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Joining variable and constant regions

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- λ light chain
  - L1-Vλ1 up to LVλ-29 --- J1-C1---J2-C2---…
  - Multiple C-λ genes, each w/ one J region
  - J defines which constant region is used
- κ light chain
  - L1-Vκ1 up to Lvκ-40---Jκa1-5--Cκ
  - Only one Cκ gene
- Heavy chain
  - LVheavy51---Dheavy1-27---Jheavy1-6---Cμ
  - D segments encode 2-8 amino acids are preceded and followed by recombination signal sequences
  - Heavy chain variable encodes amino acids 1-99, J encodes additional 14-20 amino acids
- Light Chain Transcription
  - Germline DNA
  - VJ joined
  - Primary transcript mRNA
  - Splicing to make continuous mRNA w/ LVJC
  - Translated to polypeptide to make light chain and L spliced off
- Heavy chain transcription
  - Germline DNA recombined
  - DJ regions joined
  - V and DJ regions joined
  - Transcription to mRNA
  - Splicing to make continuous LVDJC
  - Translation
- Methods to generate diversity
  - Germline
    - Use of variable region genes
    - Several D's
    - Four to ten J's
  - Combinatorial
    - Joining of any variable region to any D to any J
    - Combination of any heavy chain variable region with any light chain variable region
    - 50 V X 30 D X 6 JH = 9000 heavy variable chains
    - 40 V X 5 Jκ = 200 variable κ chains
    - 30 V X 10 Jλ = 300 variable λ chains
    - 9000 X (200+300) = 4.5 million possible binding sites
  - Junctional diversity
    - Generated during V(D)J joining by variation in exact point of recombination
    - V-D, D-J in heavy chains
    - V-J in light chains
    - RAGs cut off recombination sequences and ligates them to release them
    - Exonuclease cuts off nucleotides and releases coding sequences to be ligated together
    - The exonuclease works anywhere
      - Same number of codons but a different sequence
      - Particularly prevalent in light chain variable region
  - N region addition
    - Addition of nucleotides by terminal deoxynucleotide transferase to V, D, or J ends

        - Not encoded by a template
        - Rare in light chains
- When in B cell differentiation do Ig gene rearrangements take place?
    - In pro B cells, D is rearranged to a heavy chain J segment on both chromosomes at random
    - Heavy chain V region is rearranged to DJ on one chromosome
        - If out of frame/pseudogene, tries on other chromosome
        - If it fails again, B cell stops development
    - If μ heavy chain is expressed, becomes a pre B cell and also attempts light chain V-J rearrangement
        - Further VH-DJ joining shut off
        - κ is favored 20:1 over λ
        - Since there are four loci that could undergo VJ rearrangement, this step is usually successful
        - There are also several VJ rearrangements possible w/in a single locus
    - If light chain is produced and IgM goes to cell surface, immature B cell
        - If light chain is expressed, VL-JL joining shut off
        - Feed-back regulation is basis of allelic exclusion
        - Prevents expression of two heavy chains or two light chains
- How does B cell switch from membrane bound IgM to secreted form?
    - Alternative RNA splicing
    - Secreted μ has 20 aa sequence after C region
    - Membrane bound has 41 aa after C region
        - This sequence has n-terminal negative AA, then 26 uncharged aa (α helix) then positive charges at the C-term
        - This makes it stick in the membrane
        - 2 Poly(A) sites
            - Secreted transcription ends at first
            - Transmembrane ends at second
            - MC region in btwn the two
        - After those two poly(A) sites, Cδ genes then another poly(A) region
            - If transcription continues to this point, mainly IgD expressed
            - Cμ genes get cut out