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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OFENGINEERING & TECHNOLOGY

BCR & TCR immunological diversity

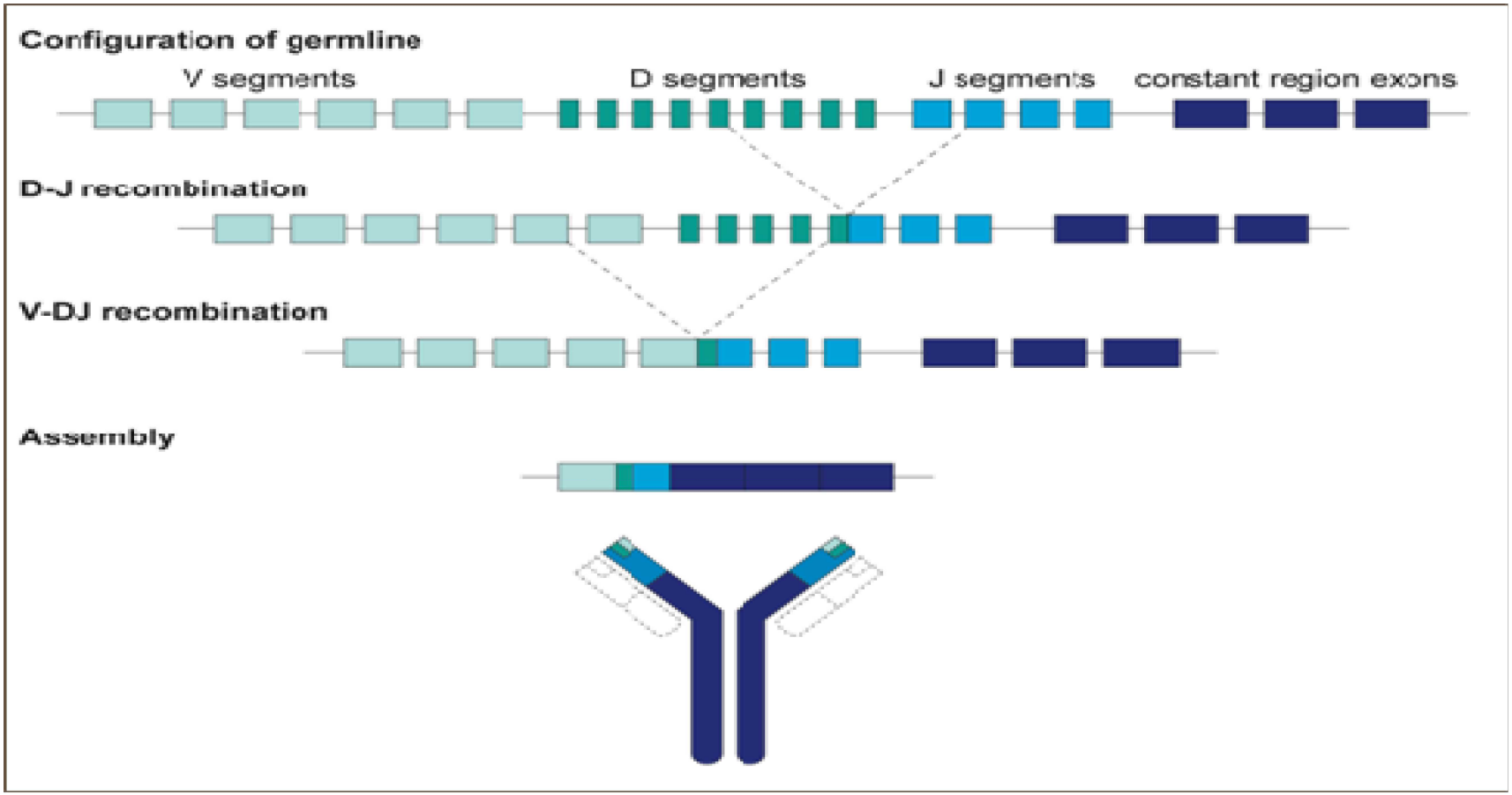
Content Outline

1. B-Cell Receptor Diversity
2. T-Cell ReceptorDiversity

BCR Diversity

•The diversity of BCRs expressed by an individual's B cells is vast, and comprises both naive receptors that are randomly generated from the germline during development, as well as receptors that are retained after successfully binding antigen during previous infections. Populations of BCRs can rapidly improve antigen binding during infection through an evolutionary process of mutation and selection known as affinity maturation.

•The initial diversity of the BCR repertoire is the result of a somatic recombination process called V(D)J recombination. This process brings together one each of the variable (V), diversity (D), and joining (J) segments of the *IGH* locus on chromosome 14 to form an exon in the heavy chain immunoglobulin gene, and one each of the V and J segments of the *IGL* (or *IGK*) locus to form the light chain. During this process, additional sequence diversity is generated by random deletion or insertion of nucleotides at segment junctions. This process combines highly variable sequence regions that determine antigen binding (the complementarity determining regions; CDRs) with more conserved framework regions (FWRs) that provide structural support. Thus each naïve B cell has its own BCR sequence, and the number of possible BCR sequences is huge, with models predicting at least 10^{18}, far greater than the number of B cells in the body.


Configuration of germline
V segments
D segments
J segments
constant region exons
D-J recombination
V-DJ recombination
Assembly

Assembly of antibody light chain

Antibody light-chains are produced by fusing three different gene segments, i.e. V, J & C. During B-Cell development, one V segment & one J segment join together with the constant region to form the gene for the antibody light chain. The gene re-arrangement occurs in the DNA, prior to gene transcription into mRNA. Transcription & translation of the assembled gene product yields light-chain polypeptide. V & J segments encode the variable domain of the light-chain polypeptide while C segments encodes for constant domain. The light-chains can be either kappa (κ) light chains or lambda (λ) light chains

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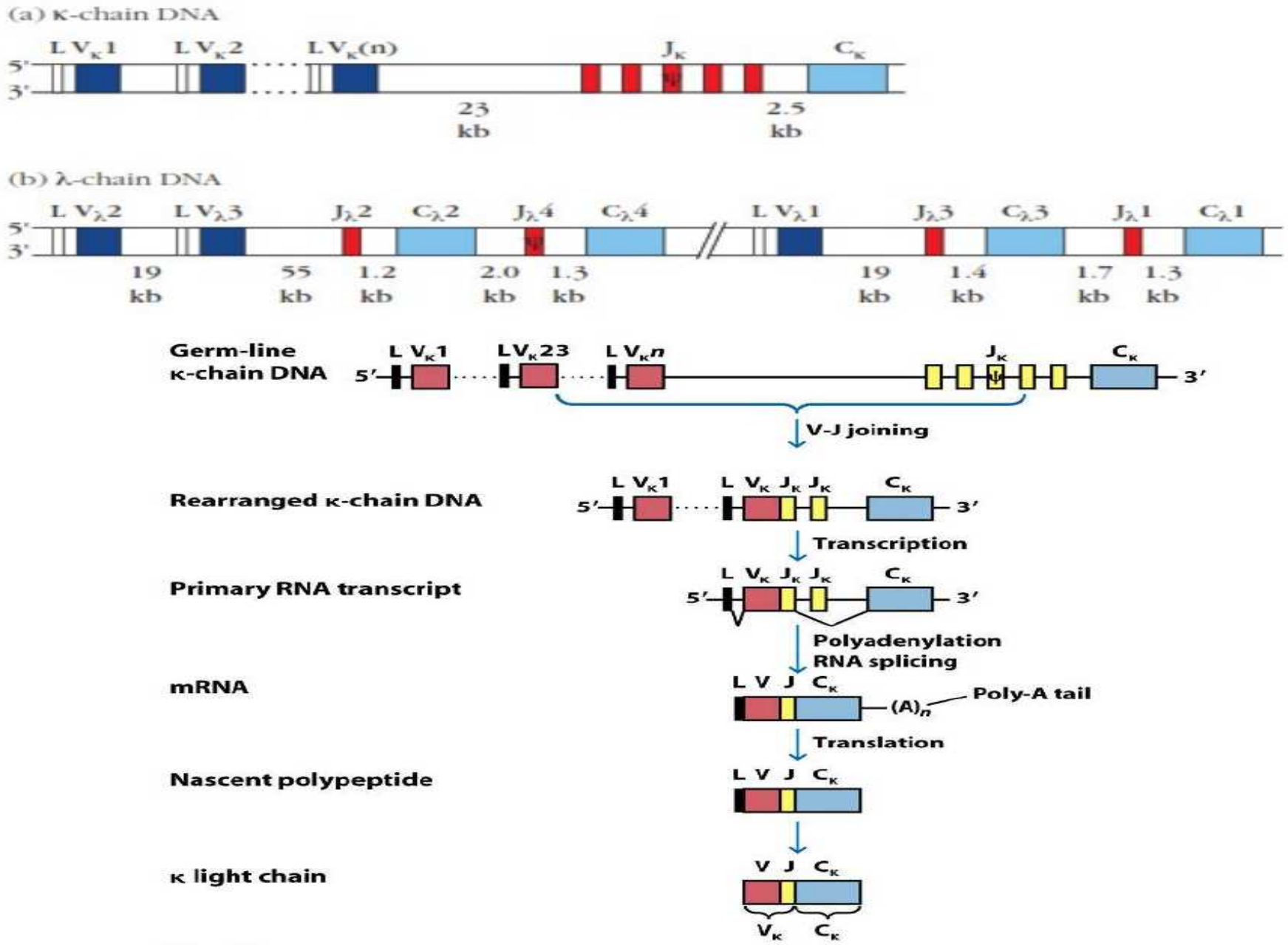
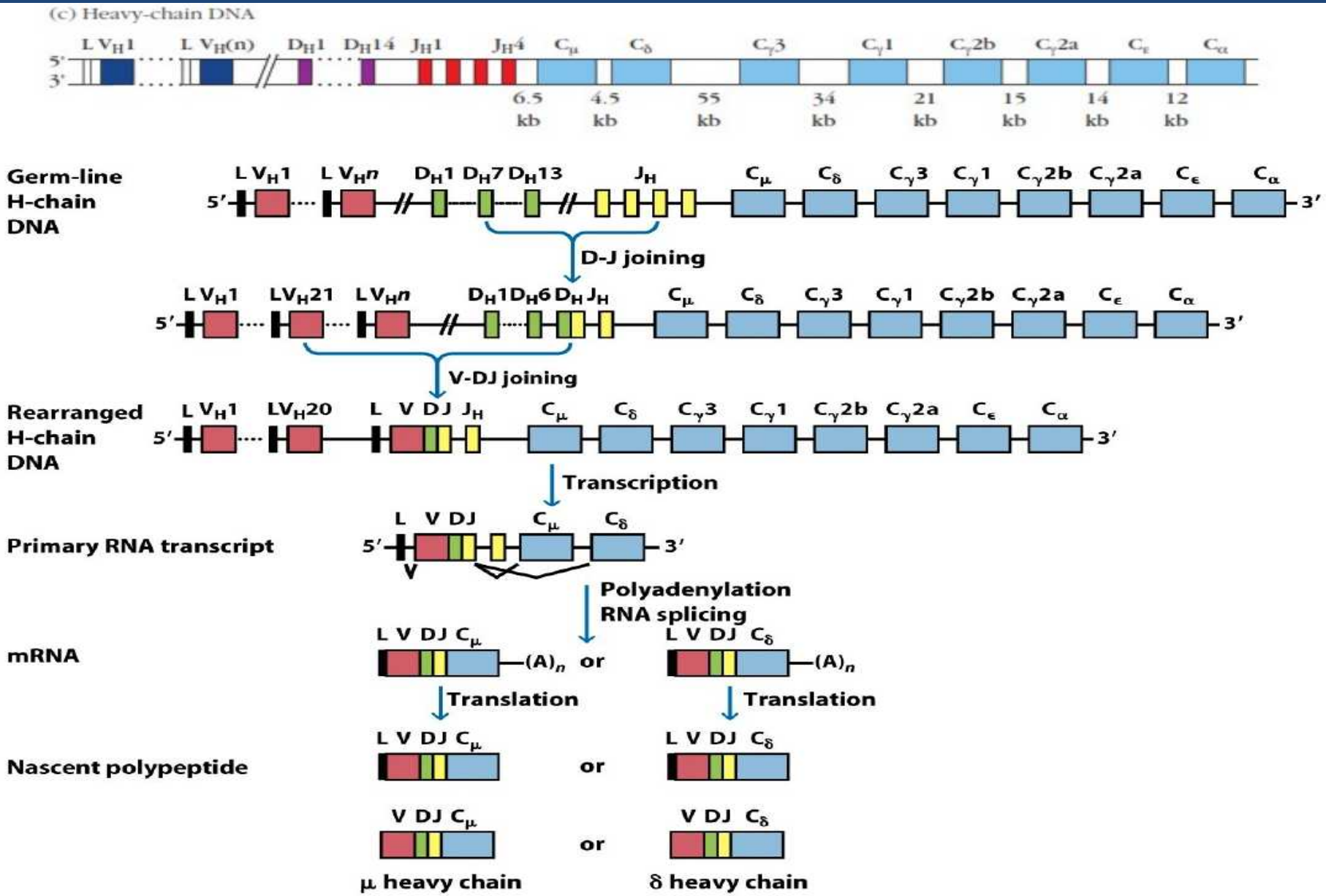


Gene arrangement for production of BCR/antibody light chains

Assembly of heavy chain

Antibody heavy-chains are produced by fusing four different gene segments, i.e. V, D, J & C. Multiple versions of each heavy chain gene segments (V, D, J & C) are encoded in the genome at one location (loci). One gene segment of each kind is joined to form a modified DNA sequence that encodes the heavy chain. During B-Cell development, generation of functional immunoglobulin heavy-chain gene requires two separate re-arrangement (recombination) events within the variable region. One D segment & one J segment joins together 5rst to form DJ segment. DJ segments then moves and joins V segments to generate a VDJ segment that encodes the variable domain of antibody heavy-chain. The VDJ segments joins together with the constant region to form the gene for the antibody heavy-chain. The gene rearrangement occurs in the DNA, prior to gene transcription into mRNA. Transcription & translation of the assembled gene product yields heavy-chain polypeptide. V, D, & J segments encodes the variable domain of the heavy-chain polypeptide while C segments encodes for constant domain. The heavy-chains can be either mu (μ; Ig M); delta (δ , Ig D); gamma (γ, IgG); alpha α;IgA); or epsilon (ε, IgE).

➢Once, assembly of both light-chain & heavy-chain gene segments is accomplished, the DNA is transcribed to mRNA & then translated to polypeptides & assembled to form a BCR or antibody molecule. Light-chains & Heavy- chains are linked together by disulfde bonds.



Gene arrangement for production of BCR/antibody heavy chains

Combinatorial Diversity

V(D)J gene segment recombination creates BCR (antibody) diversity. Combinatorial diversity from random joining of V,D & J segments creates variable domains of BCR/antibody molecules. Combinatorial diversity refers to the fact that same heavy-chain can combine with diferent light-chains, and vice-versa.

κlight chain variable domain in humans:

35 V X 5 J =175 different possibilities

λ-light chain variable domain in humans:

30 V X 4 J=120 different possibilities

Heavy chain variable domains in humans:

40 V X 23 D X 6 J =5520 different possibilities

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More combinatorial diversity from pairing of heavy-chains & light-chainsAntibody w/ κ light chain:

175 κ X 5 520 H =966000 different possibilities

Antibody w/ λ light chain:

1 20 λ X 5520 H=662400 different possibilities Hence, total possibilities of antibody molecules/BCRs: 966,000 + 662,400 = 1.6 Million possibilities

Junctional Diversity

It results from “messy” joining of V, D & J segments. Nucleotides may be deleted or added at joints, this increases the potential diversity of antigen receptors

Generation of TCR Diversity

•The capability of TCR to bind a vast array of antigens both foreign and self is due to the fact that the antigen recognition site (or variable region) is generated by a unique process of somatic recombination. A variable (V), joining (J) and constant region (C) constitute the TCR α- and γ-chains. The TCR β- and δ-chains are also made up of a V, J and C region, with an additional diversity (D) region. One segment from each region is recombined, with additional nucleotide additions and/or deletions, to generate each rearranged TCR (Figure 6). This recombination generates high T-cell diversity and enables the recognition of millions of antigens.

•Genes for the D chain segments are located completely within the region containing the α gene segments. Vα and Vγ regions of TCR proteins, like V_L regions of Ig, are encoded by V and J segments. Humans have approximately 70 different Vα, 60 different Jα, and a single Cα segment. A cluster of twelve Vγ is followed by 3 Jγ with Cγ1 and 2 Jg with Cγ2. The gene segments for TCR are flanked by the same recombination signal sequences as are the Ig gene segments, and the same RAG-1 and RAG-2 encoded recombinase and terminal **deoxynucleotidyl transferase (**TdT) are required for somatic recombination.

The joining regions for Vα and Jα and for Vβ, Dβ, and Jβ occur in CDR3, while CDR1 and CDR2 sequences are encoded within Vα and Vβ. P and N nucleotides are added to the junctions between Vβ, Dβ, and Jβ and between Vα and Jα. Generation of antigen-binding diversity for TCR, therefore, depends on the same combinatorial and junctional mechanisms used for Ig diversity. Somatic hypermutation does not seem to be an important diversity mechanism for TCR. TCR genes undergo somatic recombination in a defined sequence during T cell development in the thymus to generate overwhelming diversity.

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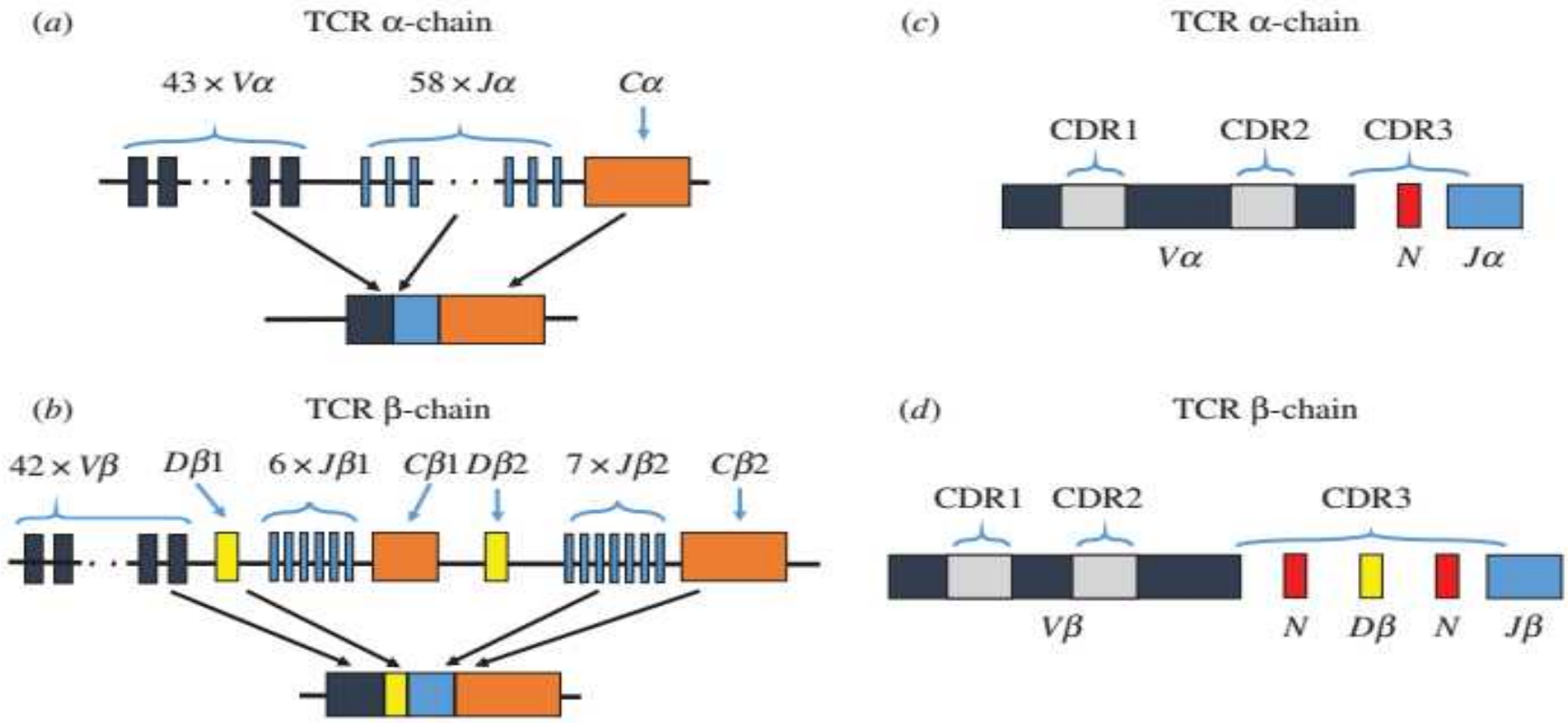
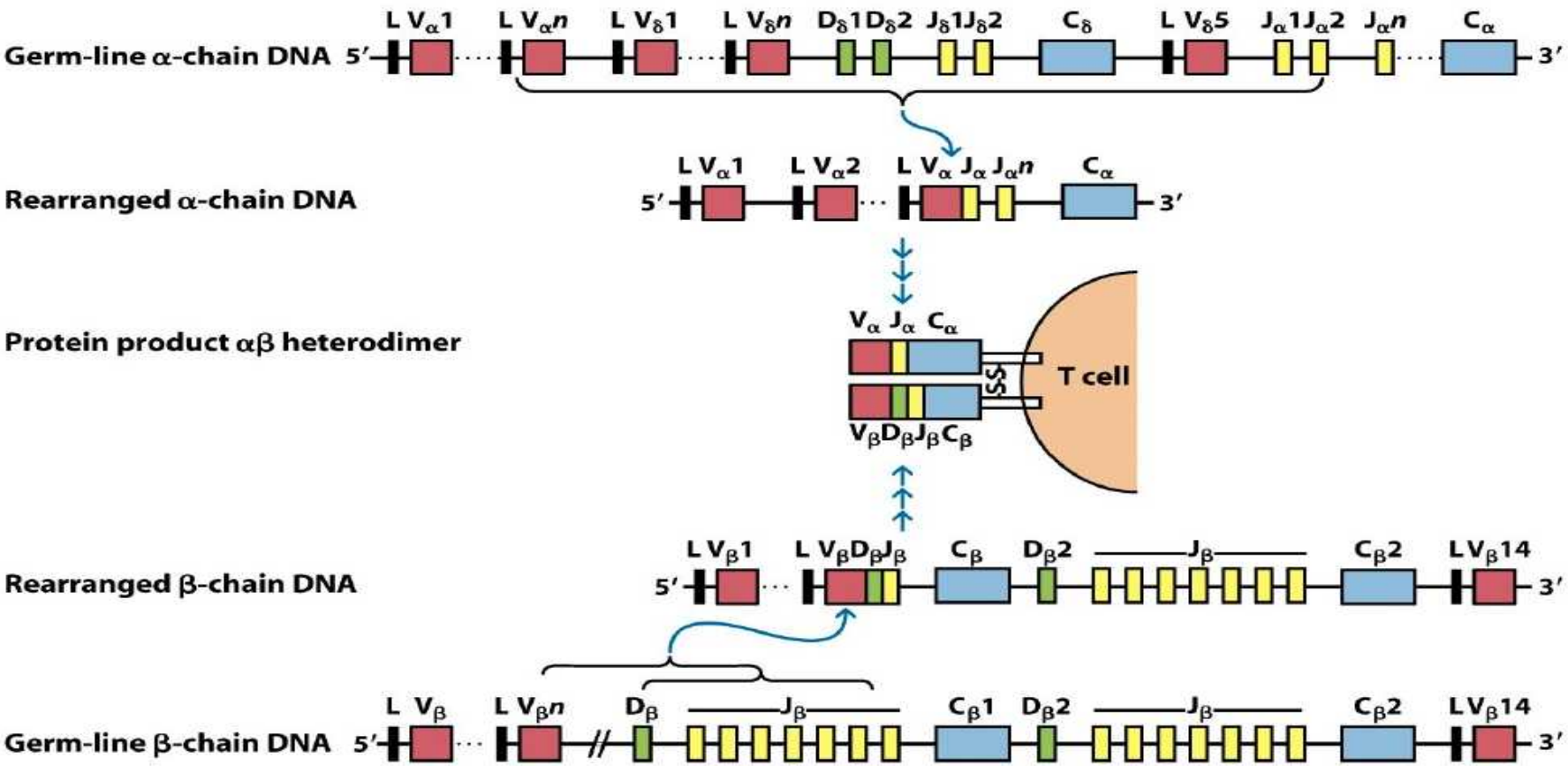


T-cell receptor gene rearrangement. (a) Variable (V), joining (J) and constant regions (C) constitute the TCR α-chain. (b) Variable (V), joining (J) and constant regions (C) constitute the TCR b-chain, with an additional diversity (D) region. Segments from each region are recombined, with additional nucleotide additions, to generate each rearranged TCR. These processes generate substantial T cell diversity. (c,d) Hypervariable complementarity-determining regions (CDR1- CDR3) of the a-chain (c) and b-chain (d). CDR1 and CDR2 regions are encoded on the V region, while the most variable CDR3 region straddles the V(D)J junction (adapted from rstb.royalsocietypublishing.org Phil. Trans. R. Soc. B 370: 20140291)



A complete view of gene rearrangement for generation of TCR diversity

References & Further reading

References

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Further reading

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