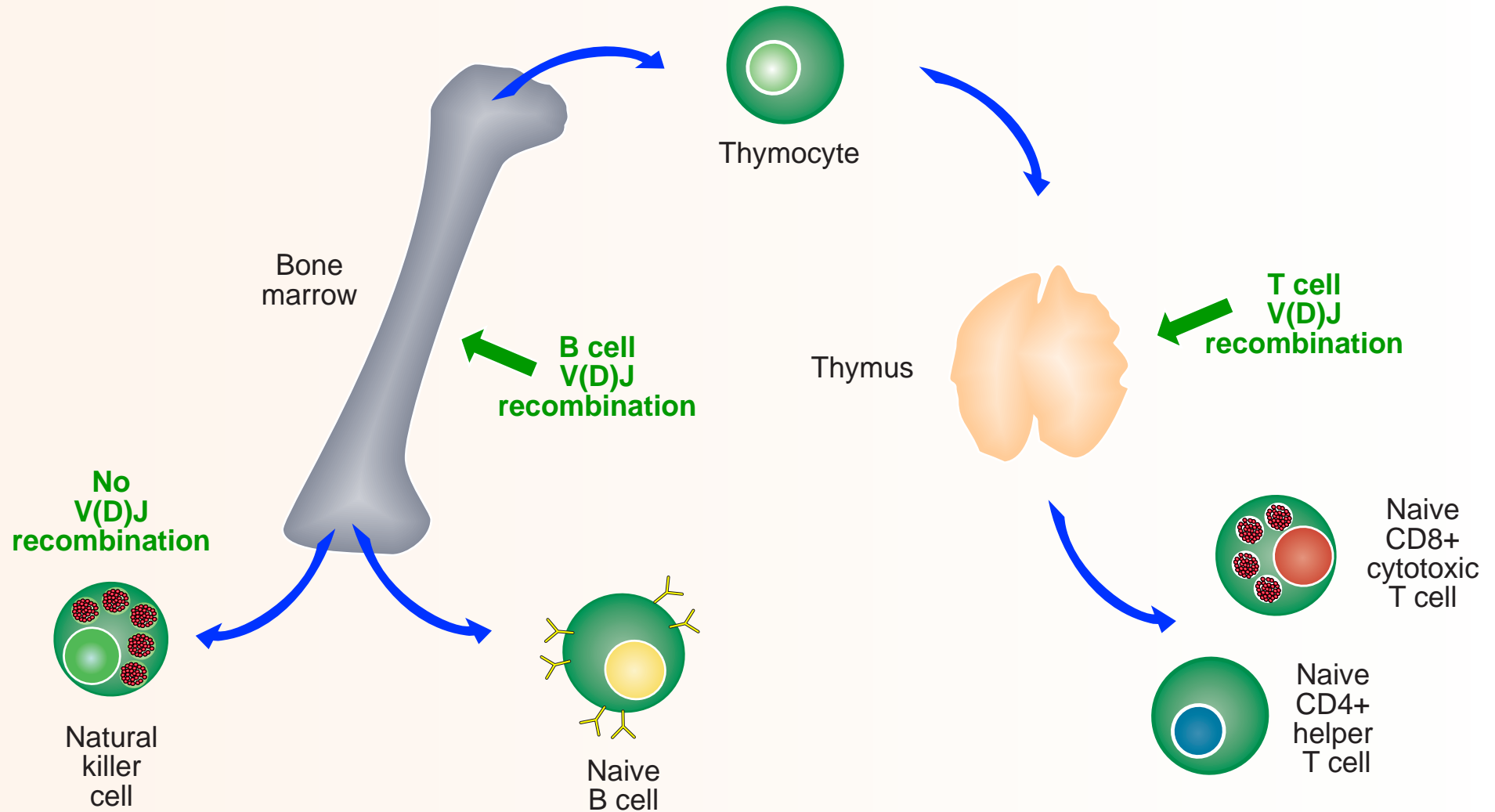
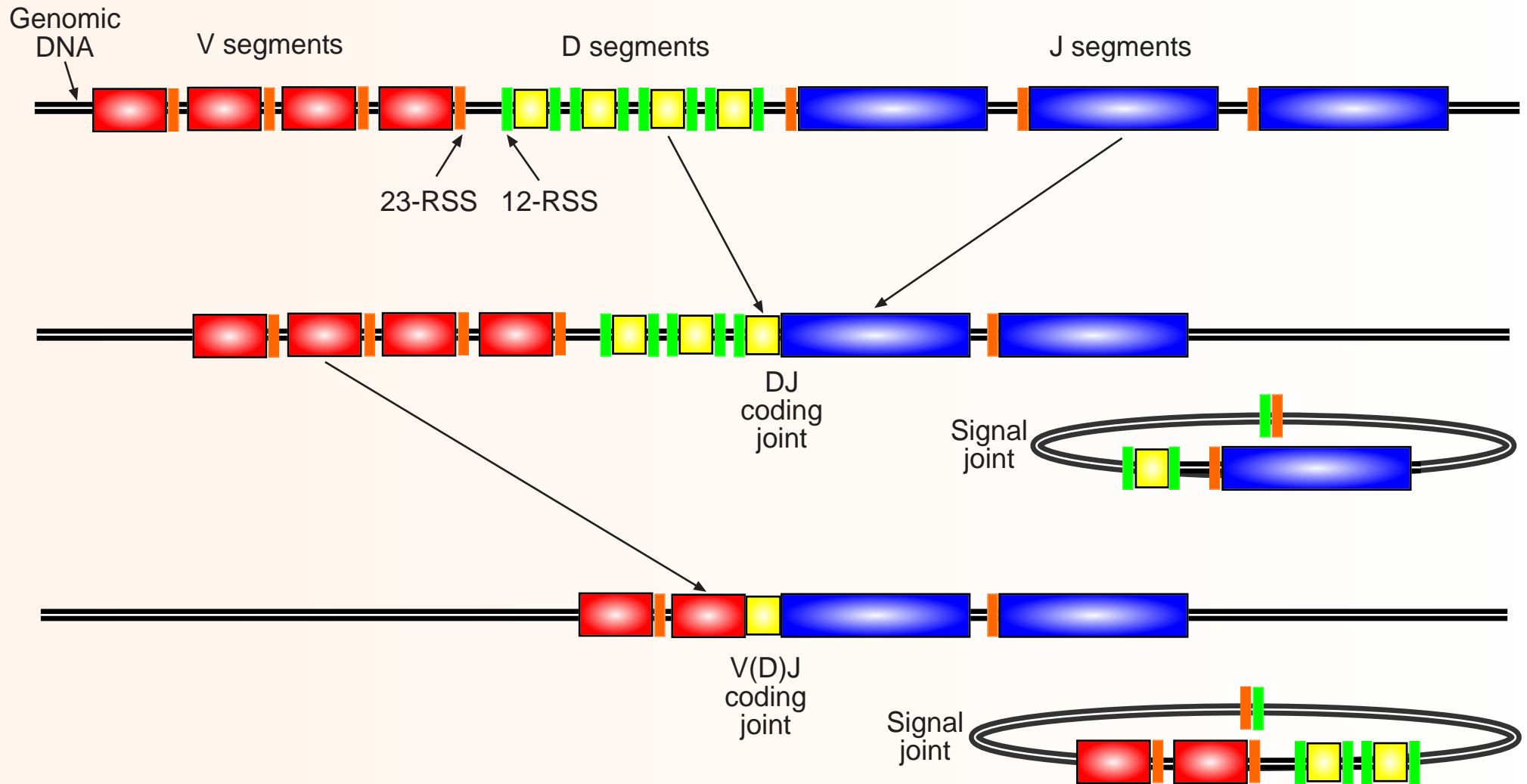


T and B cell V(D)J recombination



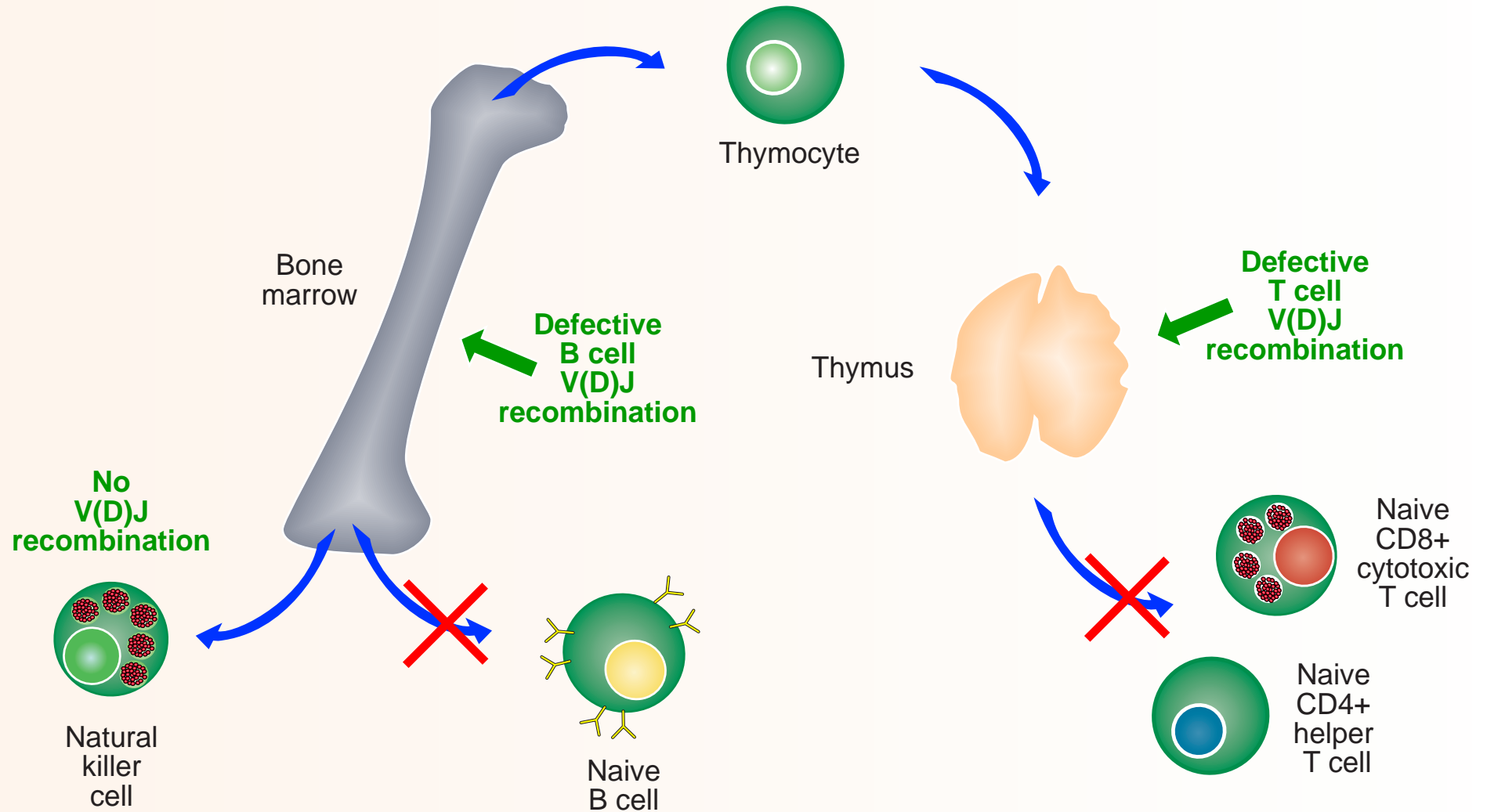
T and B cells, but not natural killer cells, undergo V(D)J recombination in order to generate diverse repertoires of T and B cell receptors (TCR and BCR) capable of recognising a wide range of pathogen epitopes. Variability in the epitope binding potential of the receptors is achieved by varying the combination of pre-existing multicopy gene segments called variable (V), diversity (D) and joining (J) segments found in the BCR-heavy and TCR- β and - δ chains. D segments are absent in the BCR-light (κ and λ) and TCR- α and - γ chains, so only V and J recombination occurs. In B cells this process takes place in the bone marrow whereas thymocytes transmigrate to the thymus where V(D)J recombination occurs.

V(D)J recombination



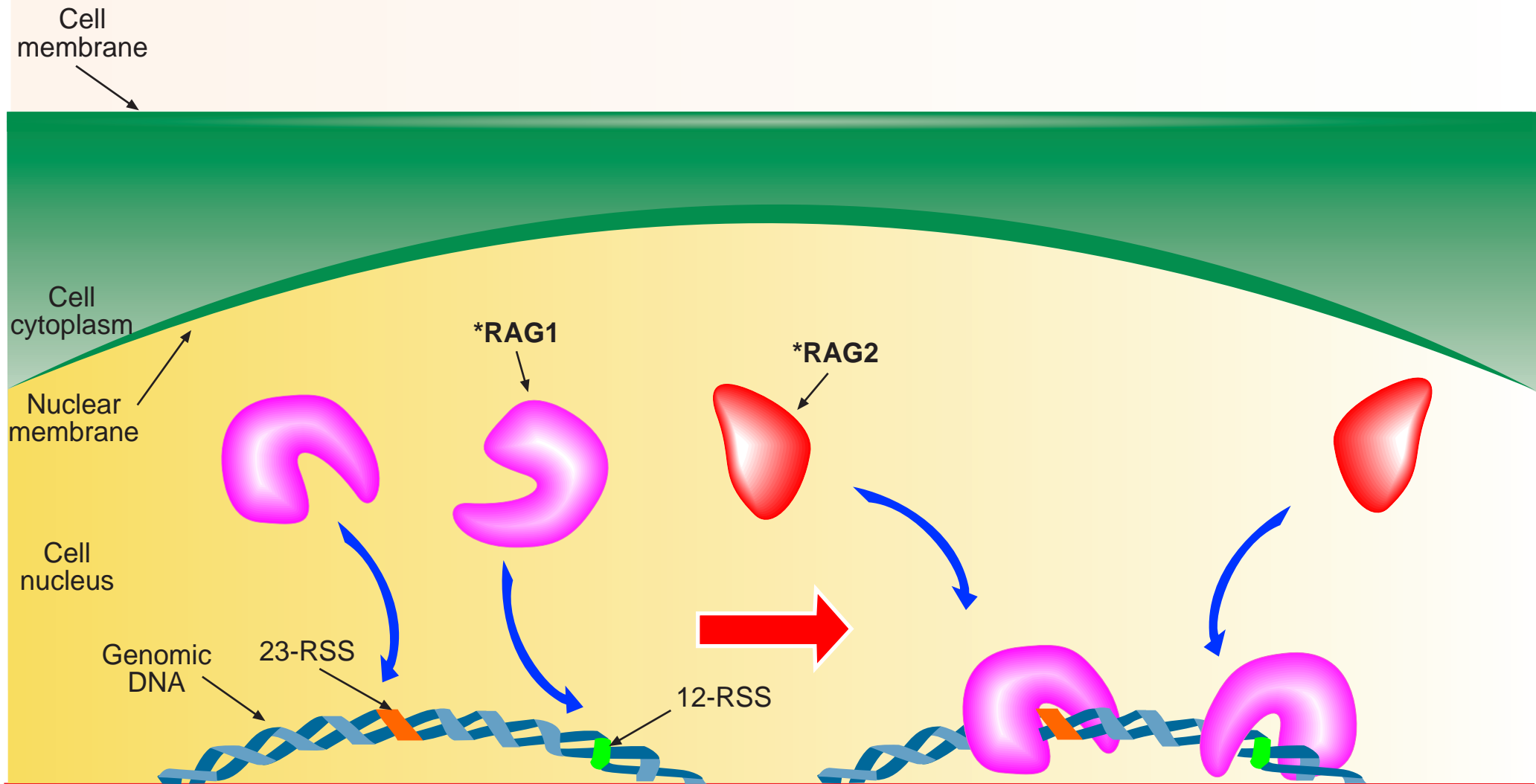
VDJ recombination occurs in the BCR-H, TCR- β and - δ chains. The BCR-L (κ and λ), TCR- α and - γ chains lack D segments and only undergo VJ recombination. Recombination activating genes encode two proteins (RAG1 and RAG2) that mediate the recognition and splicing of V, D and J segments. RAG1 binds to specific recombination signal sequences (RSS) present as 23-RSS or 12-RSS sequences flanking each gene segment and then recruits RAG2. Recombination can only occur between a 23-RSS and 12-RSS sequence (12/23 rule). A double-stranded break is introduced into DNA followed by recruitment of DNA repair enzymes that mediate non-homologous end-joining (NHEJ).

SCID: defective T and B cell development, normal NK cells



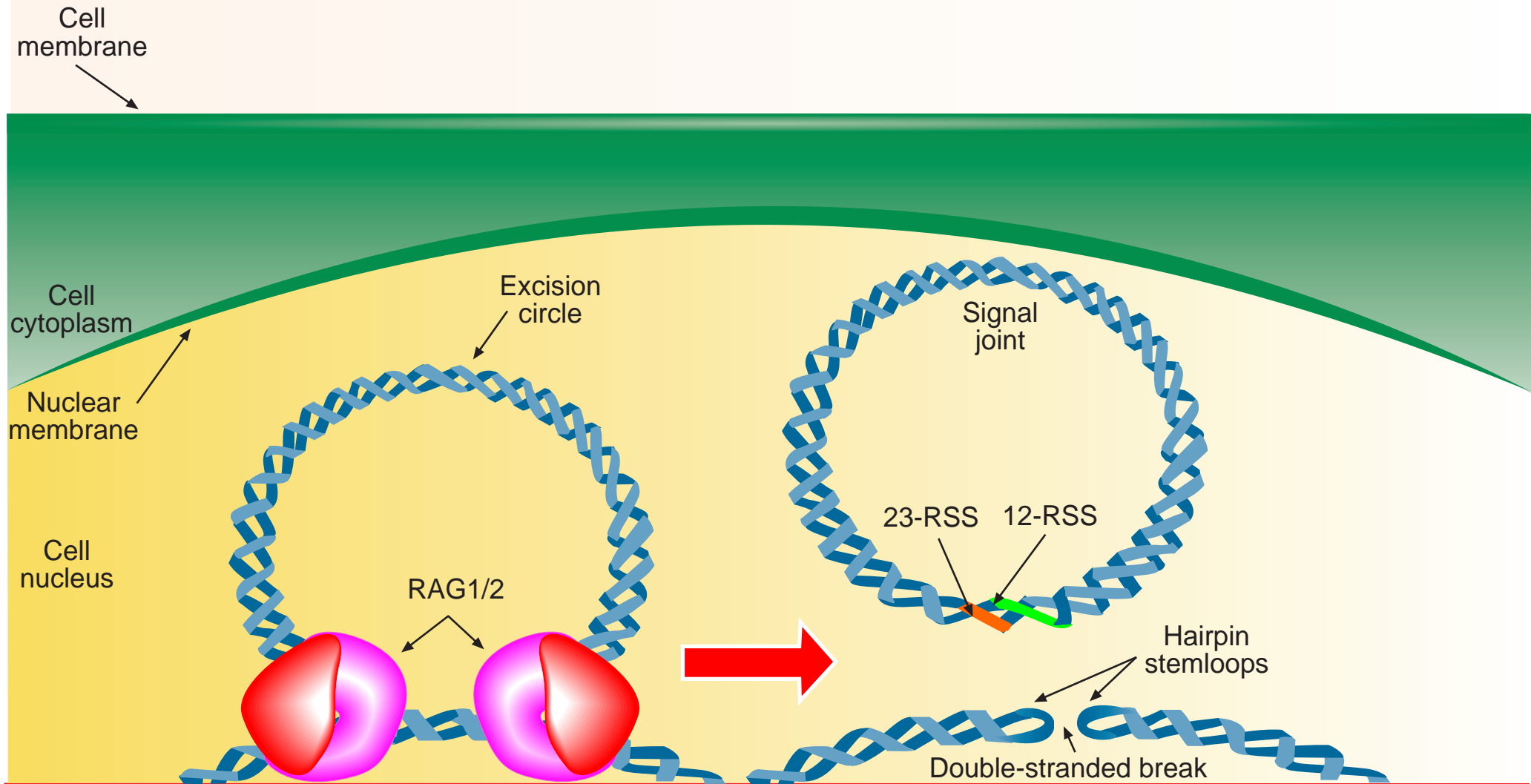
In certain types of SCID, a defect in the development of T and B cells, but normal NK cell function is evident. This stems from defects in the V(D)J recombination processes common to T and B cell maturation, but is not required for NK cell development. There are many proteins involved in V(D)J recombination and to date genetic mutations in RAG1, RAG2, DNA-PKcs, DCLRE1C (also known as Artemis), XLF (also known as Cernunnos) and DNA ligase IV have been identified in SCID phenotypes lacking T and B cells, but normal NK cell activity.

V(D)J recombination: RAG1 and RAG2 binding.



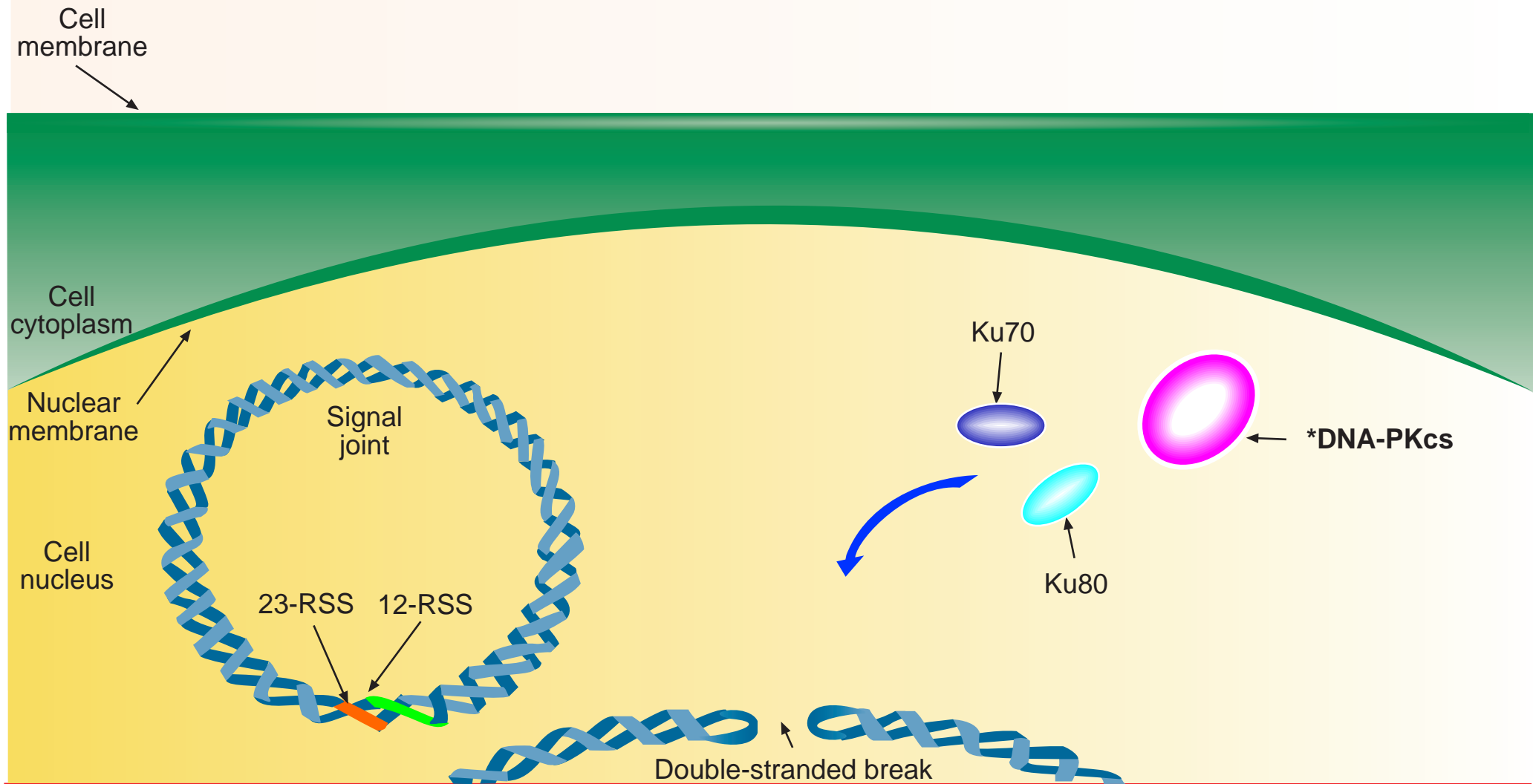
V(D)J recombination begins with the binding of recombination activating gene 1 (RAG1) proteins to specific recombination signal sequences (RSS) present in each V, D and J segment. RAG2 is then recruited and the RAG1/2 complex mediates the formation of a double-stranded break in DNA which will allow a precise fusion of a D and J or V and (D)J segment. *The most common form of SCID manifesting in a lack of development of T and B cells, but normal NK cell function, is caused by genetic mutations in RAG1 and/or RAG2.

V(D)J recombination: Formation of double-stranded break



The RAG1/2 complex mediates the random selection and alignment of a D and J or V and DJ gene segment and introduces a double-stranded break in the DNA. The blunt-ends of the excision circle are ligated between the 12-RSS and 23-RSS sequences to generate the signal joint, while the 5' and 3' ends of the genomic DNA strands are covalently linked to generate hairpin stemloop structures.

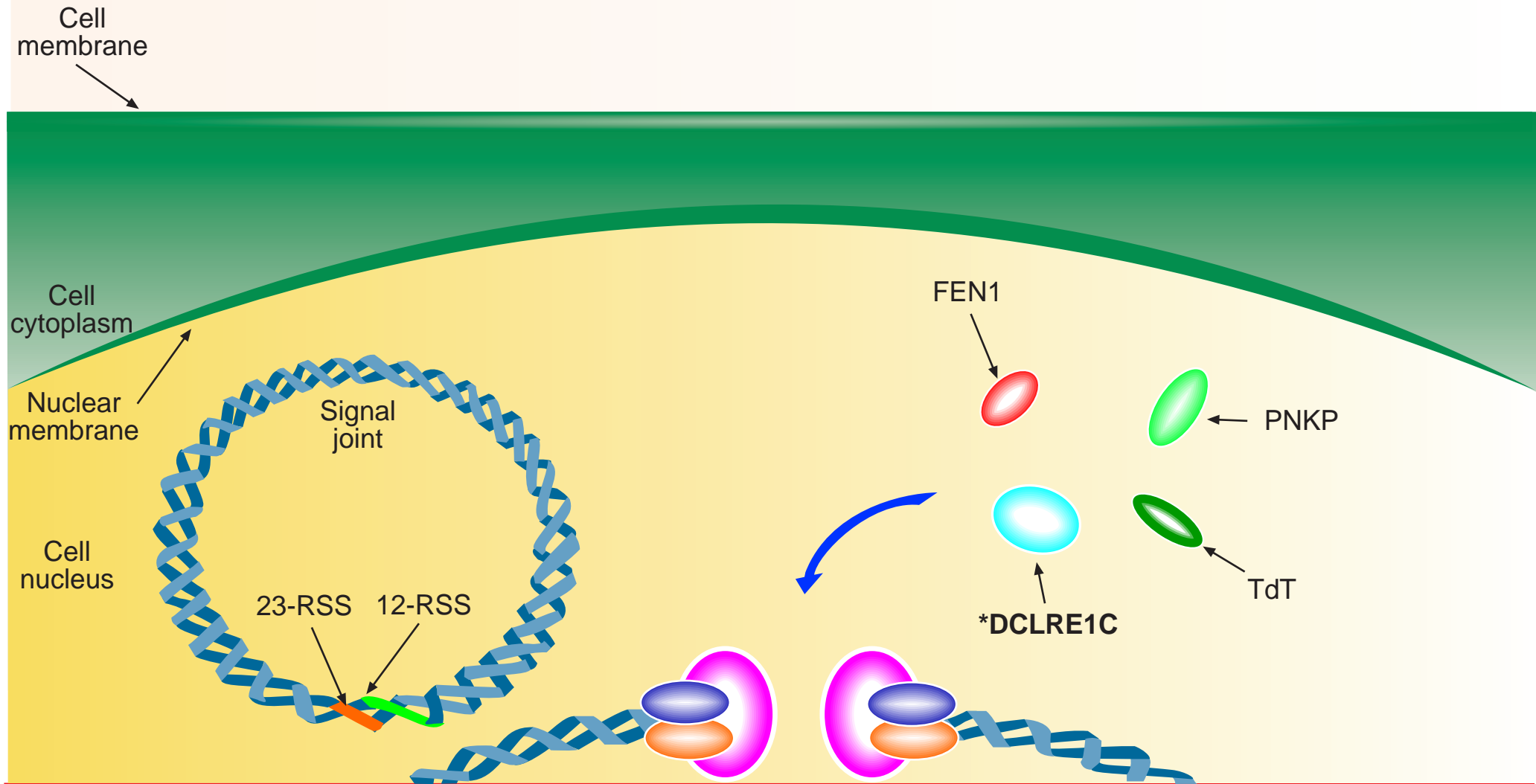
Non-homologous end-joining: recognition and end binding



Following the introduction of a double-stranded break in genomic DNA by the RAG1/2 complex, recruitment of DNA repair enzymes is initiated to mediate non-homologous end joining (NHEJ) of the DNA ends. Recognition and end-binding is first mediated by proteins Ku70, Ku80 which recruit DNA-PKcs. *In SCID phenotypes with defective T and B cell development, but normal NK cell function, genetic mutations in DNA-PKcs have been identified.



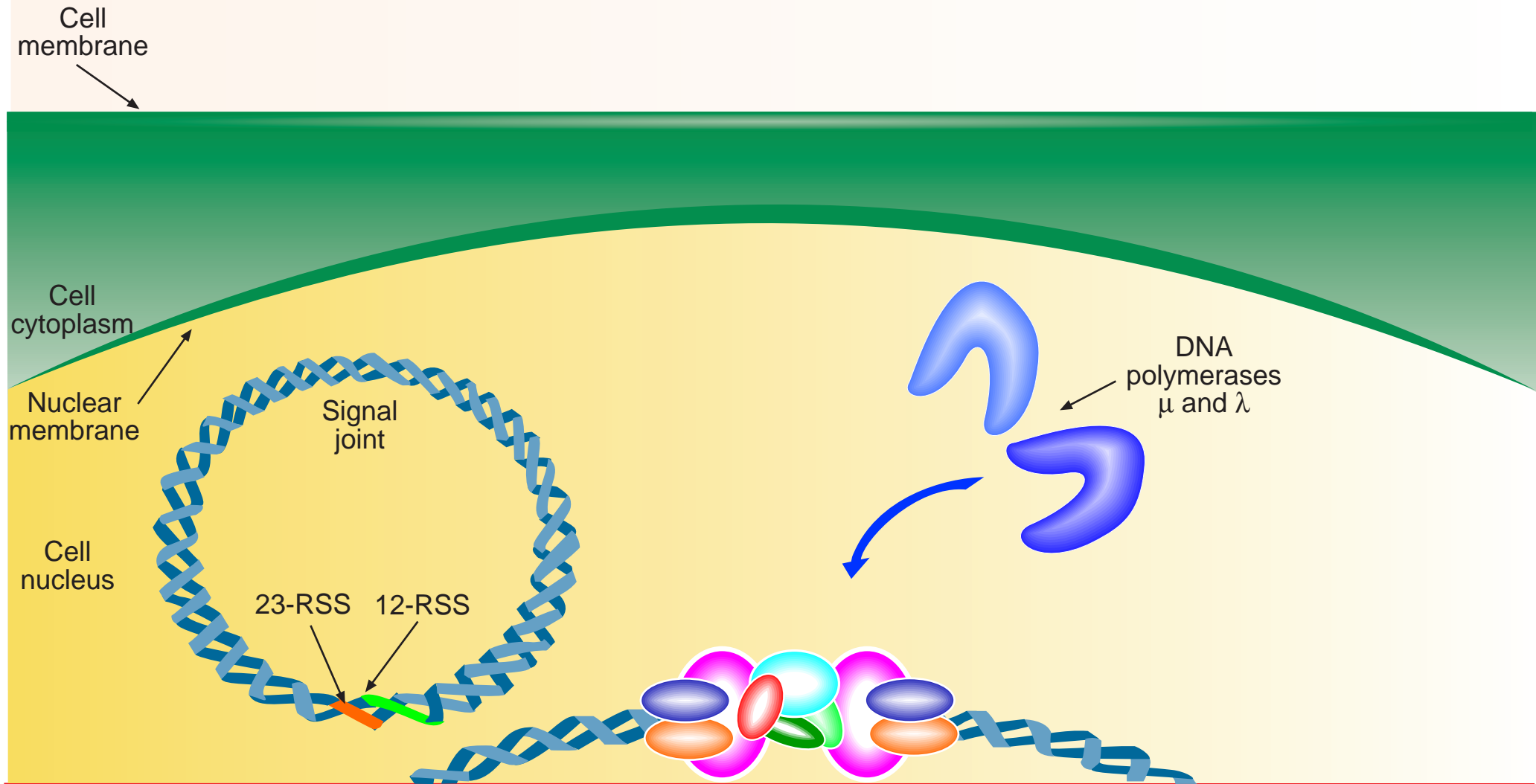
Non-homologous end-joining: end processing



Following recognition and binding of the DNA ends, recruitment of proteins FEN, DCLRE1C (also known as Artemis), TdT and PNKP occurs. DCLRE1C mediates nicking of the hairpin structures at random nucleotides while TdT adds random nucleotides to the 3' end. This process provides an additional mechanism of introducing variability into the coding regions of the TCR and BCR known as junctional diversity. *In SCID phenotypes with defects in T and B cell development, but normal NK cell function, genetic mutations in DCLRE1C have been identified.

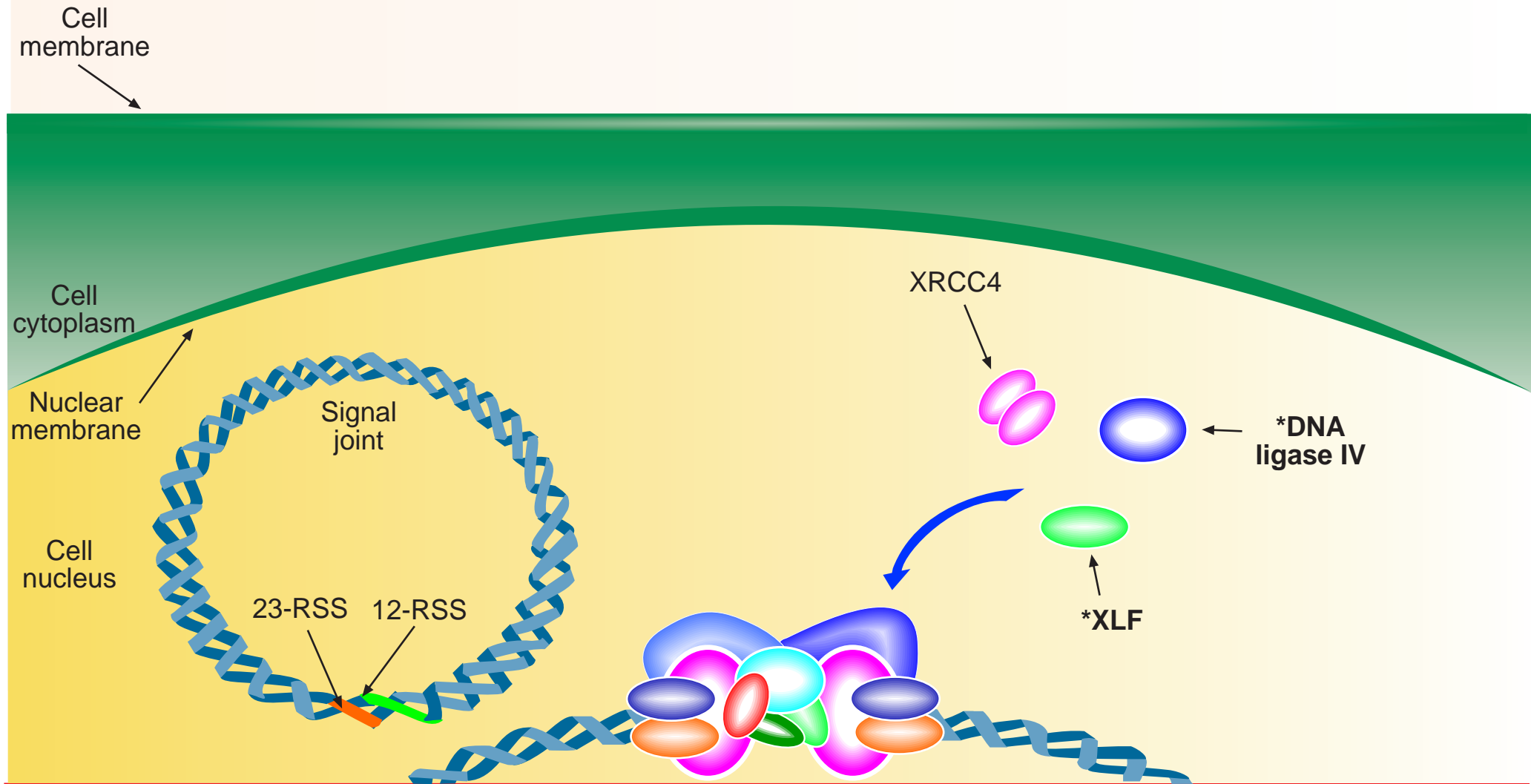


Non-homologous end-joining: DNA polymerisation



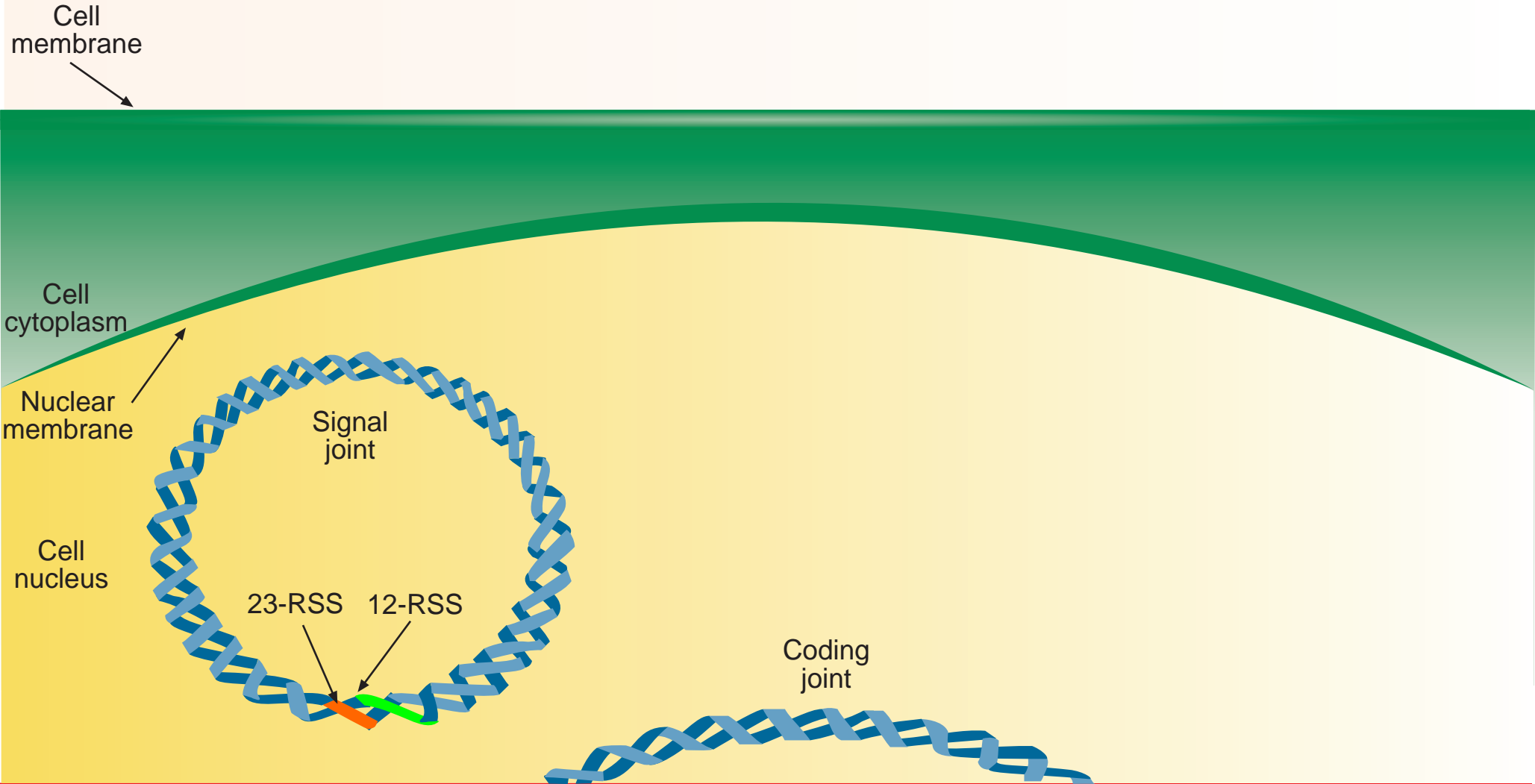
Following end-processing, DNA polymerases μ and λ are recruited to fill in single-stranded gaps in the DNA strands.

Non-homologous end-joining: DNA ligation



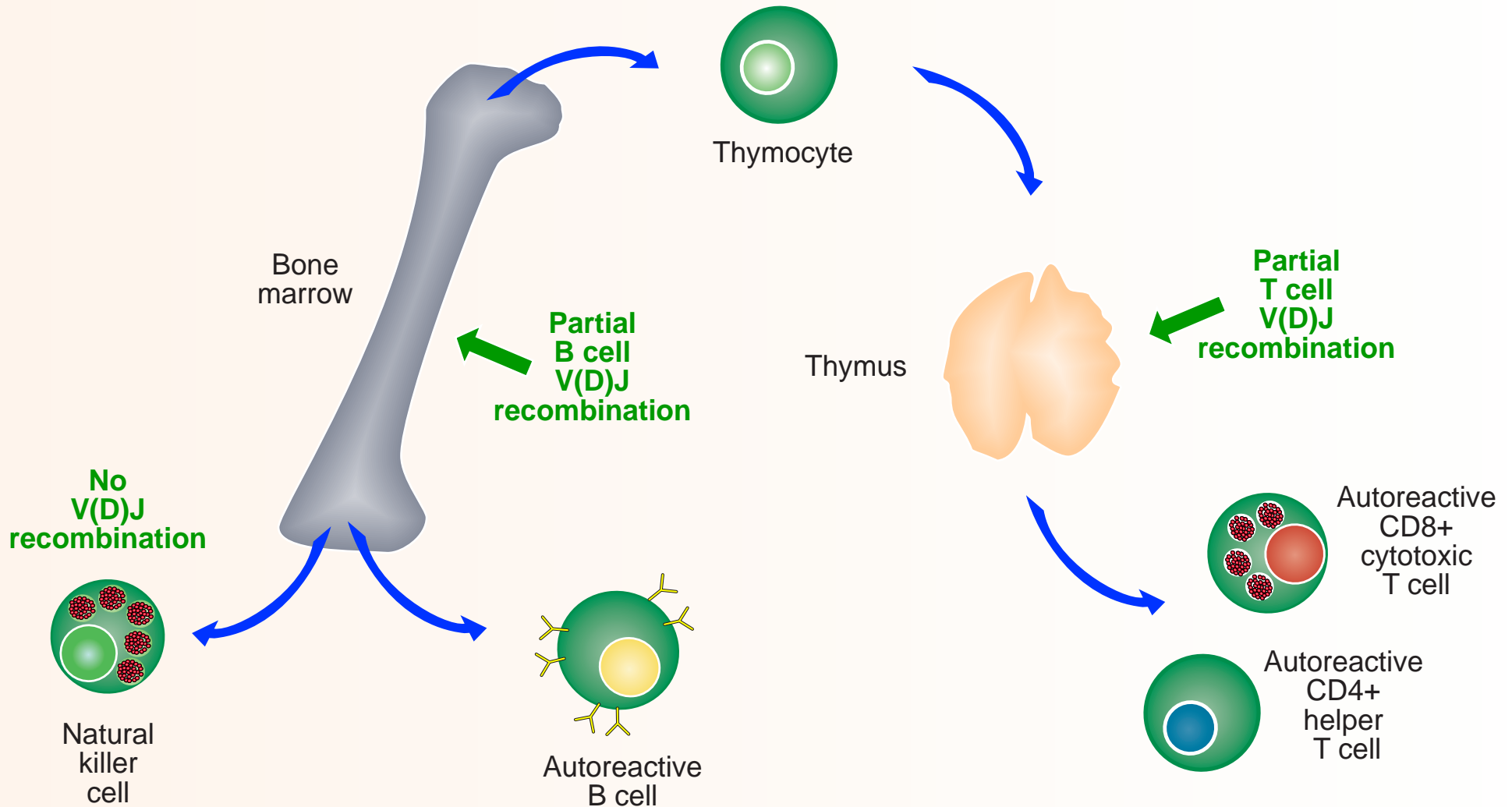
Following DNA polymerisation, a final ligation of the 3'-OH and 5'-P ends is initiated and requires recruitment of proteins XRCC4, XLF (also known as Cernunnos) and DNA ligase IV. *In SCID phenotypes with defects in T and B cell development, but normal NK cell function, genetic mutations in XLF and DNA ligase IV have been identified.

Completion of V(D)J recombination



V(D)J recombination is completed when a functional coding joint has been generated

SCID with autoimmunity: Omenn's syndrome



In certain rare forms of SCID with defects in T and B cell development, but normal NK cell function, the genetic mutations in the affected proteins may permit partial enzymatic activity, known as “leaky” SCID. This restrictive V(D)J recombination permits the development of a limited repertoire of T and B cells which may be directed towards self-antigens leading to an autoimmune response, known as Omenn's syndrome. Clonal expansion of these activated T and B cells occurs and blood levels of these cells may appear in range or elevated. Phenotypic examination of the TCR or BCR, however, reveals that these cells are oligoclonal in nature.