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ORGANIZATION & EXPRESSION OF IMMUNOGLOBULIN GENES

Antibody Diversity -

→ The variable region of both HC & LC of an antibody were observed to show wide diversity.
→ while its constant region is relatively conserved.
→ The same variable regions can be connected to diff. constant region (Class switching)

Germline Theory - If you have 10^7 diff. Abs, that means the sequences for these Abs are found in the DNA. However, this would be the DNA sequence harbours all the genes making it extremely long! Theory was dropped.

Two-gene model - For a light chain polypeptide, it can be coded by two gene segments - one for the variable region which shows high variation, while the other gene segment is relatively constant for the constant region. Before Antigen recognition, variation occurs thru two-gene model.

Somatic-variation theories - your B & T cells will undergo random mutation for genetic variation & diversity After Antigen recognition, diversity occurs via somatic-hypermutation (only in B-cells)

Hozumi & Tonegawa's Experiment:

- Two diff. cells were taken → Embryonic liver cells (Germline cells)
  → Ab-producing tumour cells (somatic cells)
- To study DNA changes before (Germline cells) & after (Somatic cells) recombination of DNA.

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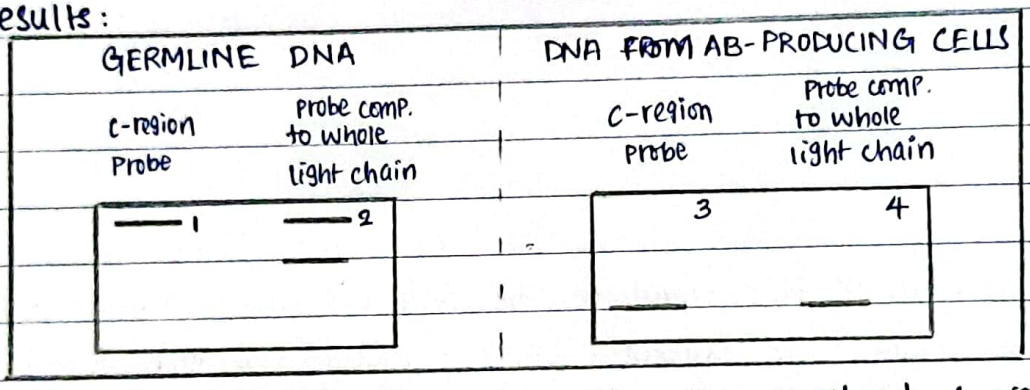
- Ran a DNA Gel Electrophoresis of both the DNA.
- RE digestion (~~the~~ using BamHI)
- Southern blotting using 2 Probes → 1 Probe was compliment to light chain region
  1 Probe to constant region of light chain
- Results:



- Conclusion: BamHI is cutting b/w the constant & variable region [germline DNA]

Conclusion: [somatic DNA]
- Both the bands have lesser bps. This indicates that the dist. b/w the constant & variable region ~~have~~ has reduced compared to that in germline DNA (which can be concluded by the MW of the bands).
- The variation is a result of genetic recombination that occurs in the ~~development of the somatic DNA from the~~ germline DNA & before differentiating into somatic DNA.

Multigene organization of Ig Genes: (In germline DNA)

(a) Light chain: V, J segments → VJ recombination } variable region
(b) Heavy chain: V, D, J segments → VDJ recombination }
(c) Lc & Hc: C segment → constant region

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NOTE: Each of these gene segments have introns in b/w.

Hozumi & Tonegawa's Experiment:

→ During recombination event, introns are removed.
→ In the somatic cells, we find the V & C fragments closer to each other.

conclusion - There are more than 1 gene segment that codes for light & heavy chains. ↳ In fact, over 2-3 genes

Light chain → κ (multigene family) : • encoded in chromosome 2 ~~(V, J, constant)~~
~~• 2 families~~
• 34-38 subfamilies (V segments)
• possess V, J & constant segment
• There is no D segments.
• There can be 5 J segments ($J_{\kappa 1}, J_{\kappa 2}, \ldots$)
• There is 1 constant region for κ chain (C_κ)

→ λ multigene family : • encoded in chromosome 22
• 29-33 subfamilies (V segments)
• ∗ J & C segments are present in pairs; b/w each of the segments, there will be introns
• There is no D segments.
• There can be 4-5 J segments ($J_{\lambda 1}, J_{\lambda 2}, \ldots$)
• There can be 4-5 constant regions ($C_{\lambda 1}, C_{\lambda 2}$)

→ Heavy chain : • encoded in chromosome 14
• 38-46 subfamilies (V segments)
• There are 23 D segments
• There can be 6 J segments
• There are 9 constant region that codes for the 5 classes of Ig (A, M, G, D, E)

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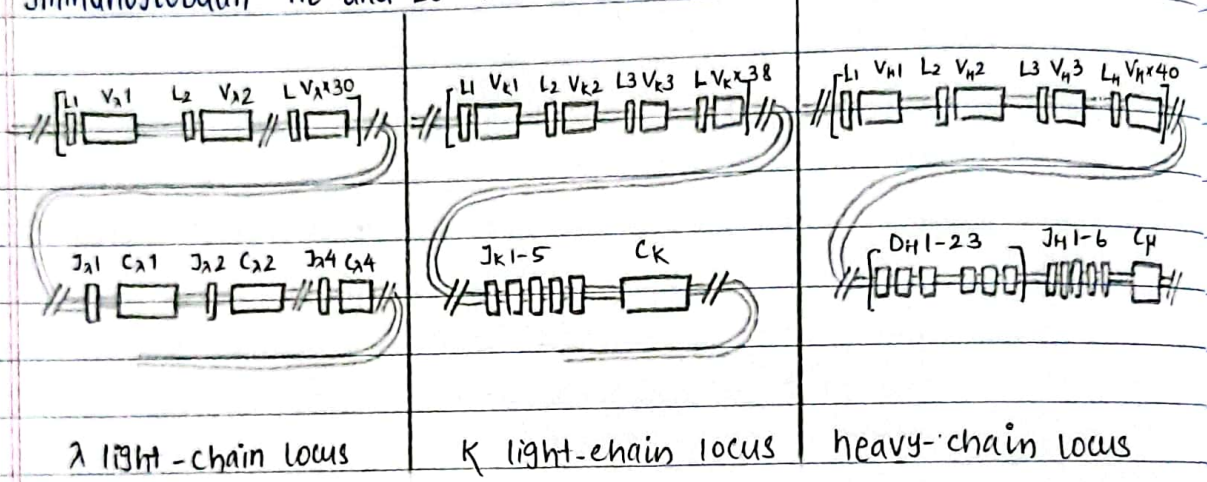
NOTE: Upstream to each V segment, with there is a leader gene present. The leader sequence (signal sequence) guides the Ig to the secretary pathway once its ~~syn~~ synthesis is over. The leader sequence is cleaved off after that, so you won't find it in the final Ab.

Immunoglobulin Hc and Lc loci in the human genome -



λ light-chain locus | κ light-chain locus | heavy-chain locus

Ig Lc & Hc gene rearrangement -

- In case of Lc, first V & J segments arrange together.
- In cas of Hc, first D & J segments are joined together. Later V segments joins DJ segment.
- ~~For~~ C segments joins later to VJ (for Lc) & VDJ (for Hc).
- In case of Lc, During recombination event, VJ recomb. will take Place (before transcription).
- C segment introns are removed after transcription (mRNA splicing) & joins VJ segment.
- Leader gene will be cleaved after translation (final Ab).
- In case of Hc, first DJ recomb. takes Place (before transcription).
- Next, VDJ recomb. will take Place (before transcription).
- C segment introns are removed after transcription & joins VDJ segment.
- Leader gene is removed in final Ab.
- The J segment helps bring C segment to come closer (join) the V segment.

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NOTE: Naive B cells will only express IgM & IgD on their surfaces (before Antigen encounter). After an encounter, class switching takes place.

- Transcription will ~~should~~ stop after C_μ or C_δ due to presence of stop codon. Therefore, the primary transcript only has C_μ or C_δ segment & depending on which C segment joins ~~VD~~ VJ segments, we get the 2 types of Ig: IgM, IgD.

05/04/22 Recombination signal sequences – (RSSs)

Each of the gene sequence possess a RSS. The RSS can ~~be~~ precede or succeed the gene segment. Since these sequences are present at gene ~~li~~ level, we find them only on variable segments (V, D, J) not in C region.

Nucleotide sequence of RSS:

(i) Two-turn RSS
→ 2 conserved regions: Heptamer + Nonamer
→ B/w the conserved region, there ~~can be~~ is 23 bp nucleotide sequence.
→ Direction of RSS – towards the Nonamer
→ λ chain: V_λ → 2 turn RSS
κ chain: ~~J_κ~~ → 2 turn RSS
H chain: V_H → 2 turn RSS
J_H → 2 turn RSS

(ii) one-turn RSS
→ 2 conserved regions: Nonamer + Heptamer
→ B/w the conserved region, there ~~can be~~ is 12 bp nucleotide sequence
→ Direction of RSS – towards the nonamer
→ λ chain: J_λ → 1 turn RSS
κ chain: V_κ → 1 turn RSS
H chain: D_H → 2× (1 turn RSS)

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During Recomb. event, a 12/23 rule is followed.

12/23 rule: A 1 turn RSS will undergo only with a 2 turn RSS. Similar RSS can't undergo recombination.

This is how cell prevents recomb. b/w the same variable segments (like J-J, V-V, D-D).

NOTE: The RSSs can be in same direction or in opp. direction.

When the recomb. takes place, it is imp. that the sequences ~~must~~ align in the same direction.

The recomb. is catalysed by V(D)J recombinase : RAG-1 and RAG-2.

Recomb. can take place in 2 ways -

(i) Loop formation + DNA excision (✓)

(ii) Coil formation - DNA excision (X)

~~For~~ During joining process, there is addn. of 8 random nucleotides b/w the joint regions. ~~There~~ If these random nts. have palindromic sequence, then they are called P addition. If its ~~not~~ non-palindromic, they are called N addition.

~~This is the first level of diversity~~

- 1st level of Diversity: Recomb. b/w V,(D),J segments
- 2nd level of Diversity: Addn. of Nts b/w the joint regions.

No. of possible Ab based on 1st level of Diversity alone:

H_L → Assuming 51 types of V sequence,
27 types of D sequence,
6 types of J sequence

Total no. = ~~8262~~ $51 \times 27 \times 6 = 8262$

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Lc → Assuming 40 types of V sequence,
(κ) 0 types of D sequence,
5 types of J sequence
Total no. = 40 × 5 = 200

Lc → Assuming 30 types of V sequence,
(λ) 0 types of D sequence,
4 types of J sequence
Total no. = ~~30×4~~ 30 × 4 = 120

$$Hc + Lc\ (\kappa + \lambda) = 8262 \times (200 \times 120) = 2.64 \times 10^6$$

Antibody Diversity :-

It can be obtained by 7 diff. ways-

(i) Multiple germ-line gene segments (no. of V, (D), J segments)
(ii) Combinatorial V-(D)-J joining (recomb. b/w V, (D), J)
(iii) P ~~ad~~ region nucleotide addition (P addition)
(iv) N region nucleotide addition (N addition)
(v) Combinatorial asso. of Light & Heavy chains (after ~~transcription~~ translation
since Lc & Hc are synthesised separately as separate polypeptides)
~~(vi) Class-switching recombination~~
(vi) Somatic Hypermutation ~~(upon Antigen in encounter)~~ (naive B cells)
(vii) Class switching recombination (upon Antigen encounter)
↳ Expression of diff. classes of Ab (IgG, IgA & IgE) but with same antigenic specificity

08/04/22 TCR Multigene families in humans :-

Gene	Chromosome location	No. of gene segments			
		V	D	J	C
α chain	14	50		70	1
δ chain	14	3	3	3	1
β chain	7	57	2	13	2
γ chain	7	14		5	2

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β chain of TCR ~ Heavy chain of Antibody.
→ ∴ There are V, D, J segments that encodes ~~for~~ for variable region.
α chain of TCR ~ Light chain of Antibody
→ ∴ There are V, J segments that encodes for variables region.

Recombination -
* V-J recombination
* After transcription, splicing will take Place.
~~V-J-C~~ VJ-C joining
* After translation, ~~so~~ TCR will have the continuous polypeptide VJC.

* D-J recombination
* ~~After transcription~~ V-DJ recomb.
* After transcription, splicing will remove introns.
VDJ-C joining.
* After translation, continuous polypeptide is formed.

~~TCR Diversity:~~
~~→ Multiple germline segments~~
TCR Diversity :-

It can be obtained through -
(i) Multiple germ-line gene segments
(ii) combinatorial V-(D)-J joining
(iii) P-region nucleotide addition (P-addition)
(iv) N-region nucleotide addition (N-addition)

NOTE : NO SOMATIC HYPERMUTATION