Joining variable and constant regions

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9:00 AM

• $\lambda$ light chain
  o L1-VA1 up to LV$\lambda$-29 --- J1-C1---J2-C2---...
  o Multiple C-$\lambda$ genes, each w/ one J region
  o $J$ defines which constant region is used

• $\kappa$ light chain
  o L1-Vk1 up to Lvk-40---Jka1-5--Ck
  o Only one Ck gene

• Heavy chain
  o LVheavy51---Dheavy1-27---Jheavy1-6---C$\mu$
  o D segments encode 2-8 amino acids are preceded and followed by recombination signal sequences
  o Heavy chain variable encodes amino acids 1-99, J encodes additional 14-20 amino acids

• Light Chain Transcription
  o Germline DNA
  o VJ joined
  o Primary transcript mRNA
  o Splicing to make continuous mRNA w/ LVJC
  o Translated to polypeptide to make light chain and L spliced off

• Heavy chain transcription
  o Germline DNA recombined
  o DJ regions joined
  o V and DJ regions joined
  o Transcription to mRNA
  o Splicing to make continuous LVDJC
  o Translation

• Methods to generate diversity
  o Germline
    - Use of variable region genes
    - Several D's
    - Four to ten J's
  o 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
    - $50V \times 30D \times 6JH = 9000$ heavy variable chains
    - $40V \times 5Jk = 200$ variable $\kappa$ chains
    - $30V \times 10J\lambda = 300$ variable $\lambda$ chains
    - $9000 \times (200+300) = 4.5$ million possible binding sites
  o 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
  o 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?
  o In pro B cells, D is rearranged to a heavy chain J segment on both chromosomes at random
  o 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
  o 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
  o 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?
  o Alternative RNA splicing
  o Secreted μ has 20 aa sequence after C region
  o 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