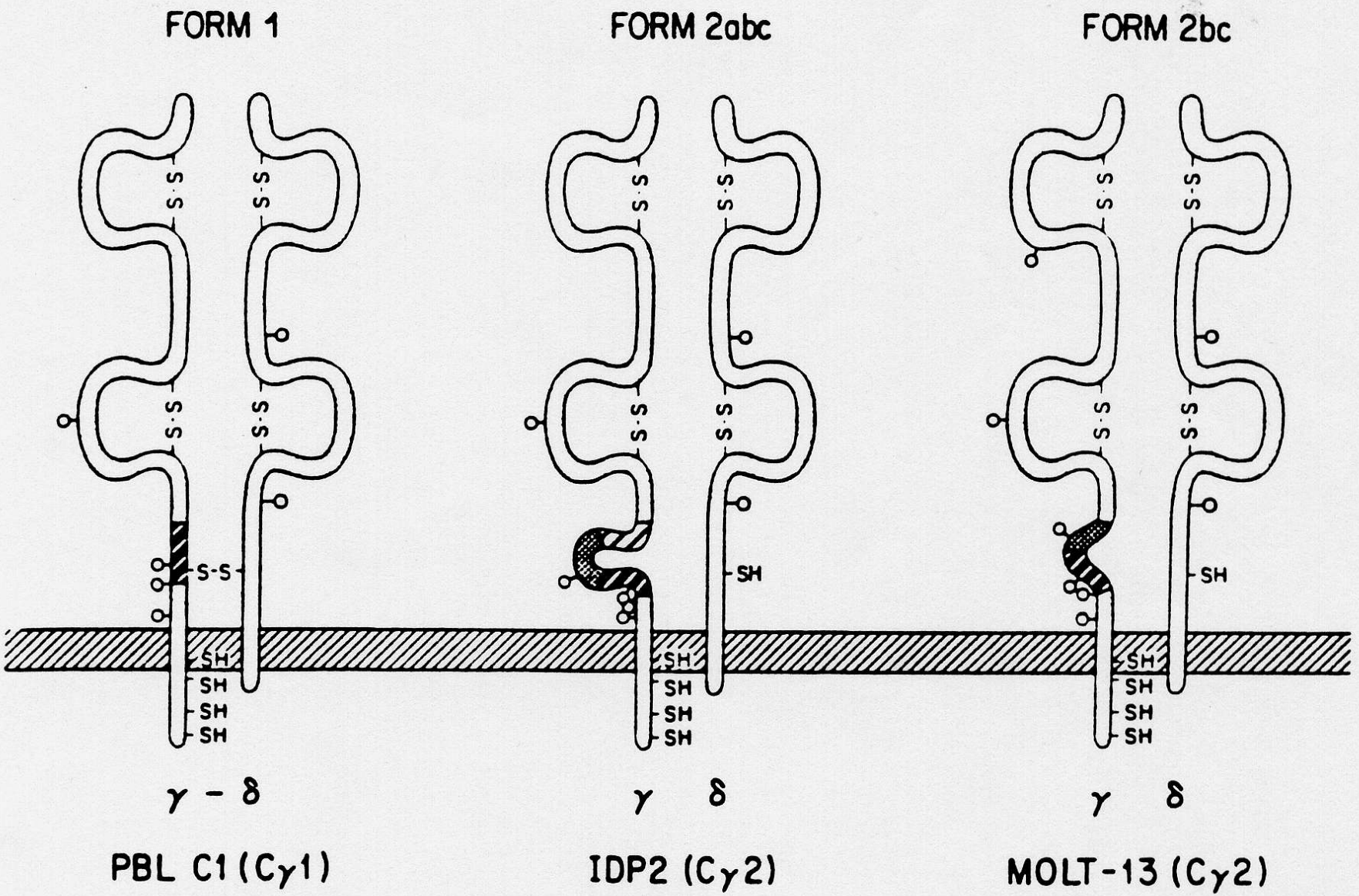
TCR β-chain cDNA

Southern Blots: evidence for rearrangement (J-region probe)





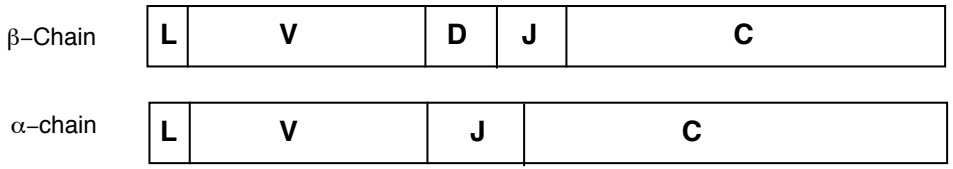
THE $\gamma\delta$ T CELL RECEPTOR



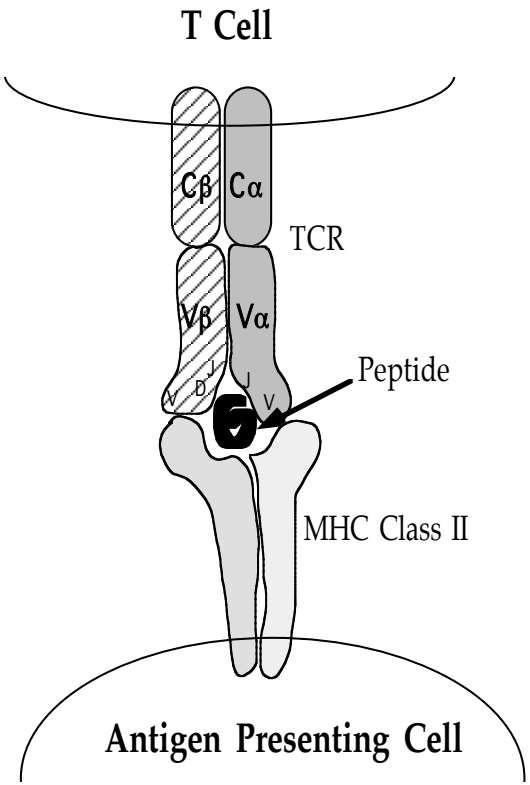
γδ T Cells

- Express γδ TCR heterodimer instead of the αβ TCR heterodimer
- Distinct lineage of T cells
- Most resting γδ T cells lack CD4 and CD8 coreceptors
- Activated γδ T cells can express CD8
- Minor subset in mouse and man (2-5%). Epithelial localization predominates.
- Expressed early in ontogeny
- MHC Restriction/recognition – little good evidence for "MHC restriction", reactivity to some non-classical MHC molecule is well-documented, but there is no evidence for requirement
- Function:
  - Secrete lymphokines and mediate cytotoxicity
  - Role in bacterial infections (mycobacterial, and others)
  - Respond to non-peptidic ligands
    i.e. bacterial phospholipids, alkylamines,
    heat shock proteins,

Both chains of the αβ TCR heterodimer are involved in antigen and MHC recognition

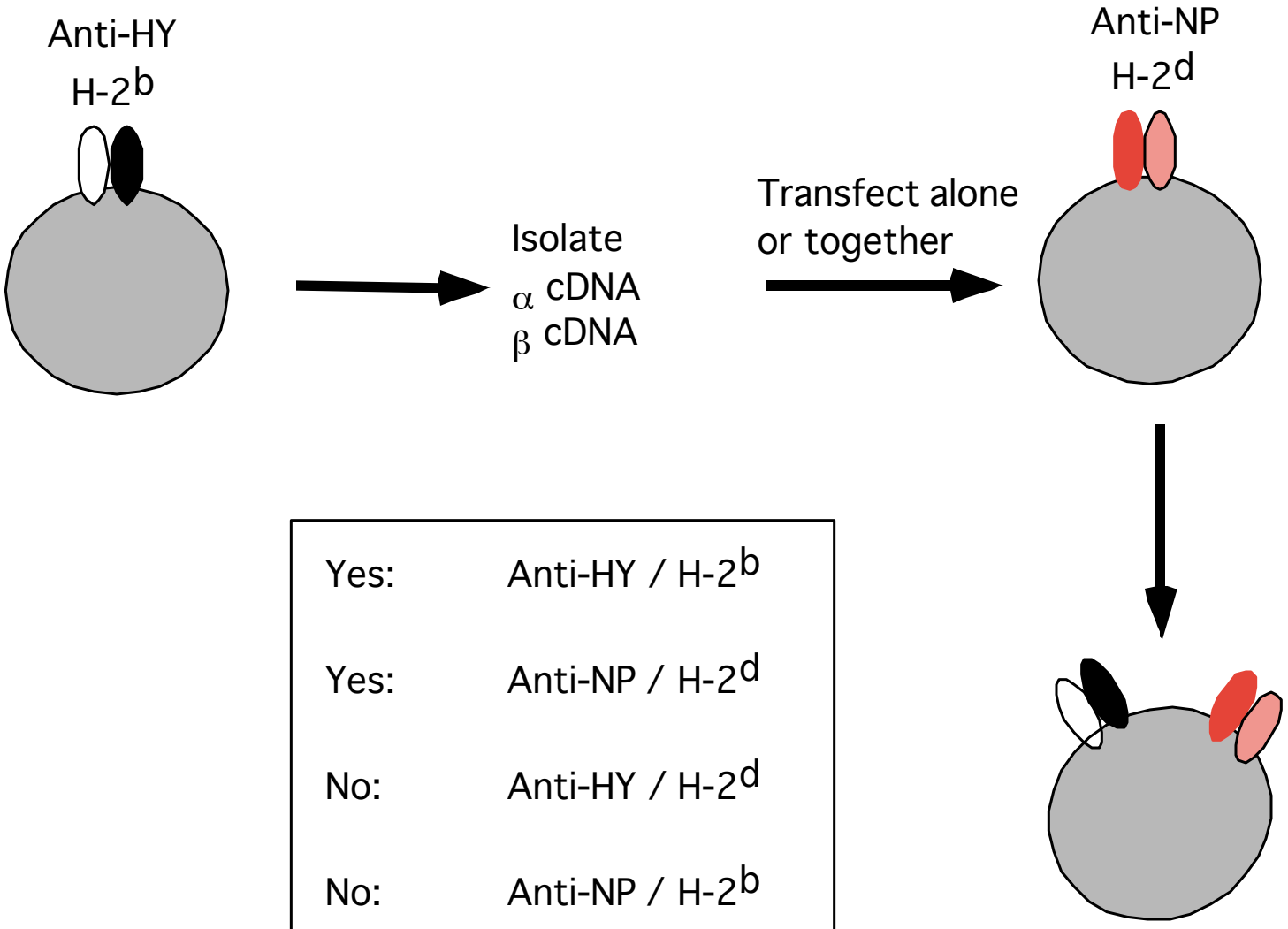


Expressed in T cells only
Clonally rearranged
Encodes a transmembrane glycoprotein
Homologous to light chains (Immunoglobulin fold)



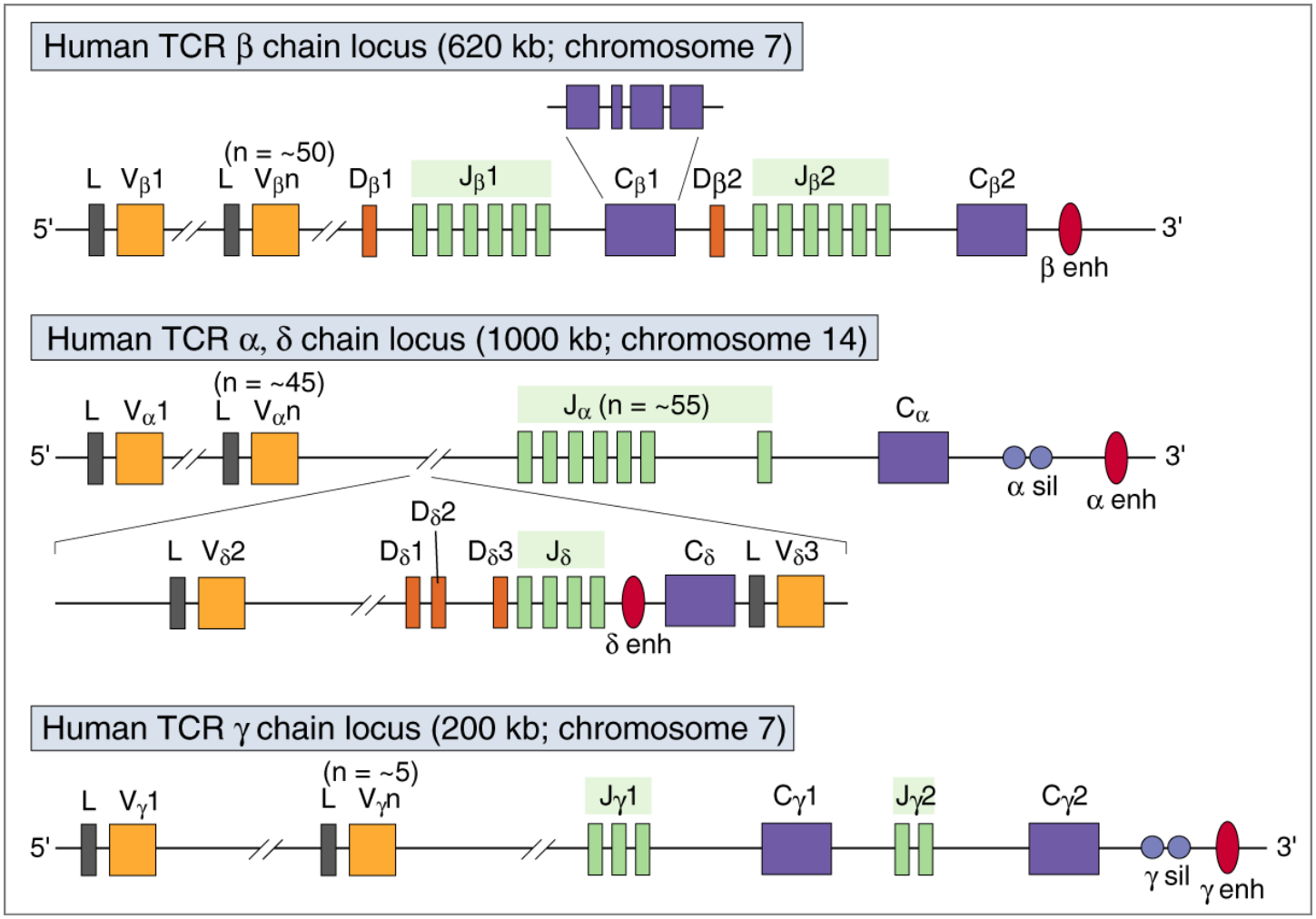
α and β chains encode all of the information necessary for peptide and antigen specificity
Transfection studies in T cell lines and hybridomas
TCR transgenic mice
α and β chains both contribute to peptide and MHC molecule recognition
Fusion of hybridomas

α and β chains of the TCR do not separately encode MHC or antigen specificity



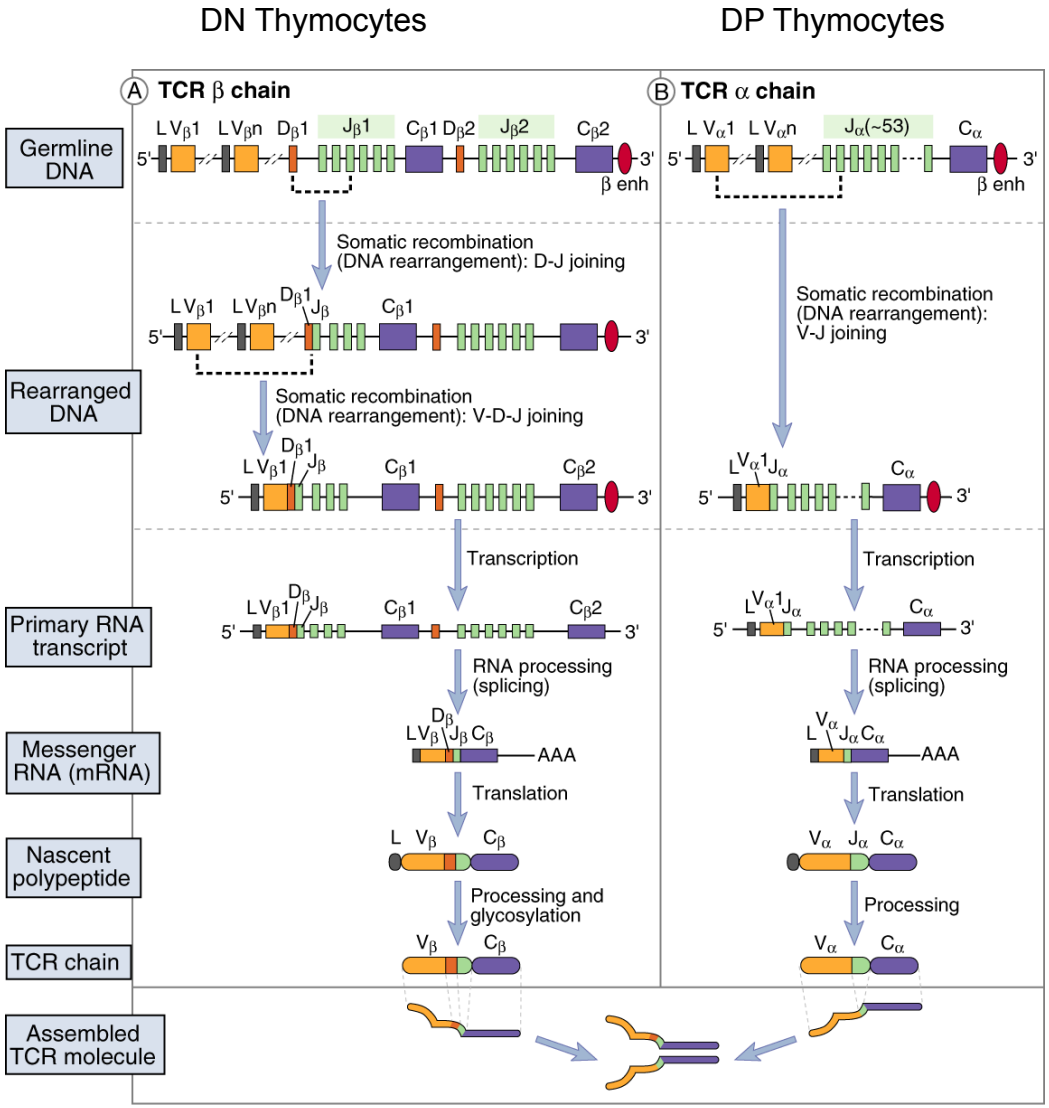
Yes:	Anti-HY / H-2^b
Yes:	Anti-NP / H-2^d
No:	Anti-HY / H-2^d
No:	Anti-NP / H-2^b

Human TCR gene loci

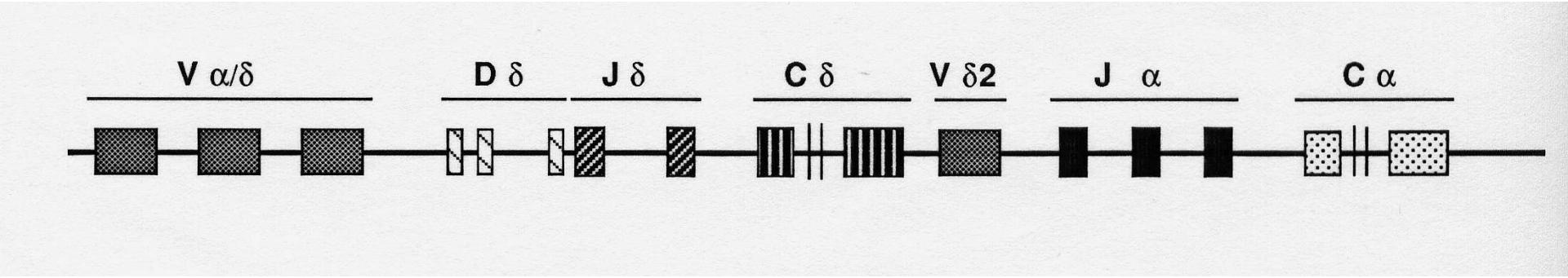


V segments	2 exons	80-250 nucleotides	CDR1 and CDR2
J segments	1 exon	47-76 nucleotides	
			CDR3
D segments	1 exon	9-16 nucleotides	

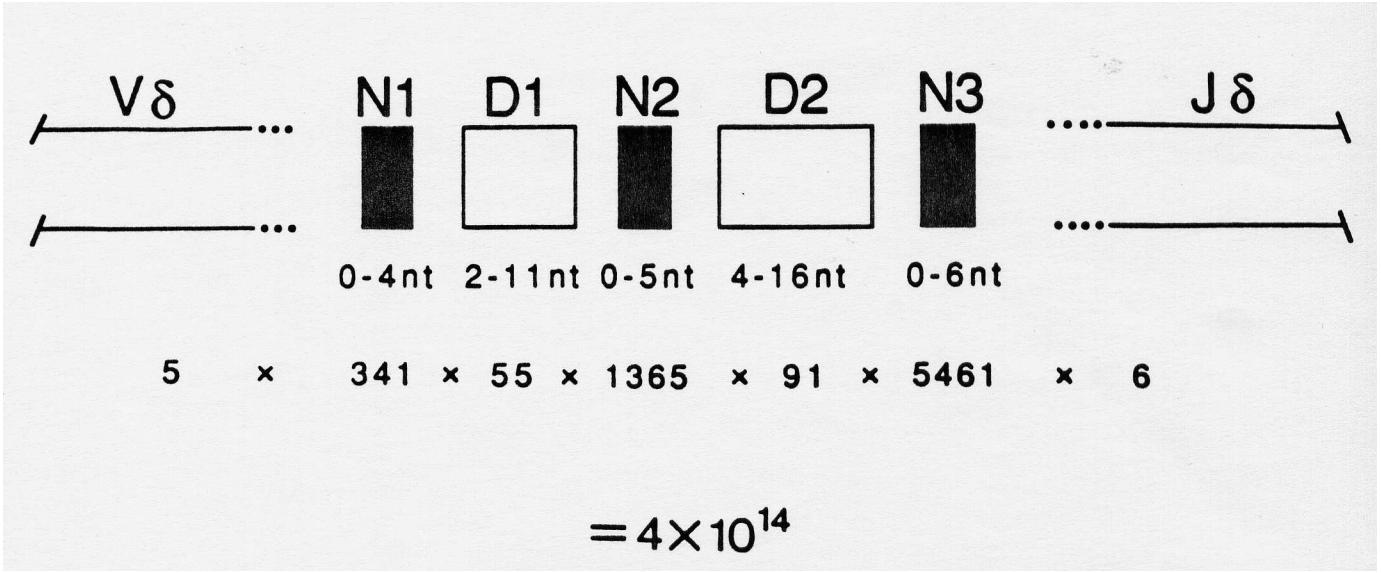
TCR gene rearrangement occurs sequentially during T cell ontogeny



Unusual organization of TCR gamma/delta genes



Enormous potential of diversity in delta rearrangements



Generating a diverse TCR repertoire

1. Recombination of different gene segments (V, D and J segments)
2. Recombination of different numbers of gene segments (δ locus)
3. Imprecise joining of gene segments
4. "P" and "N" nucleotide addition (TdT)
5. Assembly of different combinations of rearranged α and β chains

However, unlike immunoglobulin genes, ***somatic mutation*** of TCR genes does not take place.

Comparison of diversity generated in TCR and BCR assembly

	Ig		TCR αβ		TCR γδ	
	H	L	α	β	γ	δ
Variable (V) segments	45	35	45	50	5	2
Diversity (D) segments	23	0	0	2	0	3
D's in all frames	rarely	-	-	often	-	often
N-region addition	V-D, D-J	None	V-J	V-D, D-J	V-J	V-D1, D1-D2, D1-J
Joining segments	6	5	55	12	5	4
Total potential diversity	**$\sim 10^{11}$**		**$\sim 10^{16}$**		**$\sim 10^{18}$**	

Characteristics of TCR recognition of pMHC

Unusual features of TCR recognition of pMHC molecule complex

Simultaneous recognition of MHC specificity and peptide specificity

TCR affinity for peptide and MHC is very weak compared to antibodies:

Kd of 10^{-5} to 10^{-7} M for TCR – dwell time of seconds for agonists

Kd of 10^{-7} to 10^{-11} M for Ig

(Based on solution binding of monomers – flawed analysis)

Main determinant is off rate

TCR discrimination of self-pMHC vs agonist-pMHC is based on small differences in affinities (examples of only 10-fold)

Cell-Cell interaction context (avidity issues, coreceptors, particles/diffusion)

Tetramers of MHC/peptide can bind with higher *avidity*

Exquisite specificity despite low affinity:

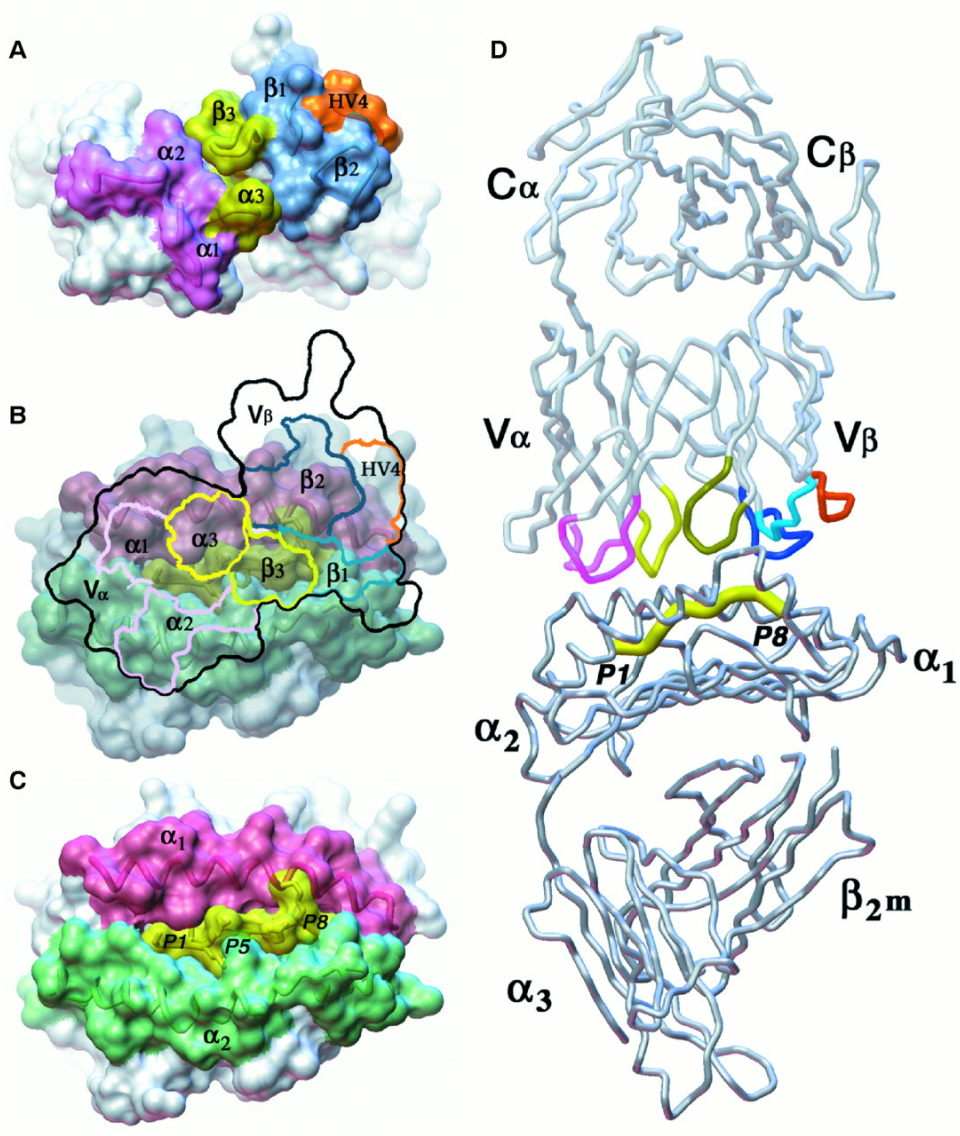
agonist peptides

altered peptide ligands

antagonist peptides

Crystal structure of an αβ TCR - Class I MHC/peptide

Garcia, et al., Science, 274:176, 1996

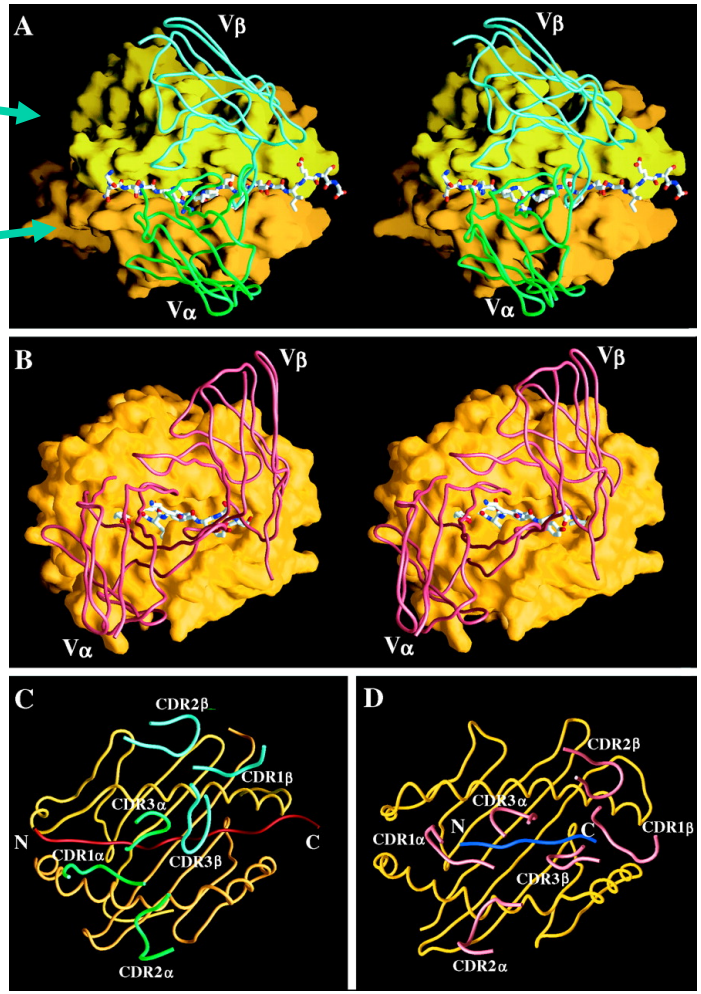


Is the TCR and Class II MHC/peptide interaction oriented differently?

Reinherz, et al., Science, 286:1867, 1999

I-A alpha chain

I-A beta chain

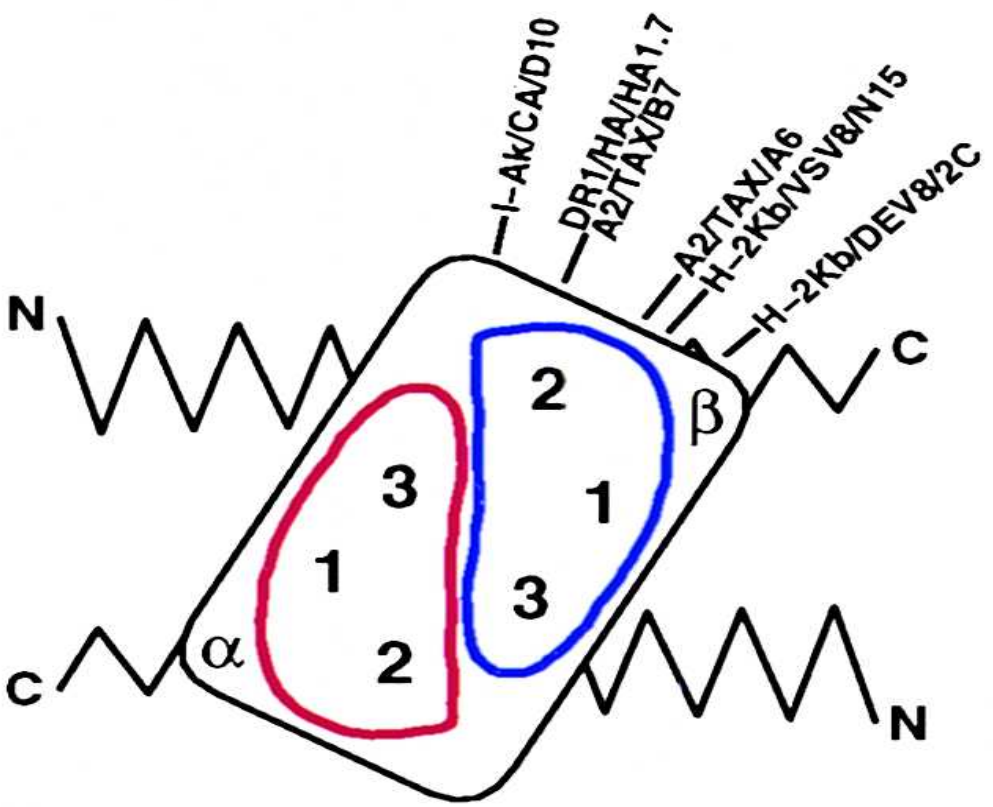


TCR-Class II peptide/MHC complex

TCR-Class I peptide/MHC complex

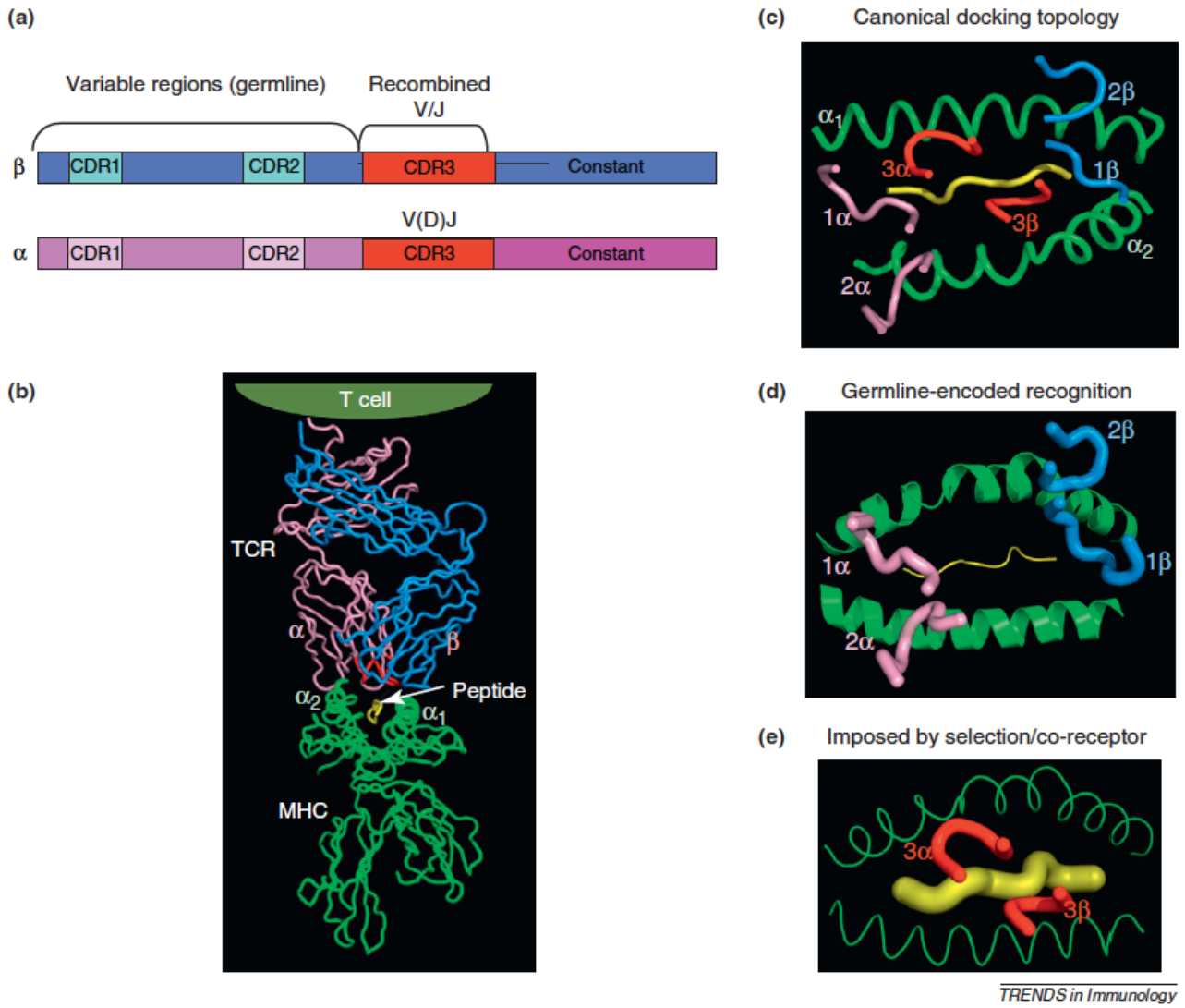
Distinct orientations of different TCR/MHC-peptide complexes

Hennecke and Wiley, Cell, 104:1, 2001



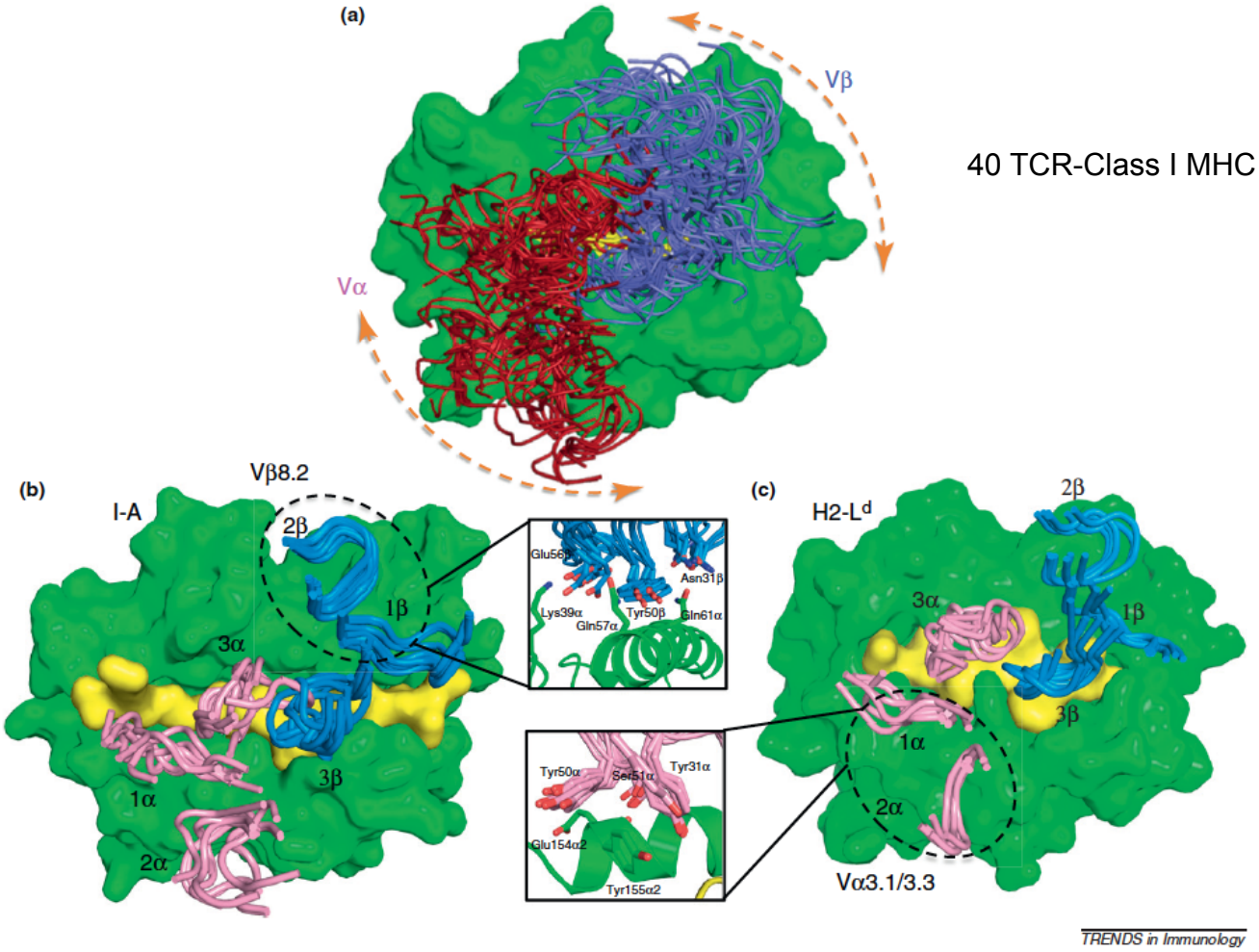
CDR loops are involved in distinct recognition functions

Garcia, Trends Immunol., 2012



αβ TCR germline bias for MHC recognition

Garcia, Trends Immunol., 2012

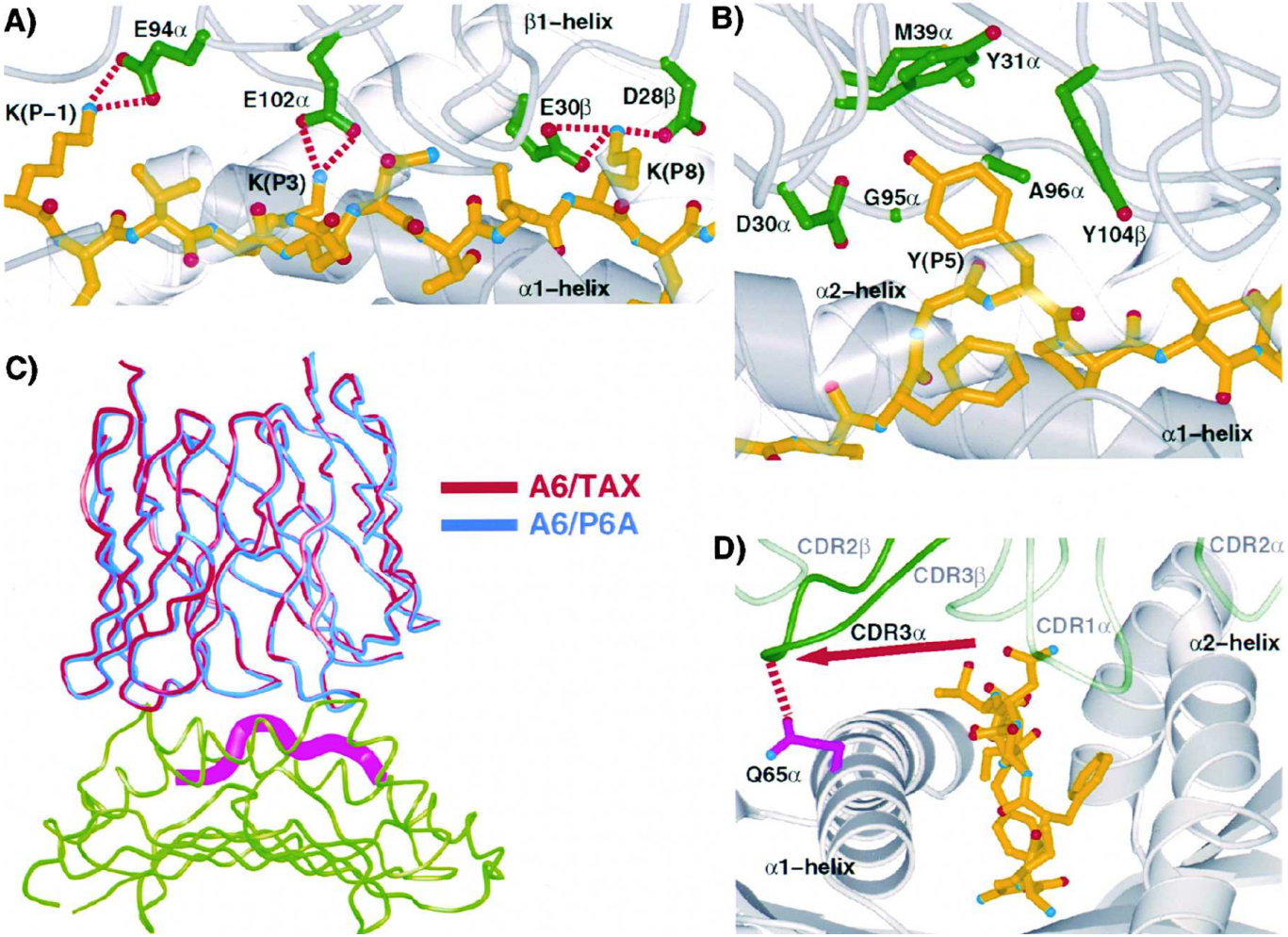


40 TCR-Class I MHC structures

Structures of limited Vβ or Vα containing TCRs with different Peptide antigen specificities show similar TCR/MHC interactions

The TCR can interact with MHC/peptide complexes via many different biochemical interactions

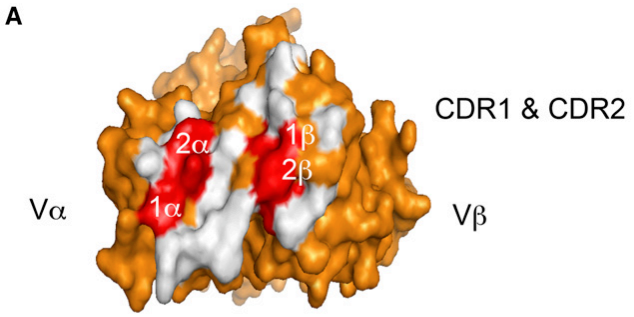
Hennecke and Wiley, Cell, 104:1, 2001



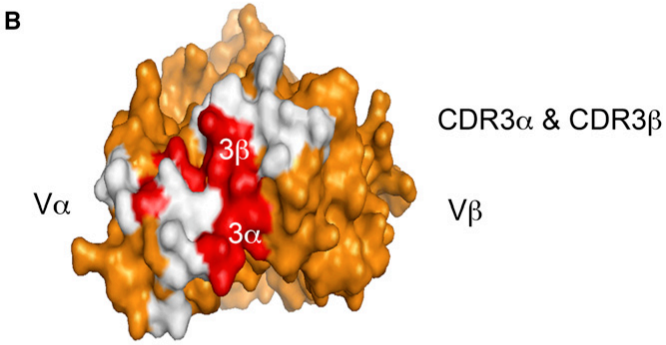
Distinct structural and energetic ways that the TCR uses for antigen recognition

Godfrey, et al, Immunity 2008

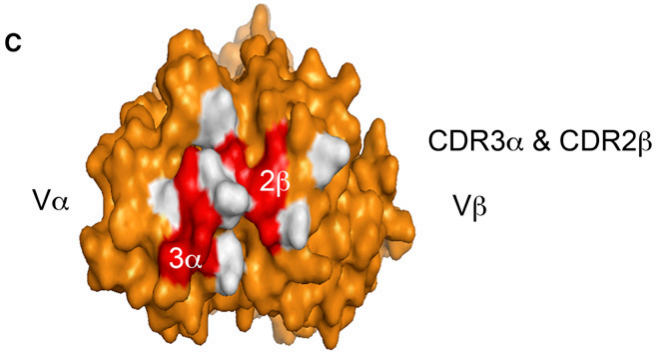
2C TCR footprint on H-2L^d



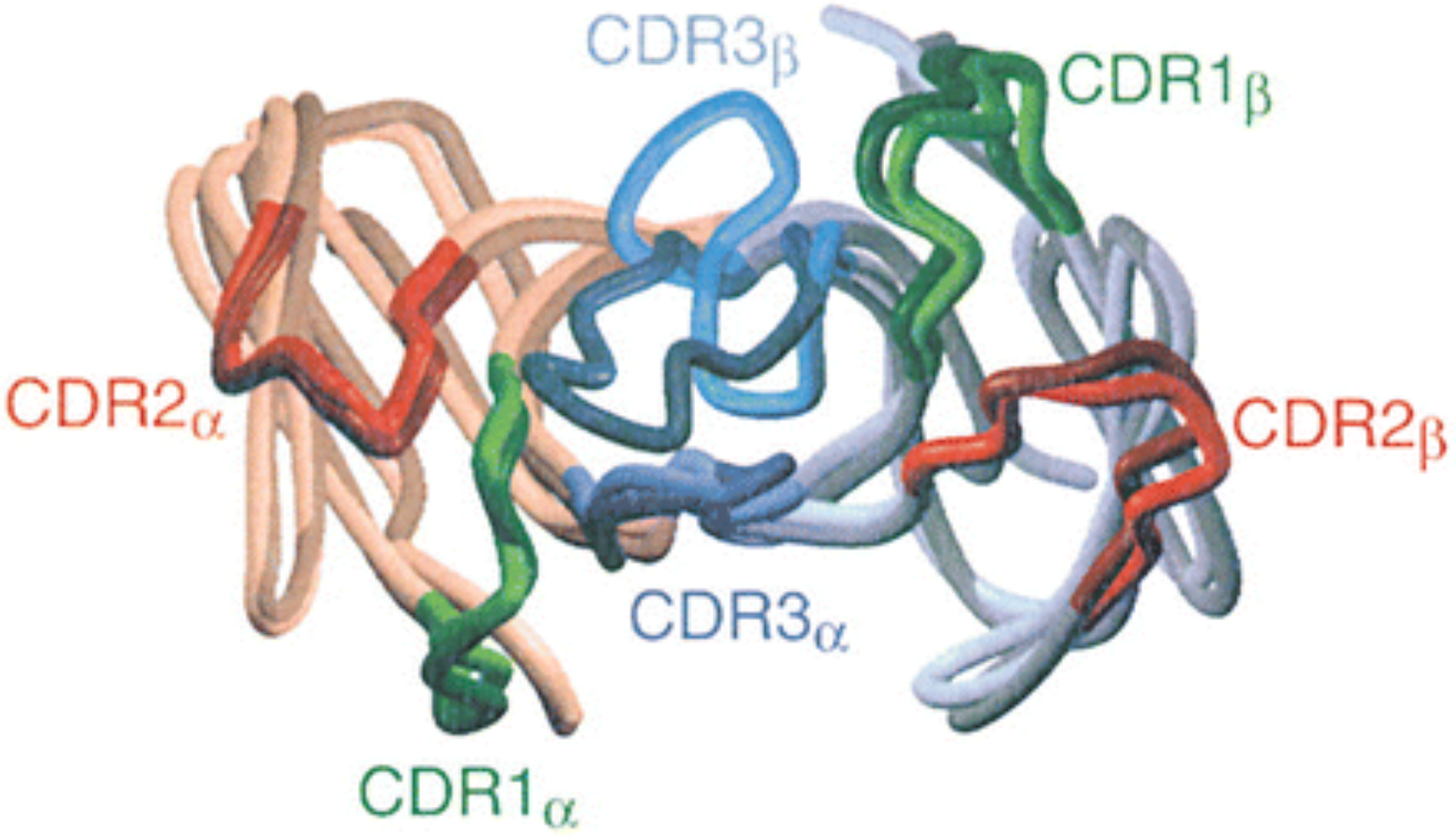
LC13 TCR footprint on HLA-B8



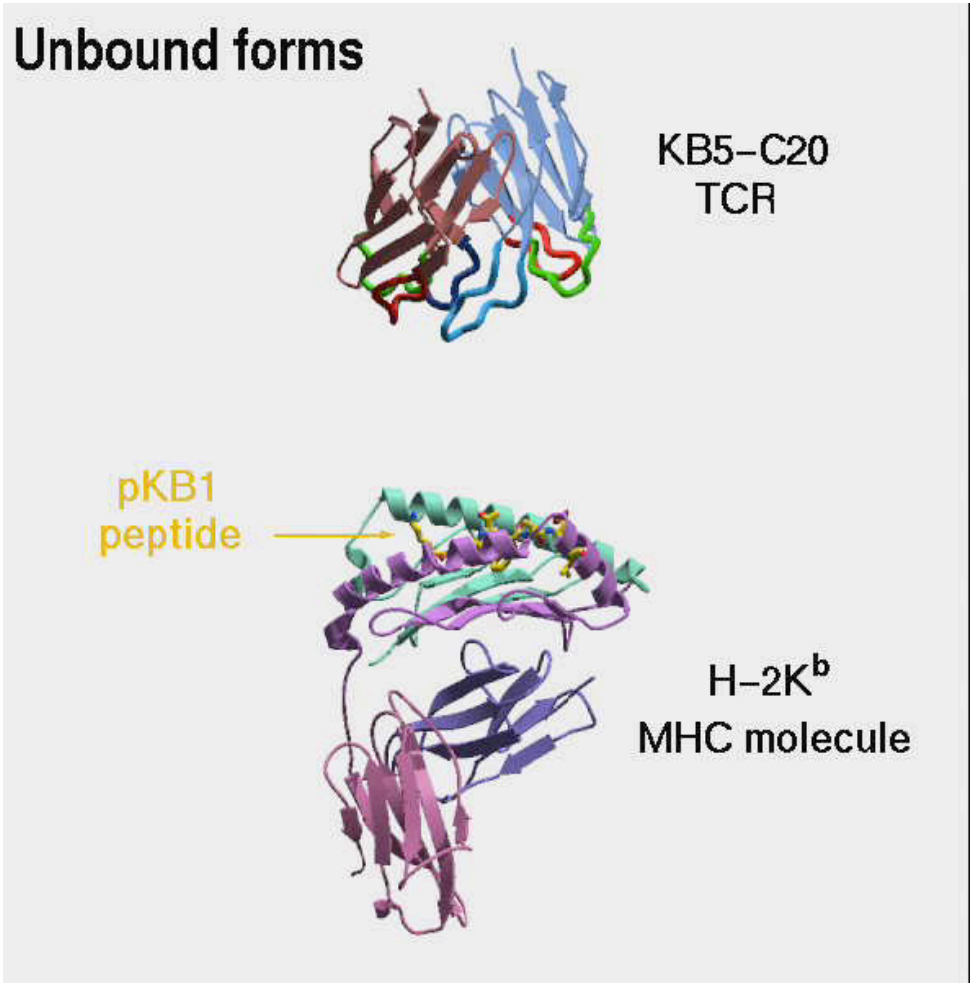
NKT TCR footprint on CD1d



Distinct conformations of the TCR CDR3 loops in the ligand-unbound and bound states



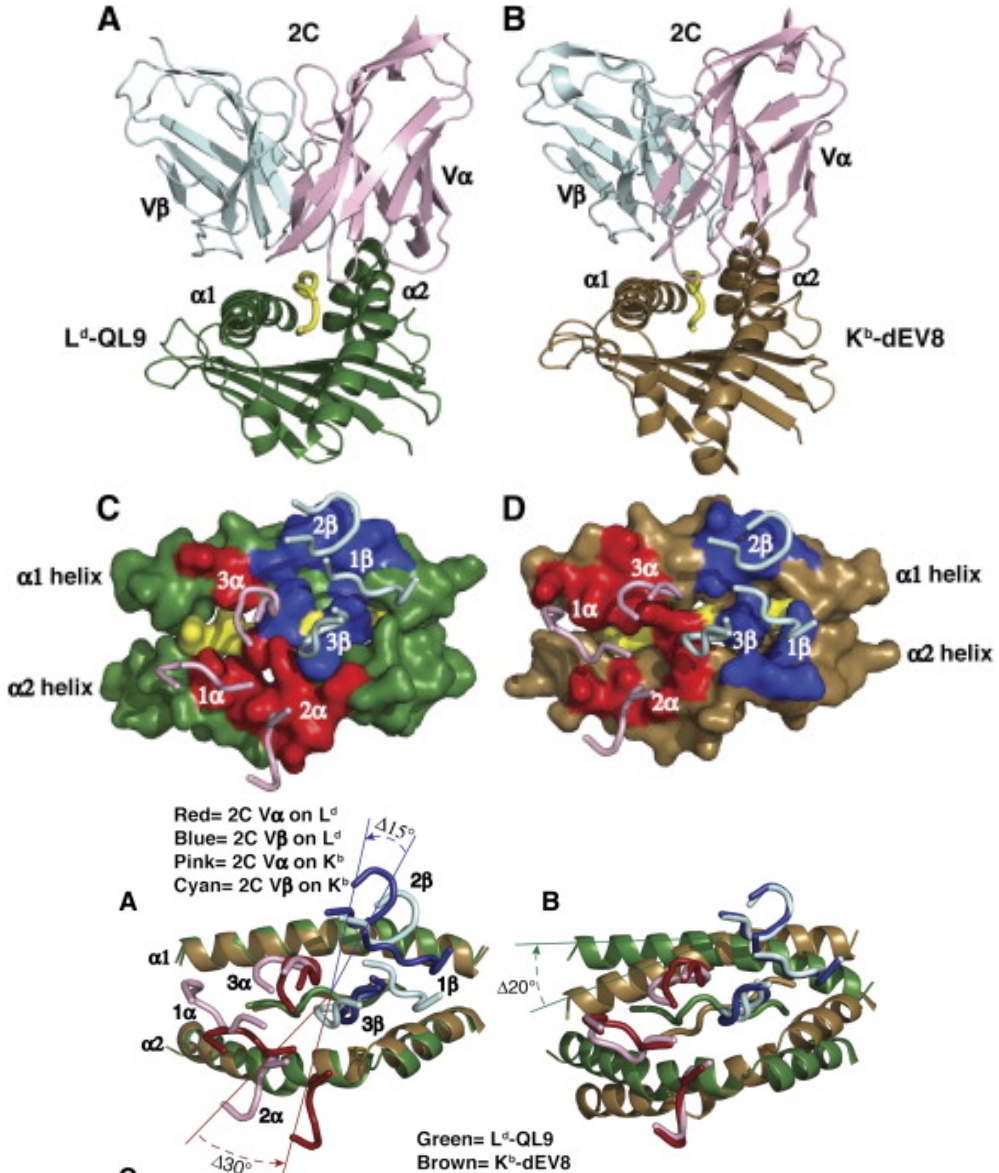
Model of a high degree flexibility in CDR3 during peptide/MHC docking



The same TCR can adopt distinct conformations to be polyspecific

Colf, et al., Cell, 2007

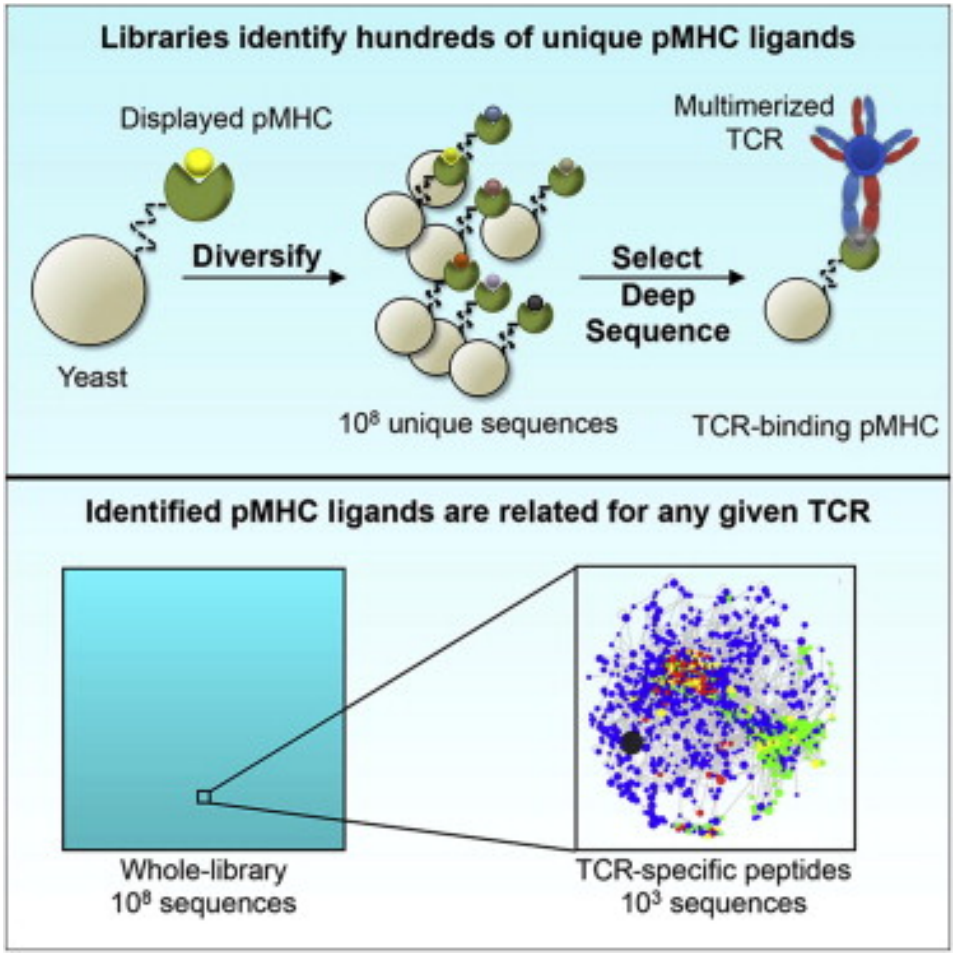
Allo-MHC plus peptide



Self-MHC plus peptide

A Single TCR can Recognize Hundreds of Different Peptides Which Share Some Common Features

Michael E. Birnbaum, et al., Cell, 2014



The TCR interacts with superantigens in a distinct manner

Superantigens

Bacterial enterotoxins
    Staphylococcal, Streptococcal and Mycobacterial

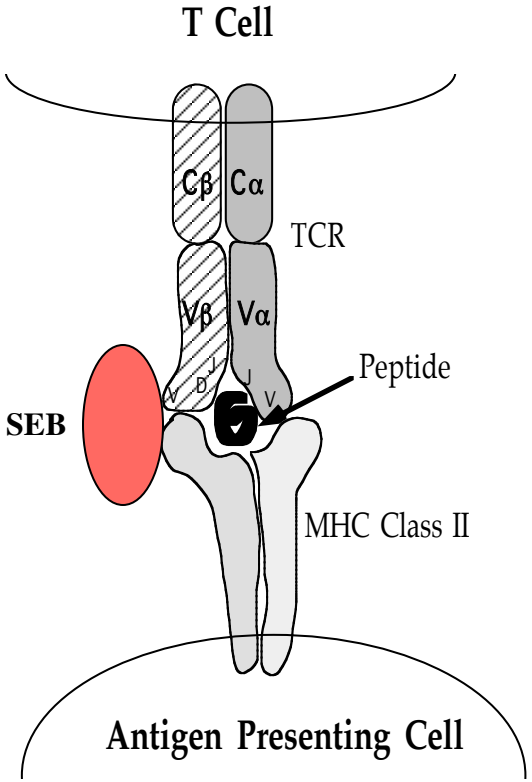
Minor lymphocyte stimulating (Mls) antigen
    Endogenous mouse retroviral products

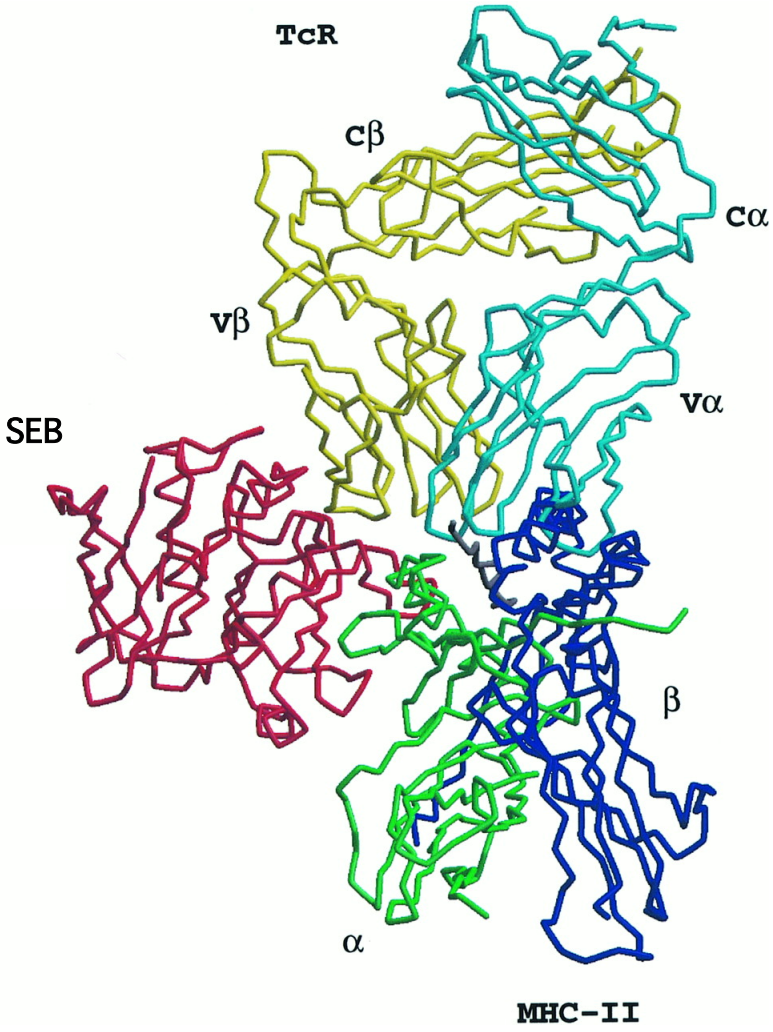
Unidentified endogenous antigens

Comparison of Superantigens and Conventional Peptide Antigens

	Conventional Antigens	Superantigens
Frequency of responsive T cells	**1 in 10^4 to 10^5**	**1 in 4 to 20**
Interaction with the TCR	+	+
Interaction with MHC	+	+
MHC restricted recognition	+	-
Requirement for processing	**+**	**-**
Binding to peptide groove in MHC	**+**	**-**

SEB/TCR/MHC structural model





Superantigens have relative specificity for Vβ segments

Toxin	Vβ specificity	
	Human	Mouse
SEA	?	1, 3, 10, 11, 17
SEE	5.1, 6.1-3, 8, 18	11, 15, 17
SED	5, 12, ?	3, 7, 8.1-3, 11, 17
SEB	3, 12, 14, 15, 17, 20	3, 7, 8.1-3, 17
TSST1	2	3, 15, 17
ExFT	2	3, 10, 11, 15, 17
MAM	?	6, 8.1-3

Adapted from Marrack and Kappler, Science, 248:705, 1990

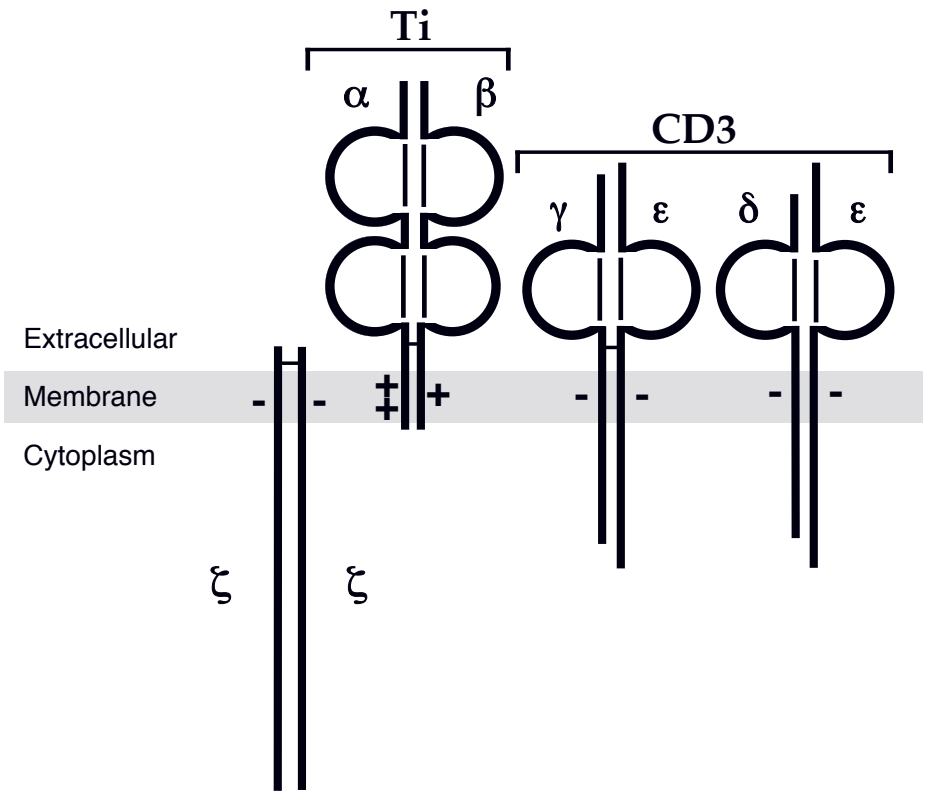
Diseases Caused by Superantigens

Toxin	Organism	Disease
Staphylococcal enterotoxins (SE) A, B, C1, C2, C3, D and E	S. aureaus	Food poisoning, Shock
Toxic Shock Syndrome Toxin (TSST1)	S. aureus	Toxic Shock Syndrome
Exfoliating Toxins A and B	S. aureus	Scalded Skin Syndrome
Pyrogenic exotoxins A, B, C	S. pyogenes	Fever, Rash, shock
M. arthritides mitogen	M. arthritides	Shock

Adapted from Marrack and Kappler, Science, 248:705, 1990

The TCR is an oligomer

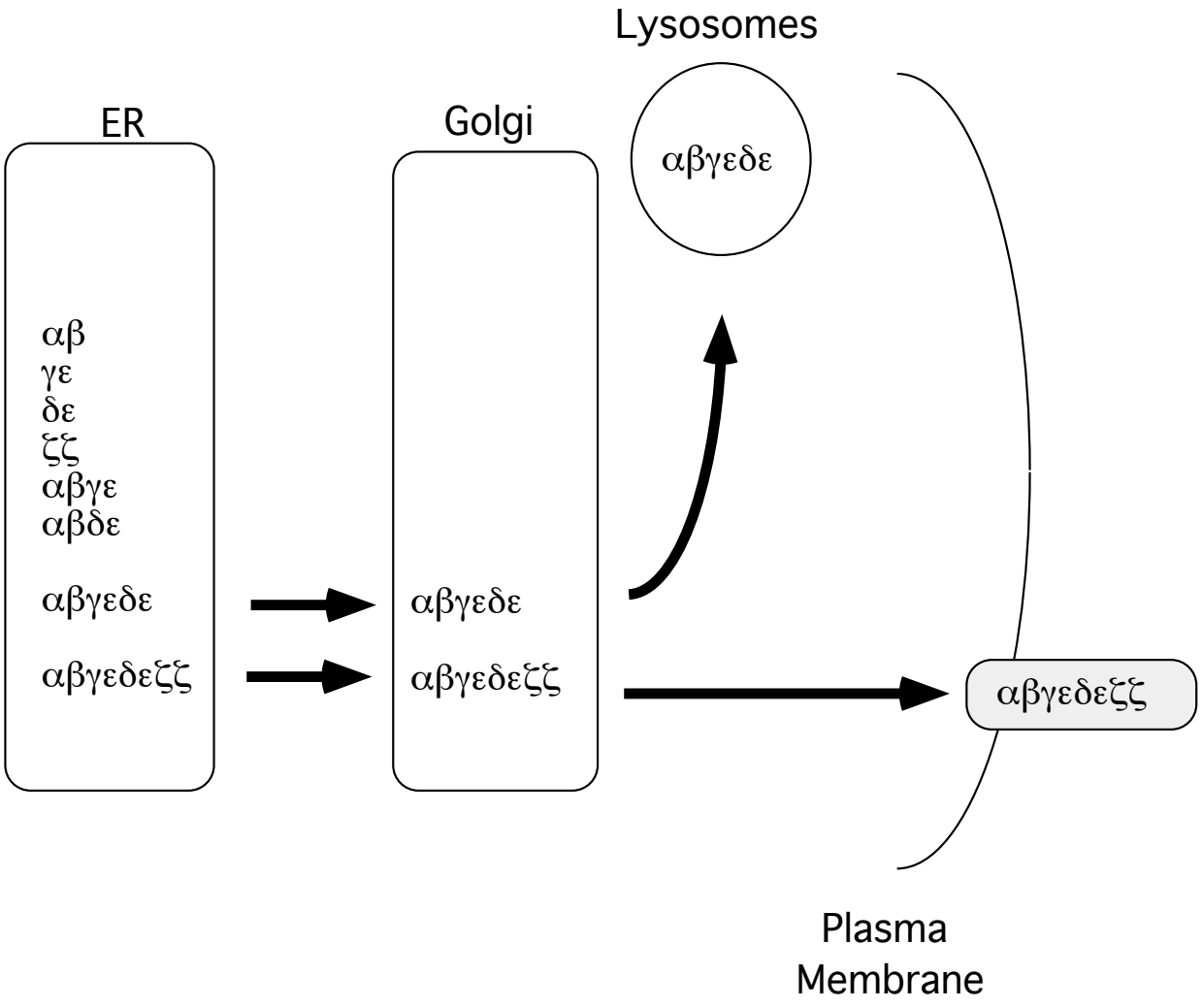
The TCR is an oligomer



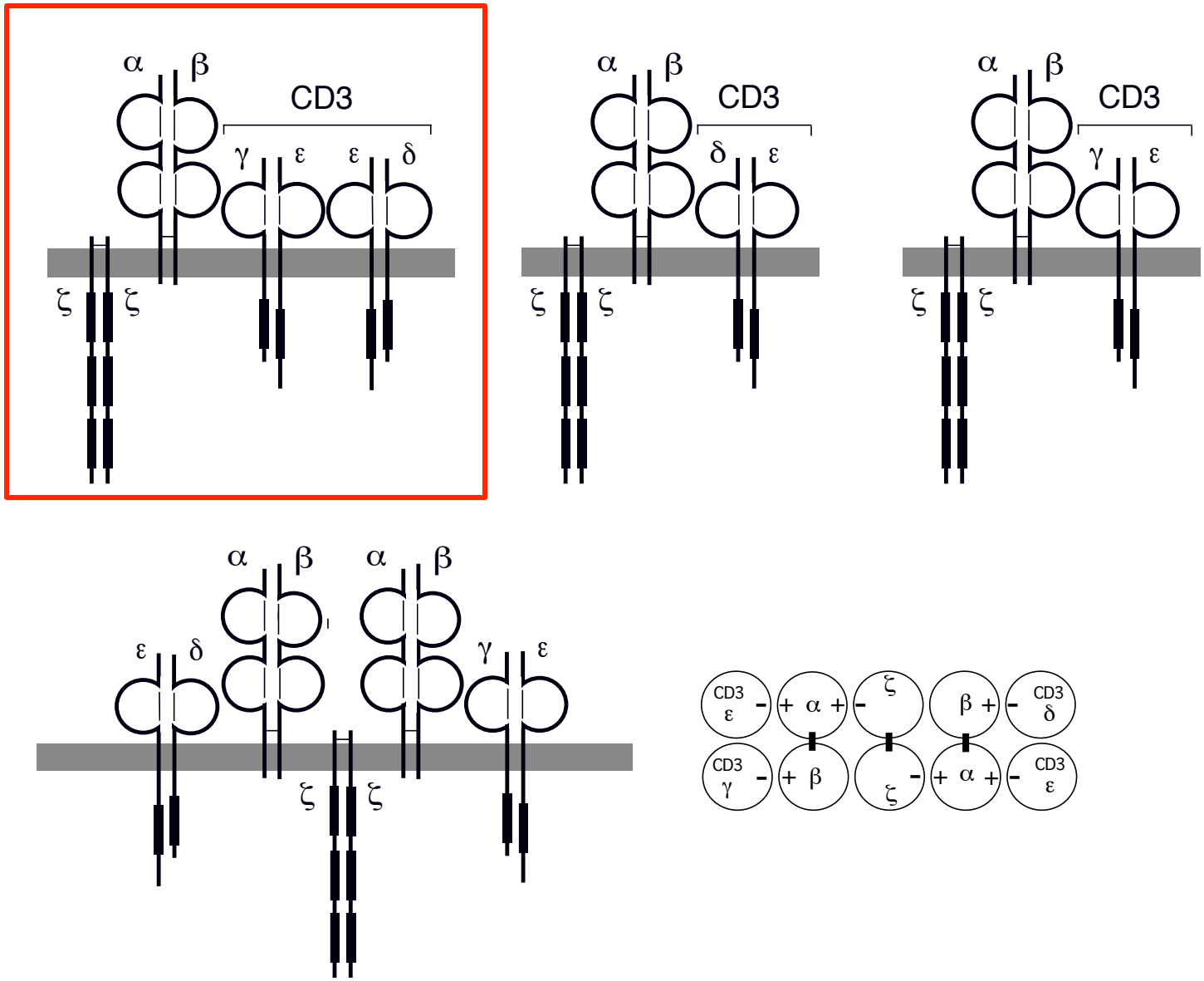
Evidence:

1. Cointernalization of the CD3 and αβ heterodimer
2. Coimmunoprecipitation (very detergent dependent)
3. Chemical cross-linking (β and CD3 γ)
4. Mutants (high CD3 expression requires αβ, γδ or pre-TCR)
5. In vitro assembly studies

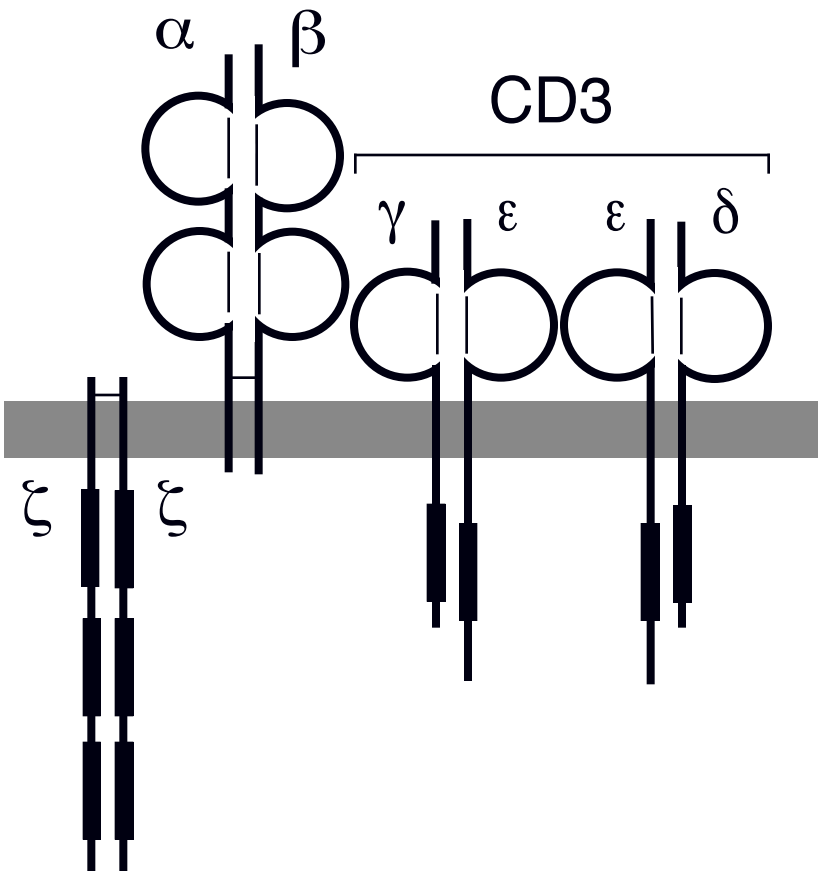
TCR Assembly:
Ordered Interactions and Quality Checkpoints

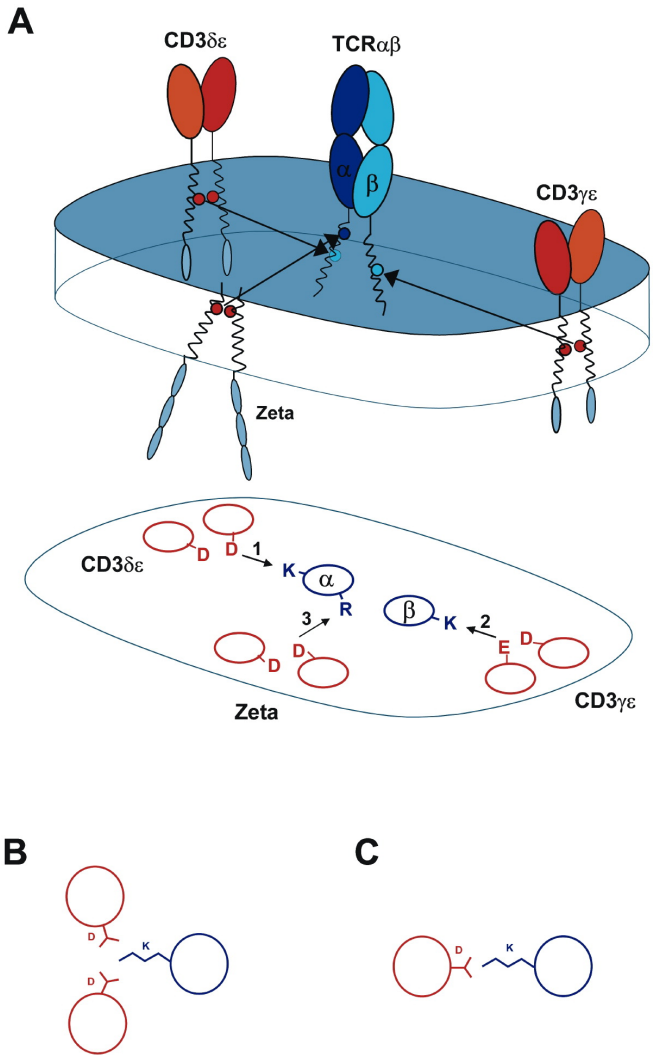


TCR Stochiometry: Models



In Vitro Assembly Favors a Single αβ Heterodimer per TCR and Unusual Transmembrane Interactions

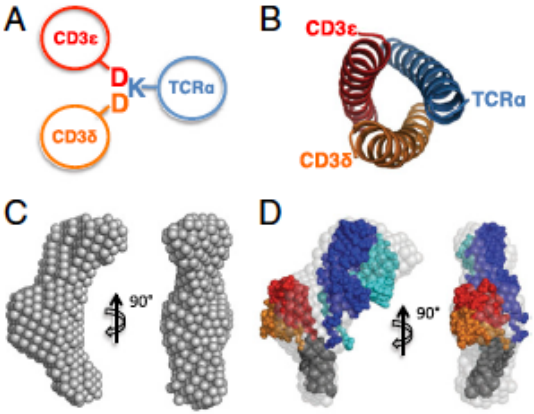




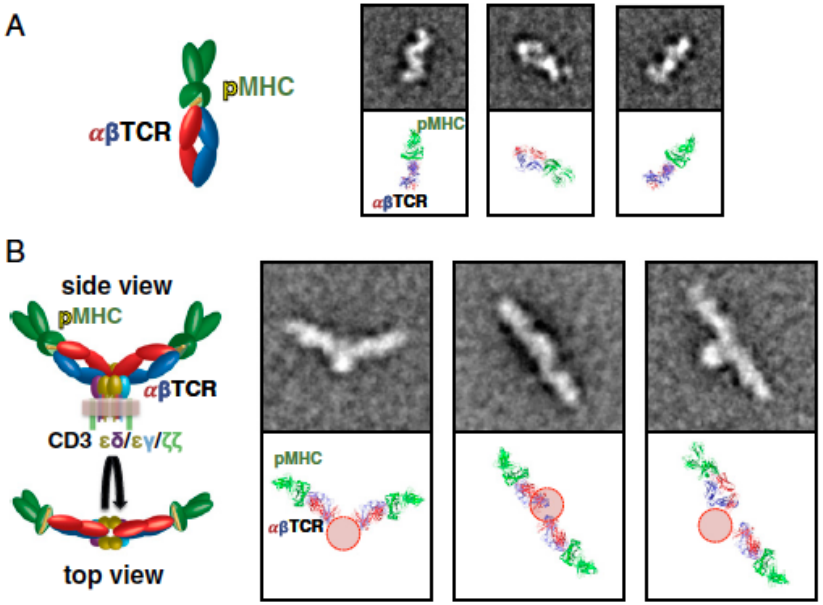
Call, et al., Cell, 111:967, 2002

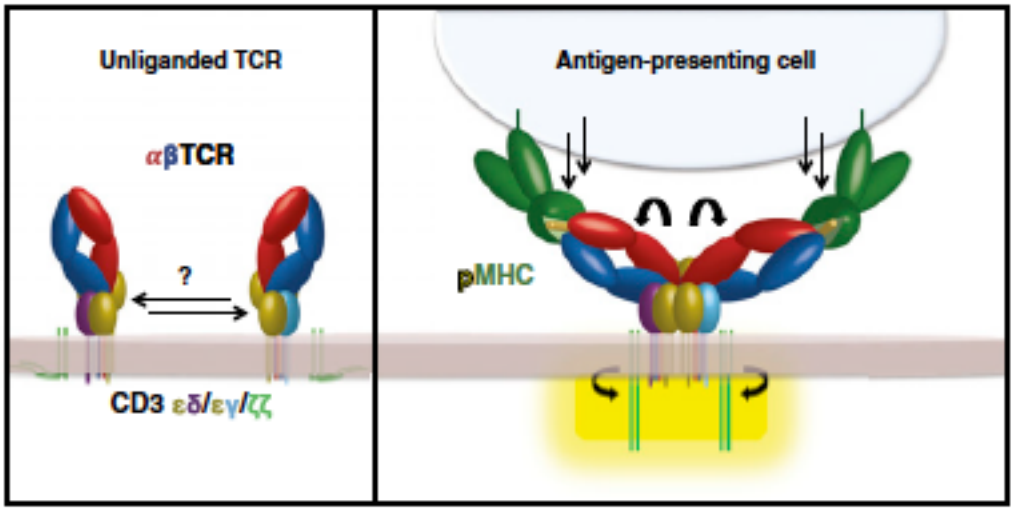
Recent Biophysical Studies Suggest CD3 Chains Sit Below TCR αβ Chains and the ligated TCR may Dimerize

Small angle X-ray scattering (SAX)



Negative Stain EM



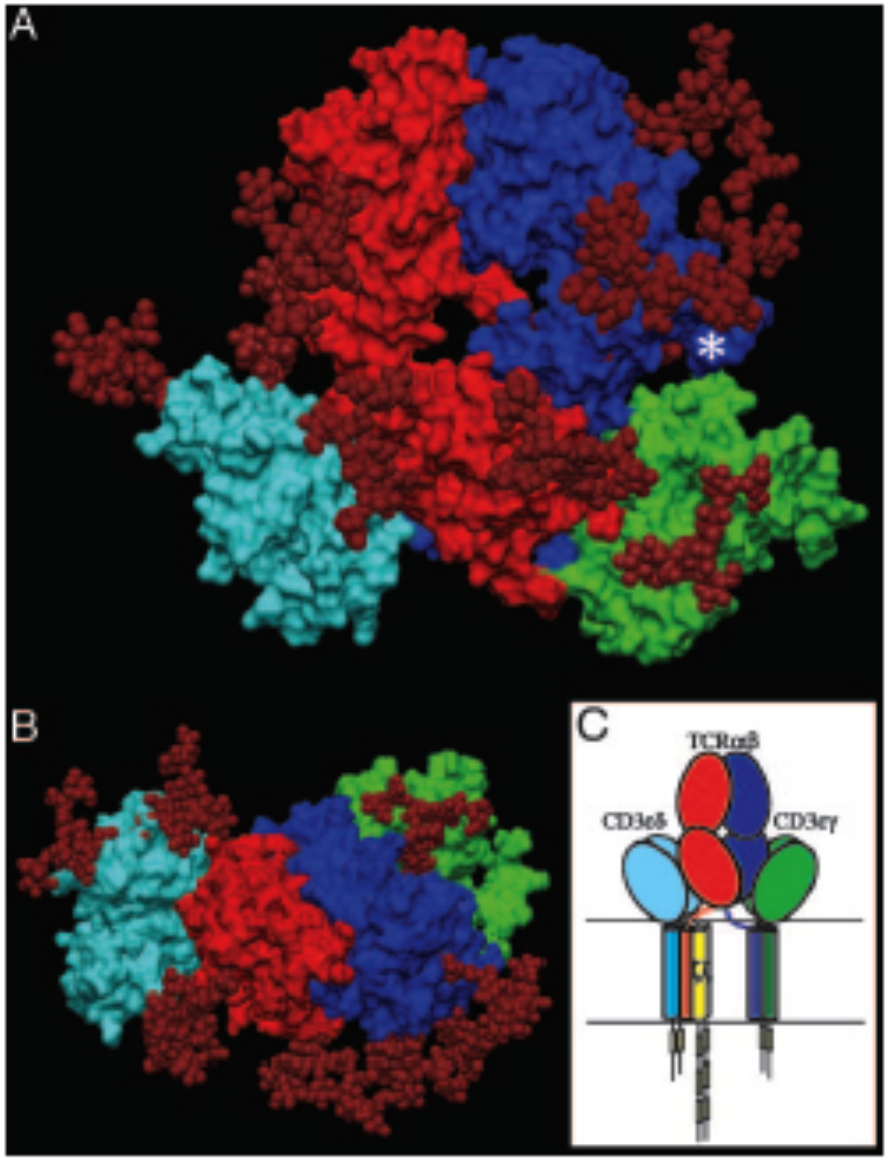


Birnbaum, et al., PNAS, 2014

Model of TCR αβ Heterodimer - CD3 complex

Sun, et al, PNAS, 2004

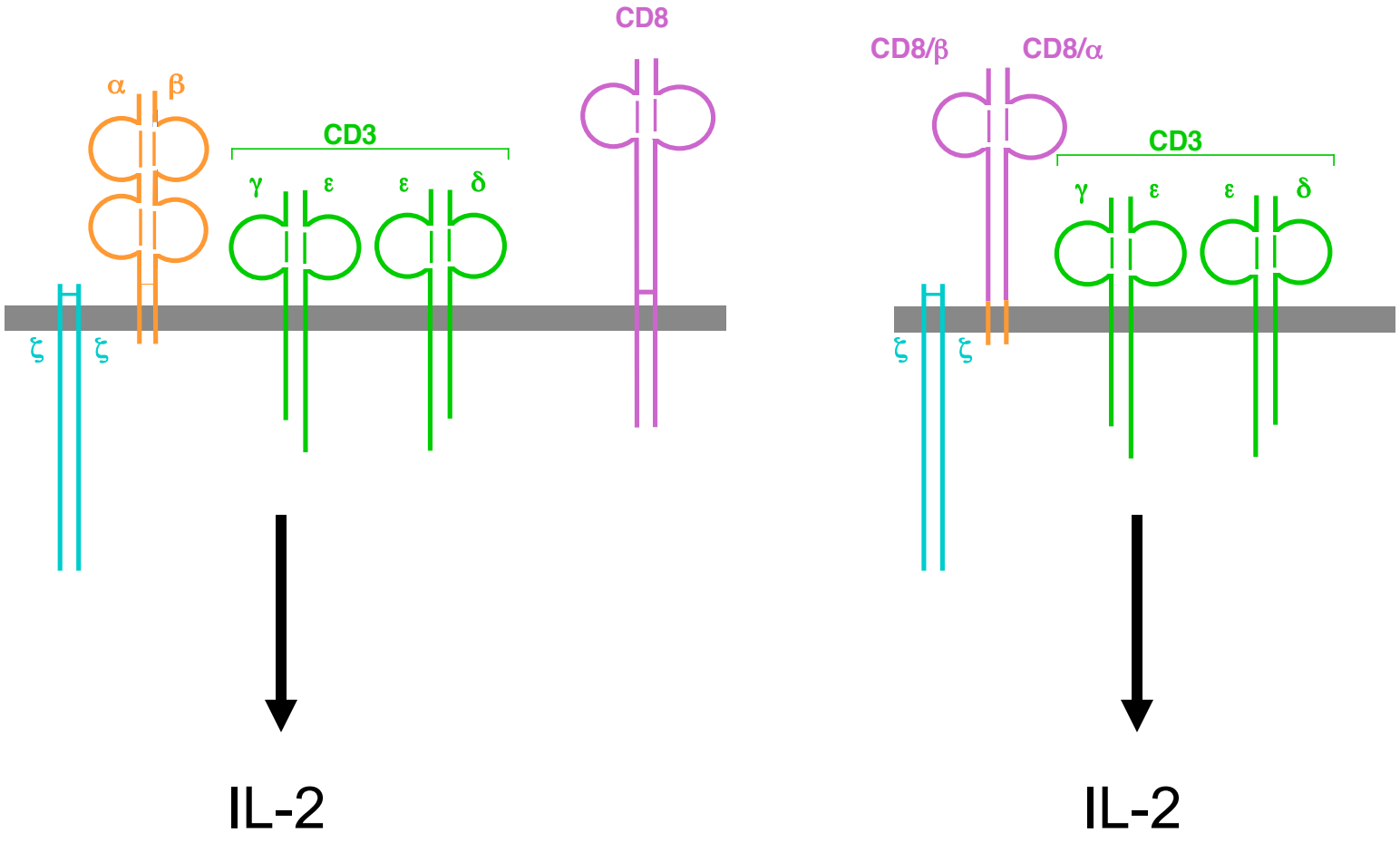
CD4 and CD8 would be on this side - based on TCR and MHC interactions



The CD3- and ζ-chains are the signaling components of the oligomeric TCR

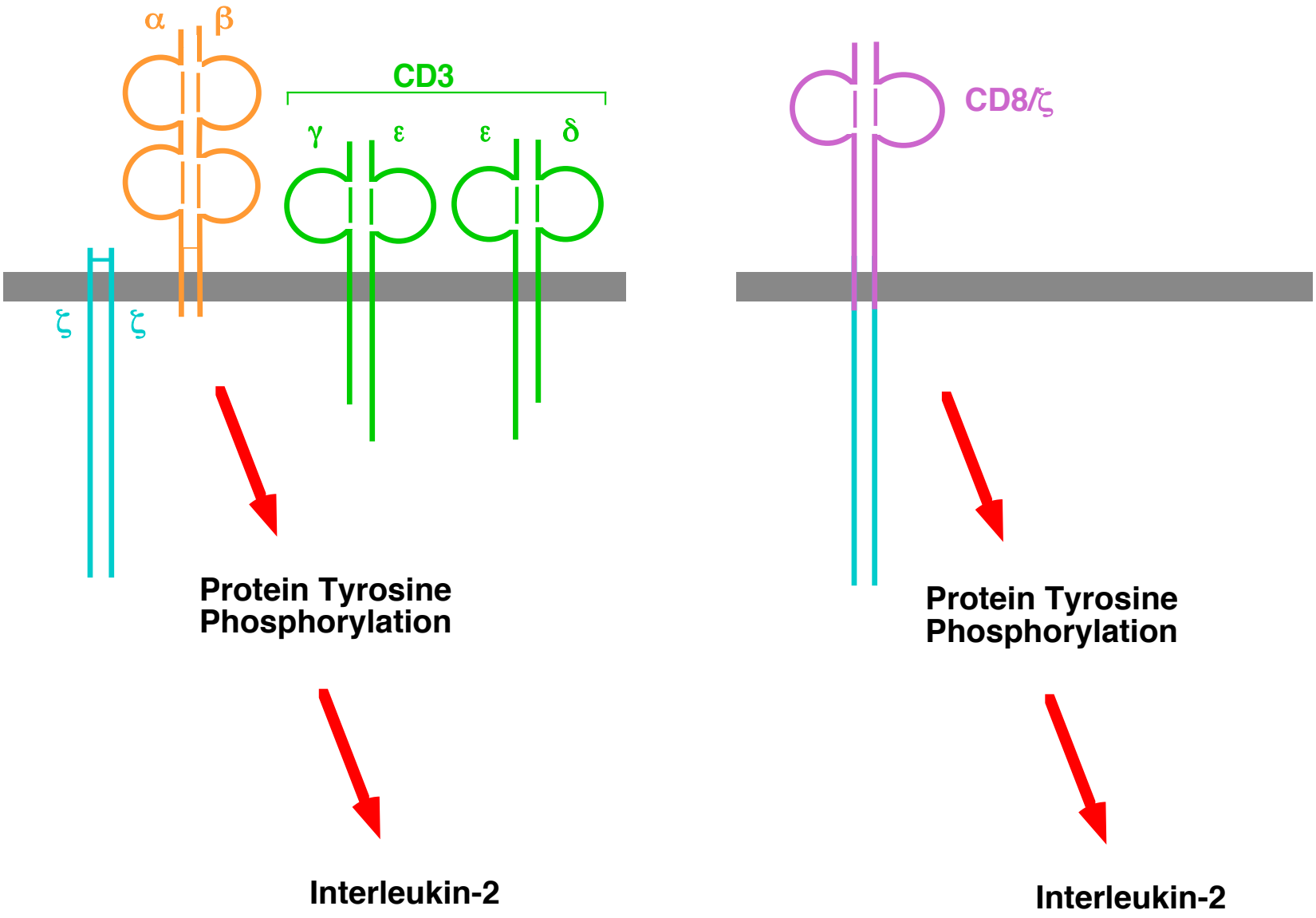
Transmembrane domains allow structural and functional coupling of the αβ heterodimer to CD3 chains

Tan and Weiss, J. Exp. Med, 1991



TCR and CD8/ζ Chimera Induce Similar Signals That Can Lead to T Cell Activation

Irving and Weiss, Cell, 1991

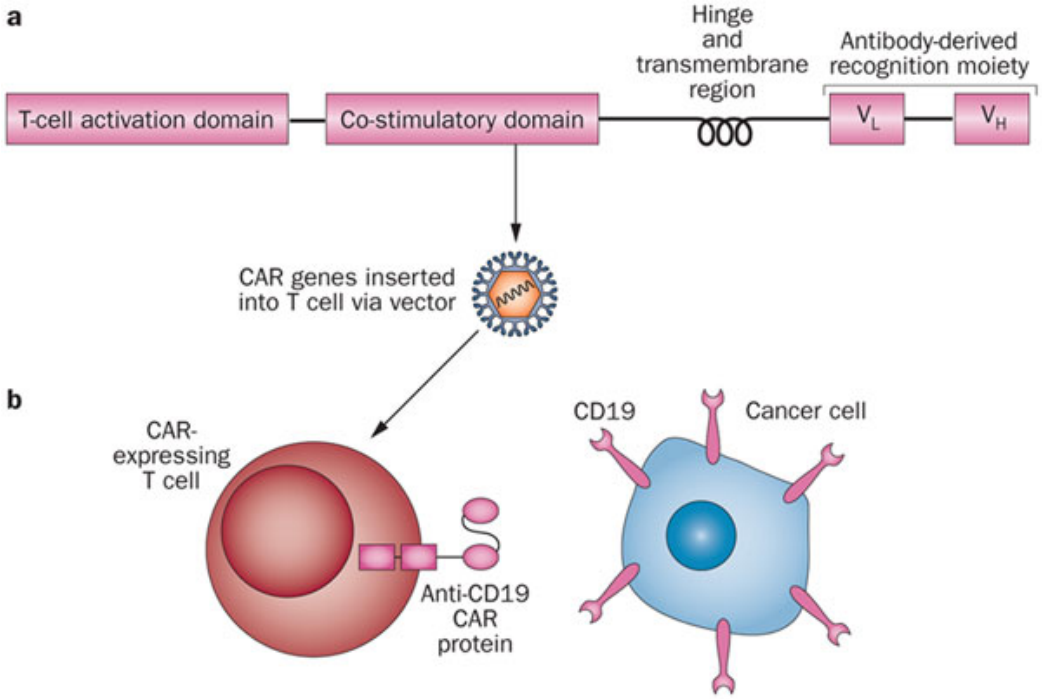


BRIEF REPORT

Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia

David L. Porter, M.D., Bruce L. Levine, Ph.D., Michael Kalos, Ph.D., Adam Bagg, M.D., and Carl H. June, M.D.

N Engl J Med 2013;368:1509-18.
DOI: 10.1056/NEJMoa1215134

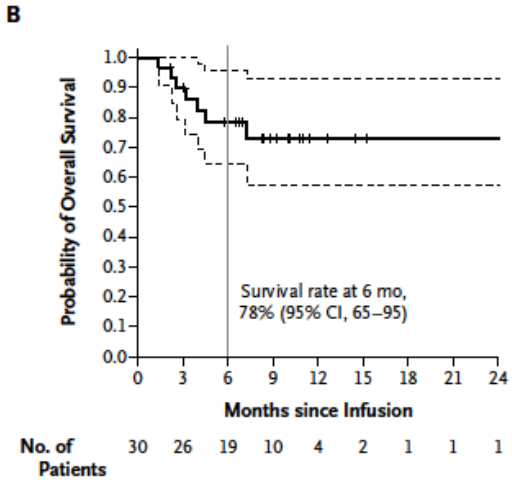


ORIGINAL ARTICLE

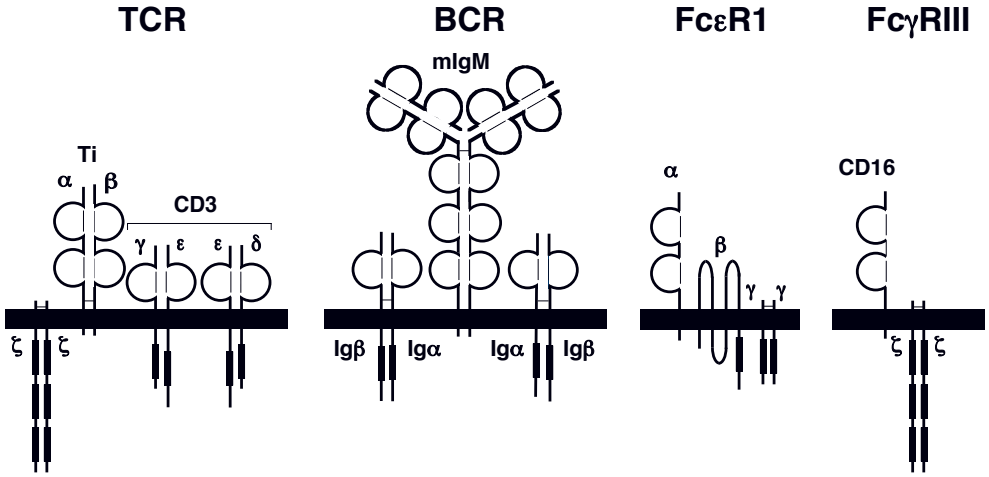
Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia

Shannon L. Maude, M.D., Ph.D., Noelle Frey, M.D., Pamela A. Shaw, Ph.D., Richard Aplenc, M.D., Ph.D., David M. Barrett, M.D., Ph.D., Nancy J. Bunin, M.D., Anne Chew, Ph.D., Vanessa E. Gonzalez, M.B.A., Zhaohui Zheng, M.S., Simon F. Lacey, Ph.D., Yolanda D. Mahnke, Ph.D., Jan J. Melenhorst, Ph.D., Susan R. Rheingold, M.D., Angela Shen, M.D., David T. Teachey, M.D., Bruce L. Levine, Ph.D., Carl H. June, M.D., David L. Porter, M.D., and Stephan A. Grupp, M.D., Ph.D.

N Engl J Med 2014;371:1507-17.
DOI: 10.1056/NEJMoa1407222



ITAM-containing receptors



ITAMs
(Immunoreceptor Tyrosine-Based Activation Motif)

hζ1	N Q L Y N E L N L G R R E E – Y D V L
hζ2	E G L Y N E L Q K D K M A E A Y S E I
hζ3	D G L Y Q G L S T A T K D T – Y D A L
hCD3γ	D Q L Y Q P L K D R E D D Q – Y S H L
hCD3ε	N P D Y E P I R K G Q R D L – Y S G L
hCD3δ	D Q V Y Q P L R D R D D A Q – Y S H L
hDAP12	E S P Y Q E L Q G Q R S D V – Y S D L
rIgE FcR γ	D A V Y T G L N T R N Q E T – Y E T L
rIgE FcR β	D R L Y E E L – H V Y S P I – Y S A L
mIg α	E N L Y E G L N L D D C S M – Y E D I
mIg β	D H T Y E G L N I D Q T A T – Y E D I
BLV gp30	D S D Y Q A L L P S A P E I – Y S H L
EBV LMP-2	H S D Y Q P L G T Q D Q S L – Y L G L
SIV Nef	G D L Y E R L L R A R G E T – Y G R L
KSHV	L Q D Y Y S L H D L C T E D – Y T Q P
Consensus	– – – Y – – L – – – – – – – – Y – – L

The ITAM as a Conserved Signal Transduction Module

ITAM can confer signal transduction function to heterologous receptors,
17 aa are enough

ITAMs are encoded on 2 exons, evidence for evolutionary conservation

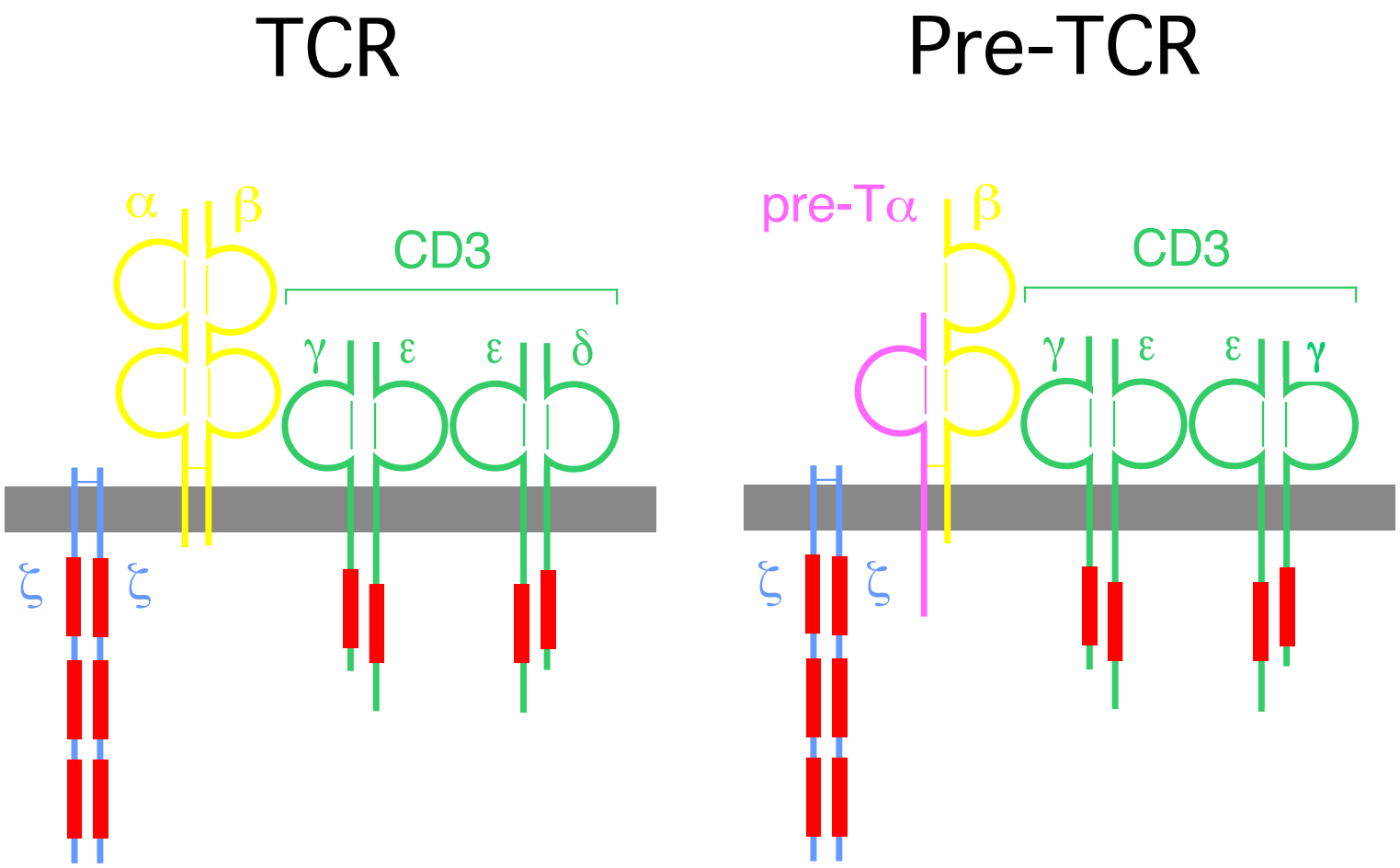
Tyrosines and Leucines (or Isoleucines) are critical, as is spacing between YXXL
residues 7 and 8 aa spacer are OK, 6 is not

Function of redundancy: Signal Amplification vs Distinct Functions
Multimers signal better
Effector binding differences

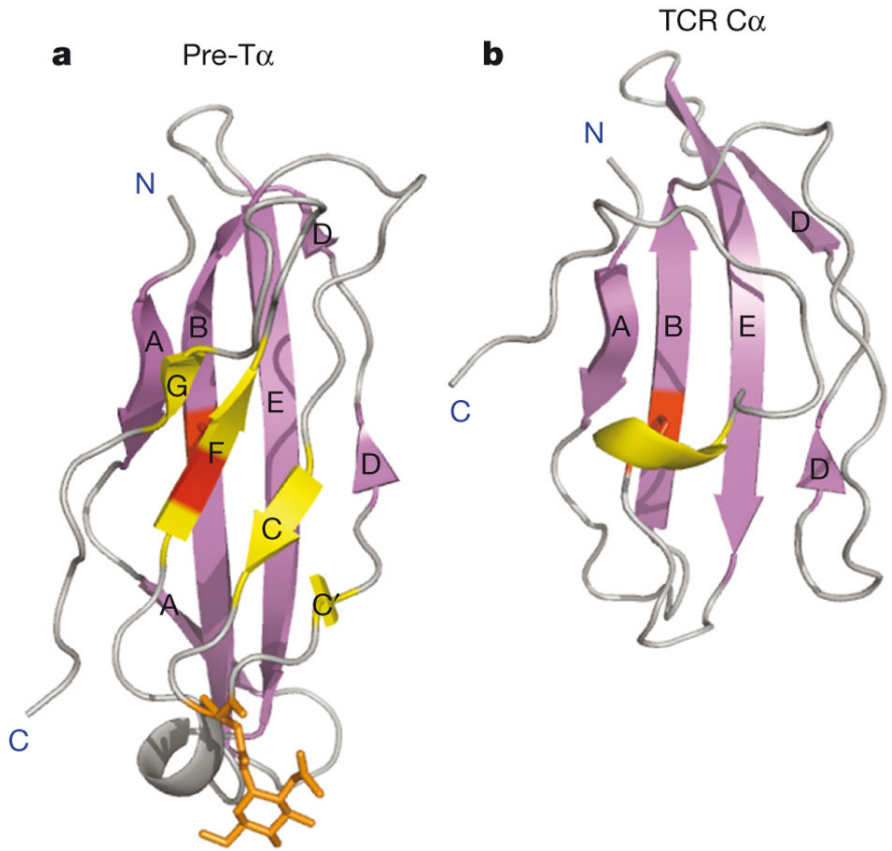
Viruses usurp signaling function

The Pre-TCR, an immature receptor to assess functional β-chain rearrangement during thymus development

An immature form of the TCR has a surrogate for the α Chain, Pre-Tα



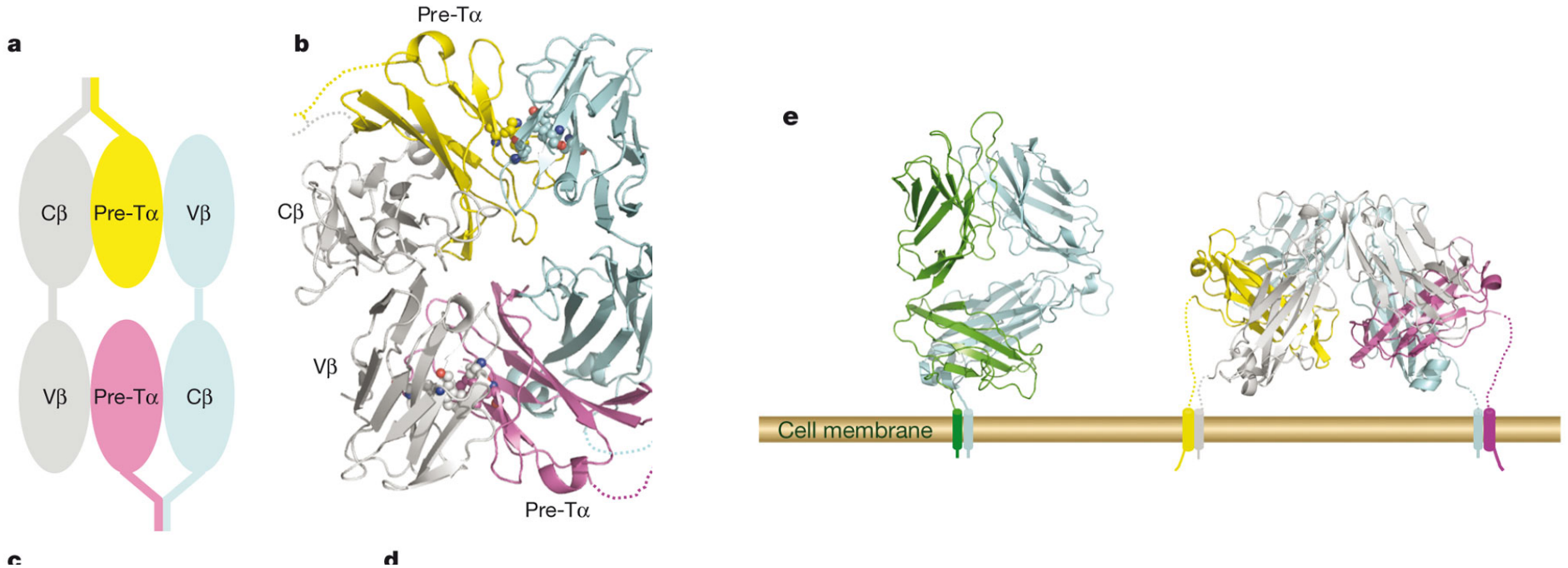
The pre-Tα structure



SS Pang *et al. Nature* **467**, 844-848 (2010) doi:10.1038/nature09448

nature

The pre-TCR dimer forms a constitutive dimer



SS Pang *et al. Nature* **467**, 844-848 (2010) doi:10.1038/nature09448