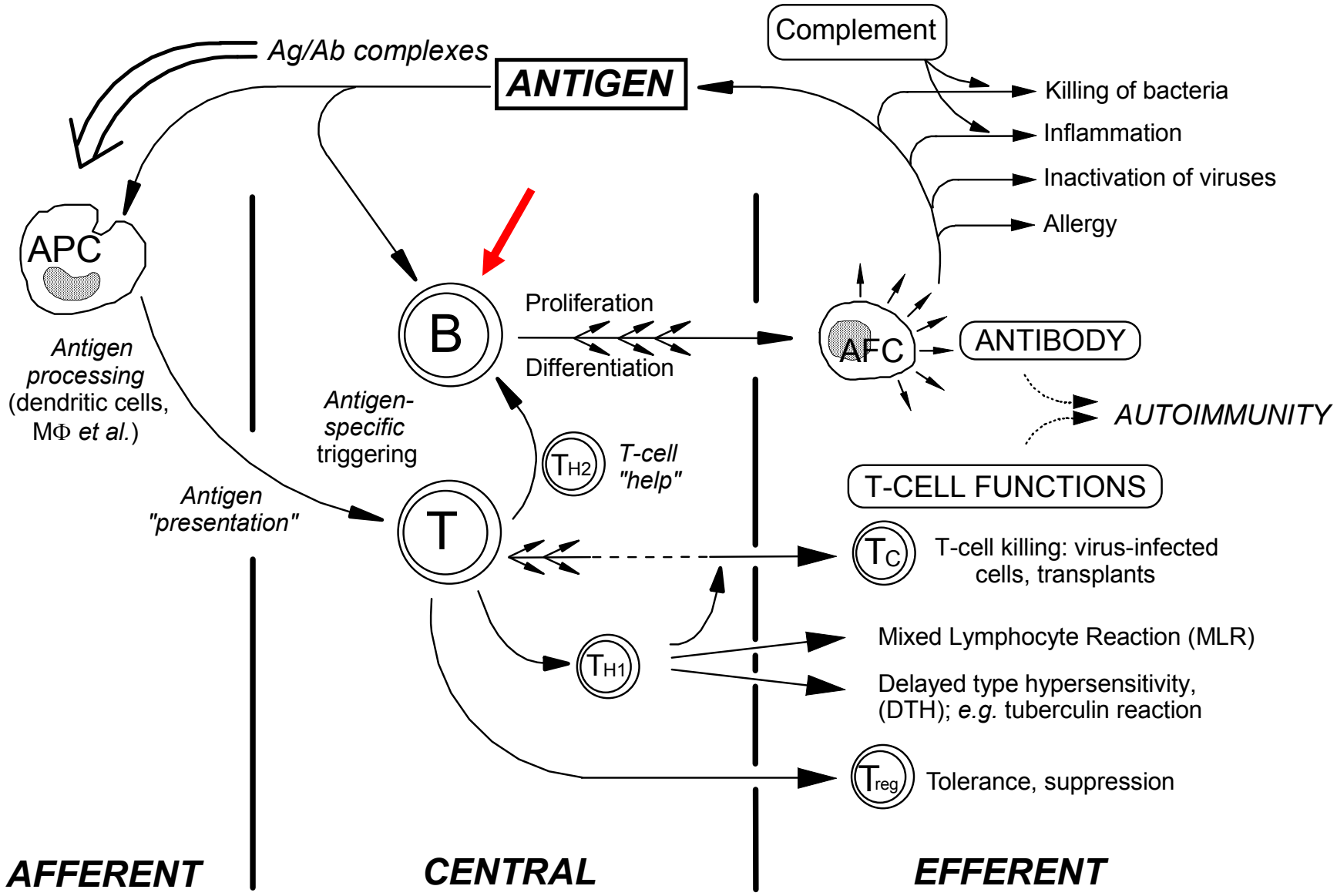
Immunology 2011
Lecture 8
Genetic Basis of Antibody Diversity
30 September



THREE "LIMBS" OF THE IMMUNE RESPONSE

Genetic Basis of Antibody Diversity

OUTLINE

PROBLEM OF ANTIBODY GENE DIVERSITY

GERMLINE *vs.* SOMATIC THEORIES

THREE GENE FAMILIES (*H-chains, kappa, lambda*)

- *GENE ORGANIZATION*
- *V/J/D REARRANGEMENT*
- *CLASS SWITCHING*
- *SOMATIC MUTATION*

SOUTHERN BLOT ANALYSIS OF GENE ORGANIZATION

How to Account for Antibody Diversity?

- An Ab combining site is made up of one V_L *and one* V_H
- The specificity of any combining site is determined by its amino acid sequence.
- There exist *many* unique combining sites – let's say at least 10^6

How many V-region genes must exist?

If each V-region requires a unique gene, we might need only 10^3 *L +* 10^3 *H-chains (combinatorial association of* V_L *and* V_H*)*

How to Account for Antibody Diversity?

Germ-Line *versus* Somatic Theories

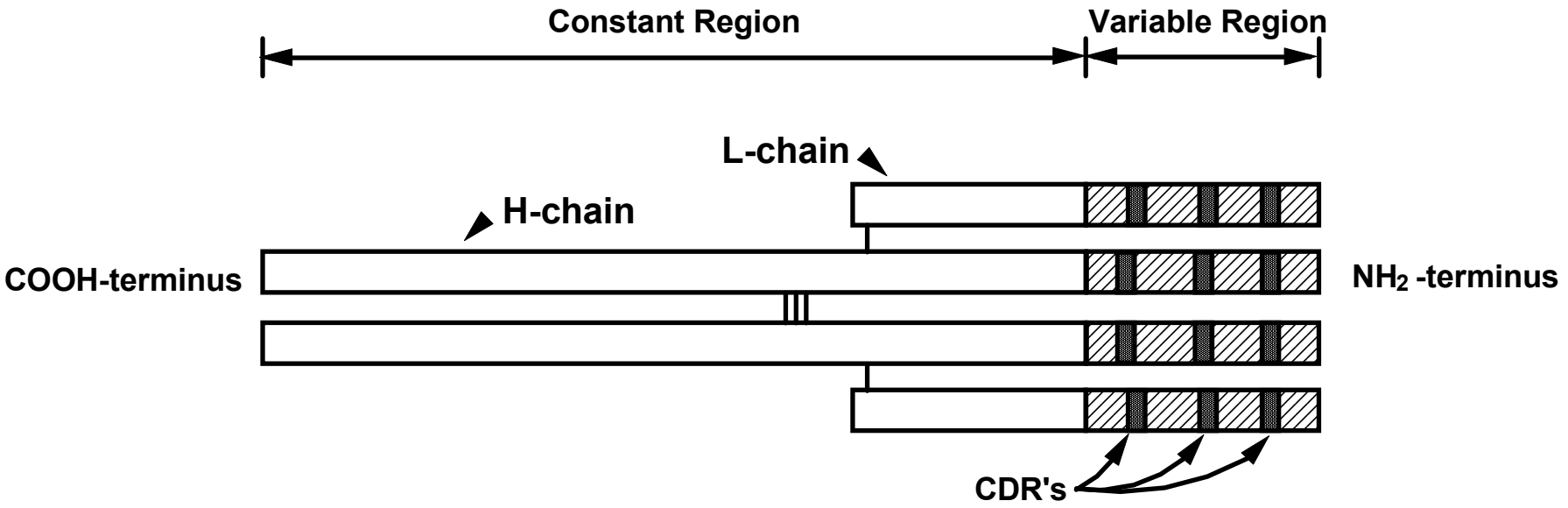
Germ-line: One germ-line gene exists for every V-region
i.e. Need *thousands* of genes

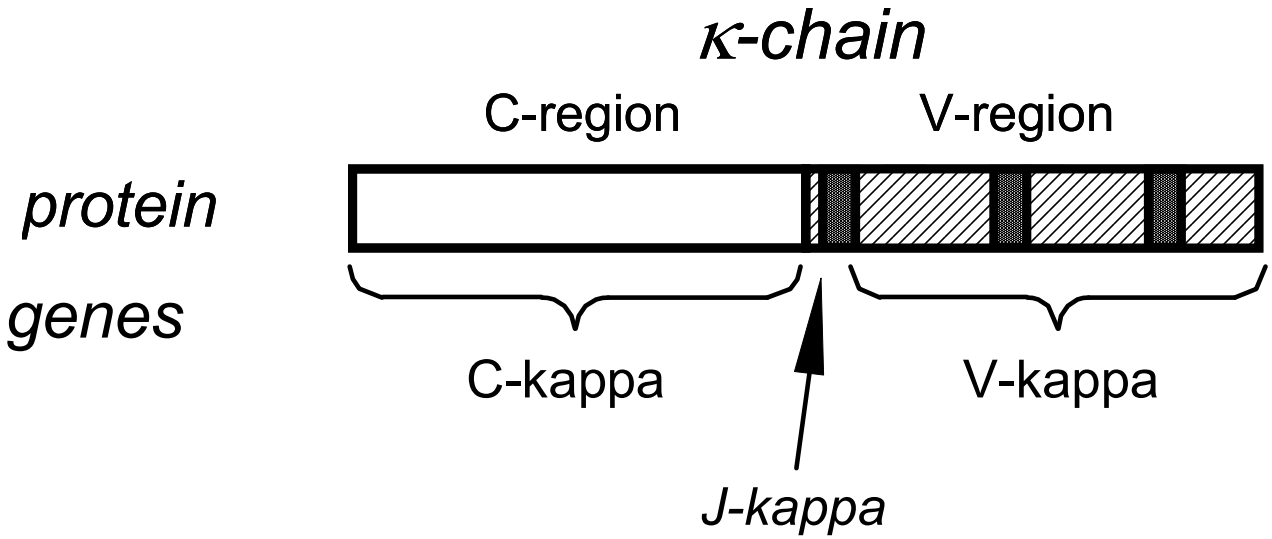
Somatic: One germ-line gene diversifies during development.
i.e. Need *as few as 3* genes (V_H, Vκ, Vλ)

So…who's right?
Everybody is!

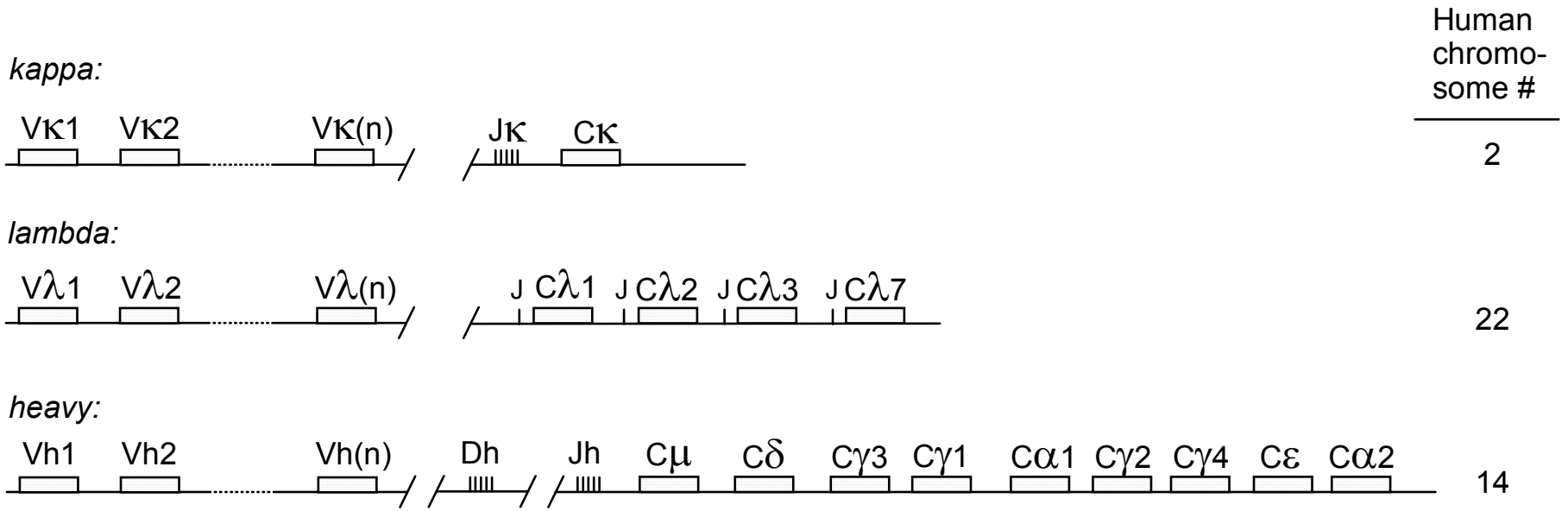
1) *Many germ-line genes exist* (Germ-Line diversity)

2) *These genes are diversified B-cell development* (Somatic diversity)


Constant Region
Variable Region
L-chain
H-chain
COOH-terminus
NH2 -terminus
CDR's


κ-chain
C-region
V-region
protein
genes
C-kappa
V-kappa
J-kappa

Organization of Human Immunoglobulin Genes



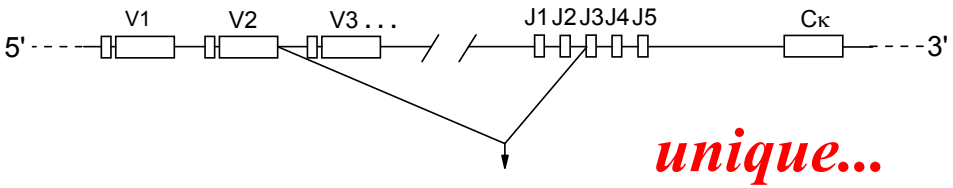
Three Ig Gene Families:

H-Chains, κ-Chains, λ-Chains

Each family has V-(D)-J-C, varying numbers of each gene and differing details of organization

MOLECULAR BASIS OF KAPPA GENE EXPRESSION

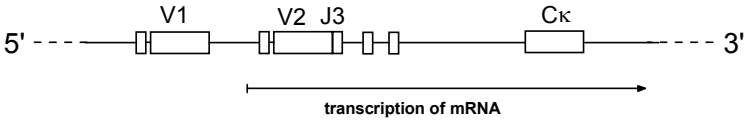
Germ-line configuration:



1) *Germ-line DNA*

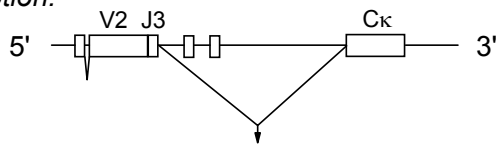
unique...

Following DNA rearrangement:



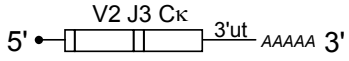
2) *Rearranged DNA*

Following transcription:



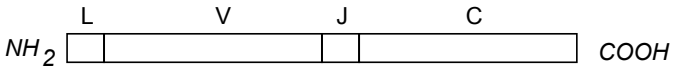
3) *Precursor mRNA*

Following RNA splicing:



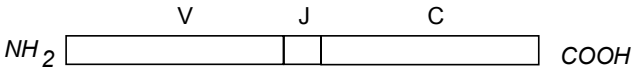
4) *Mature mRNA*

Following translation:



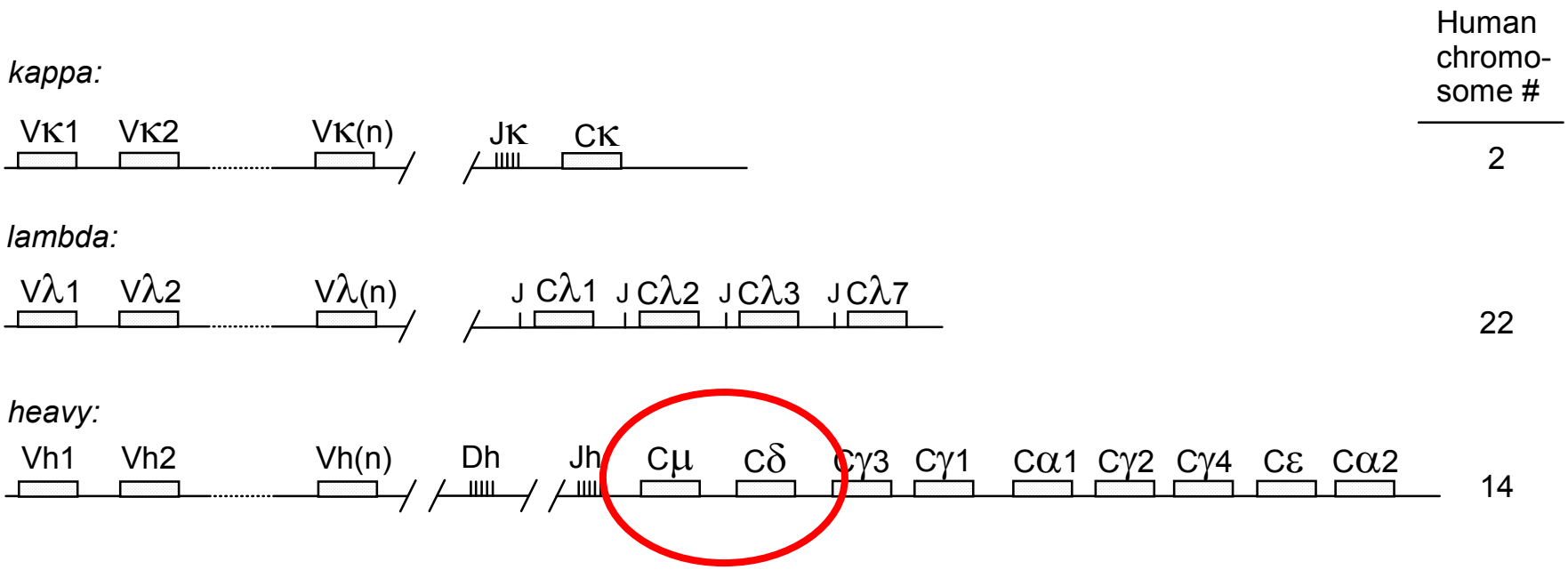
5) *Precursor PEPTIDE*

Following peptide processing:

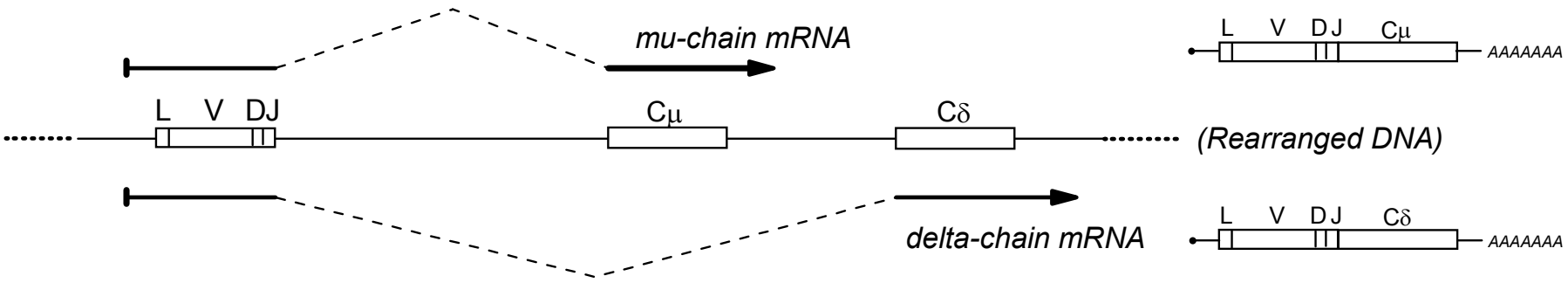


6) *Mature PEPTIDE*

Organization of Human Immunoglobulin Genes



Some B-cells express both membrane IgM and IgD

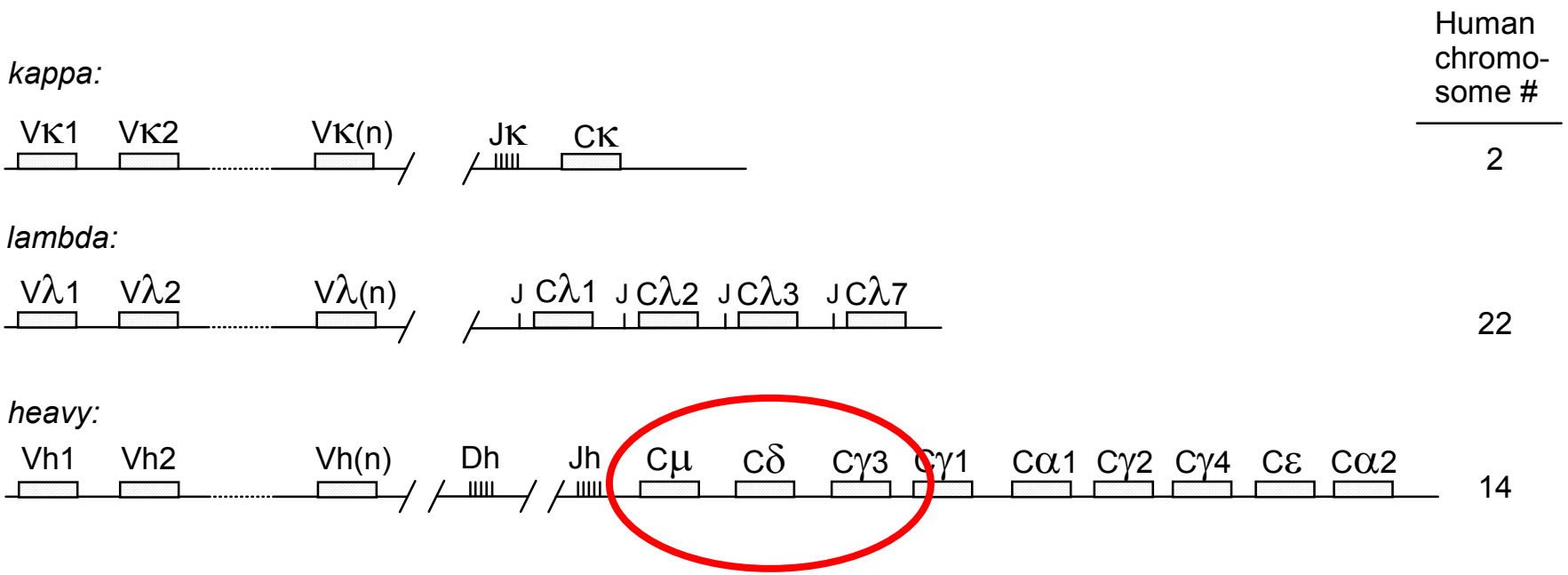


Dashed lines in RNA indicate an intervening sequence removed during RNA processing

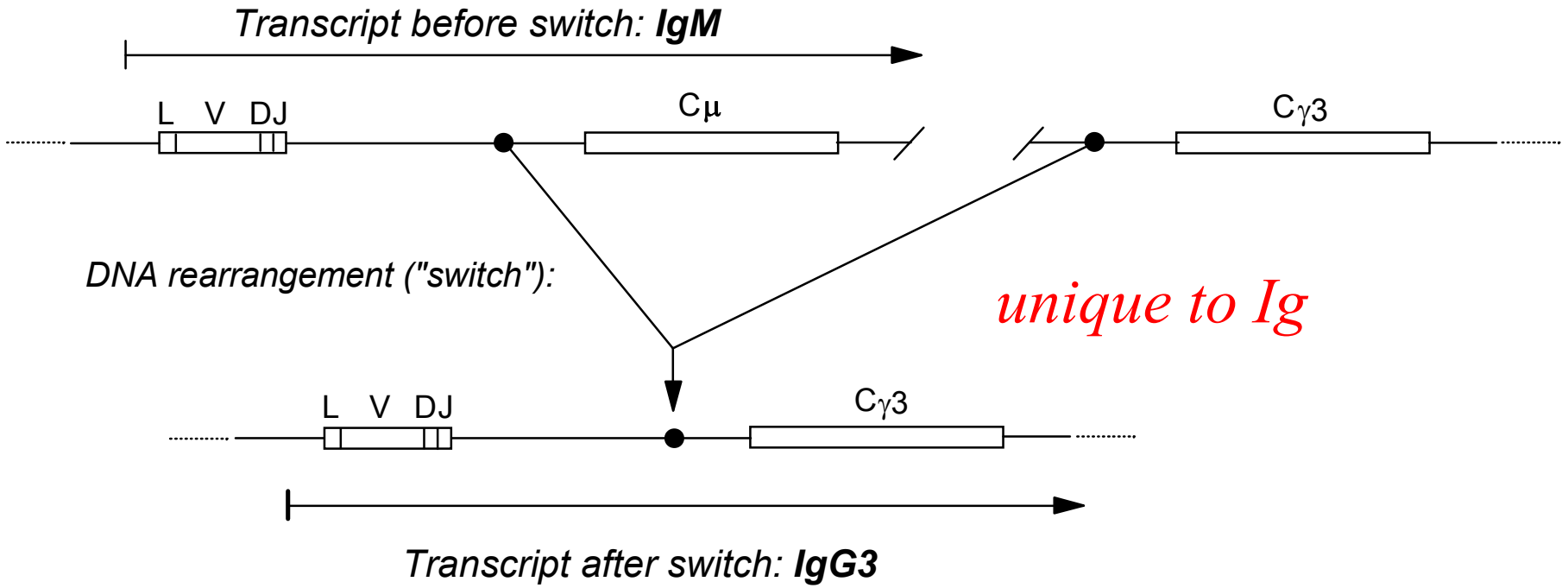
SIMULTANEOUS SYNTHESIS OF IgM AND IgD IN B-CELLS BY ALTERNATE RNA SPLICING

common to many other genes

Organization of Human Immunoglobulin Genes



Memory: Ab-secreting cells switch from IgM to IgG…



MOLECULAR BASIS OF CLASS SWITCHING

Summary: Sources of Antibody Diversity

1) ***~50 germline V_k-genes***

2) ***5 germline J_k-segments***
   "Combinatorial Joining" of V_k & J_k
   50 V_k x 5 J_k = *250*

3) ***DNA rearrangement is imprecise***
   250 x 10 = *2,500*

4) ***Somatic mutation of rearranged V-genes***
   2,500 x 10 = *25,000* different V_k- regions

5) ***Heavy and light chains associate randomly***
   "Combinatorial Association"
   25,000 X 25,000 = *~$6x10^8$* combining sites.

The Immune system as a defence organization

1. Its function is selective destruction.
2. It is large, complicated and elaborate.
3. It is expensive.
4. It is wasteful.
5. It has distinct components performing apparently identical functions.
6. It is slow to react.
7. It is prepared for events that never happen.
8. It fights today's threats with the solutions of past problems.
9. It is susceptible to corruption.
10. It can destroy that which it protects.

Peter Parham, 1990

T-CELL ANTIGEN RECEPTOR

another rearranging DNA...

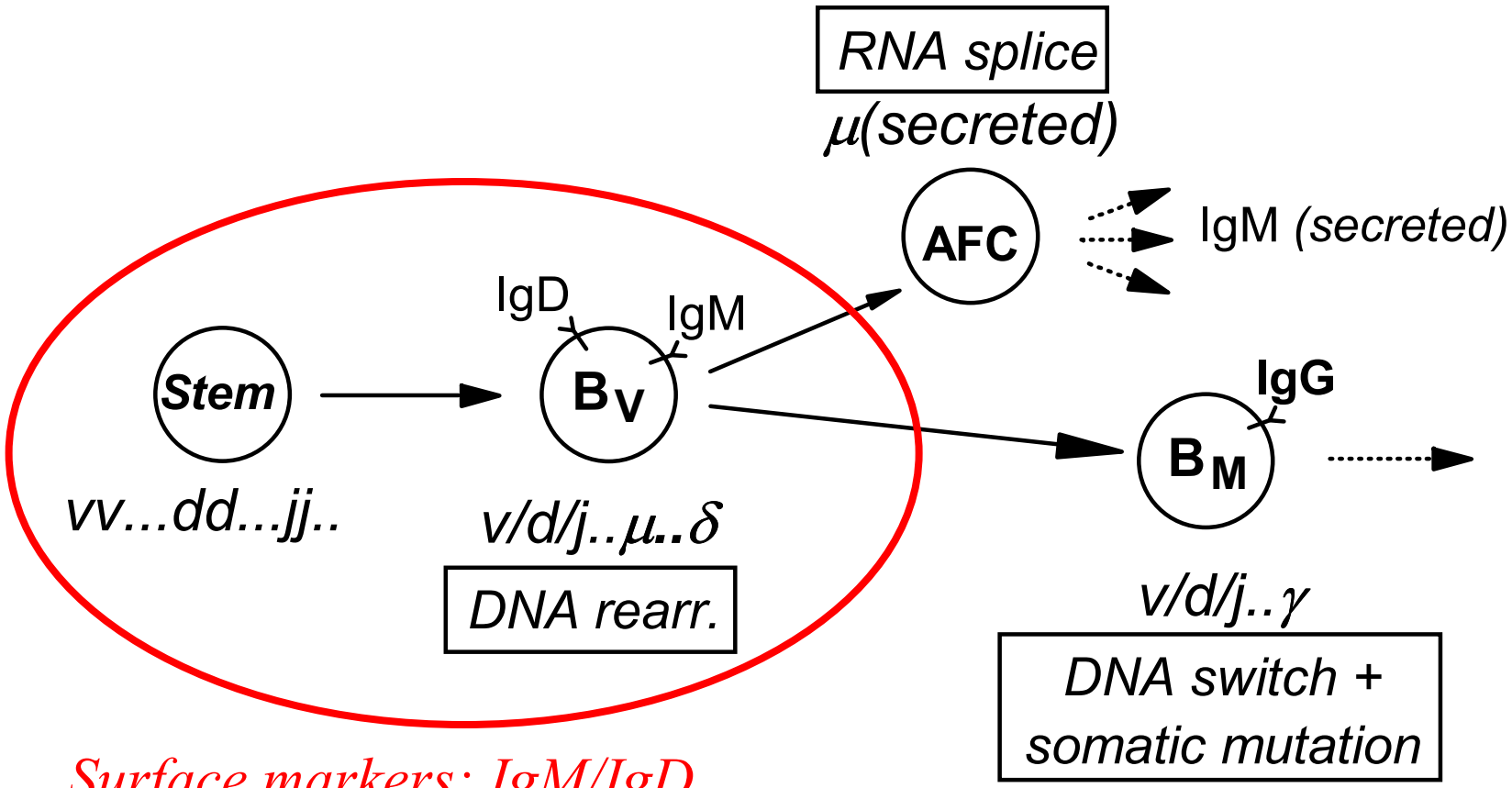
- ***Members of Ig Superfamily***
- ***α/β chains*** (homologous to Ig L & H-chains)
- ***Similar gene structure/rearrangement***
  (VVV-[DDD]-JJJ-C...)

However: ***(1) TCR is <u>only</u> membrane-bound***

(2) Monovalent

(3) No somatic mutation

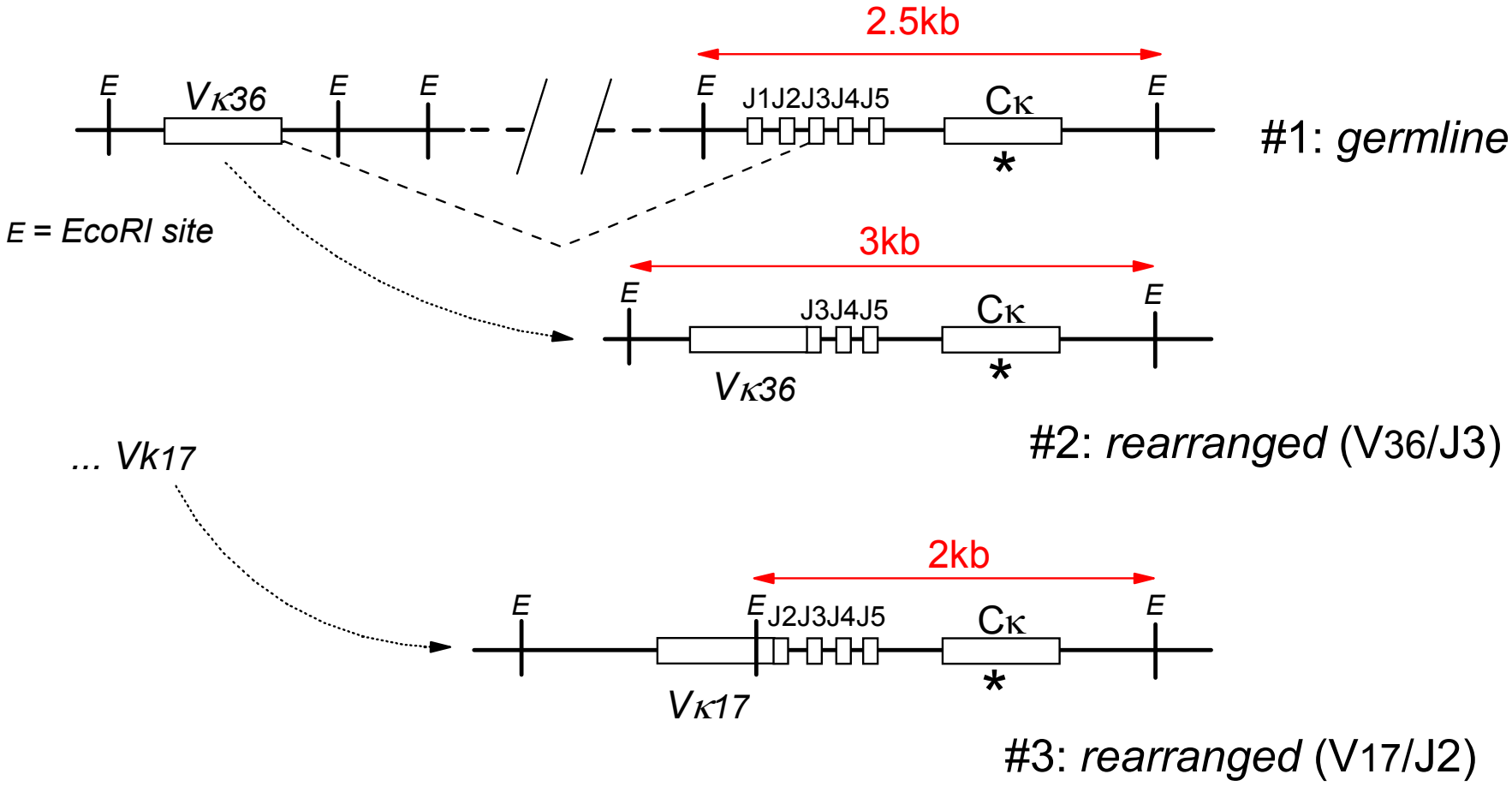
Genetic Events in B-Cell Differentiation



Surface markers: IgM/IgD…

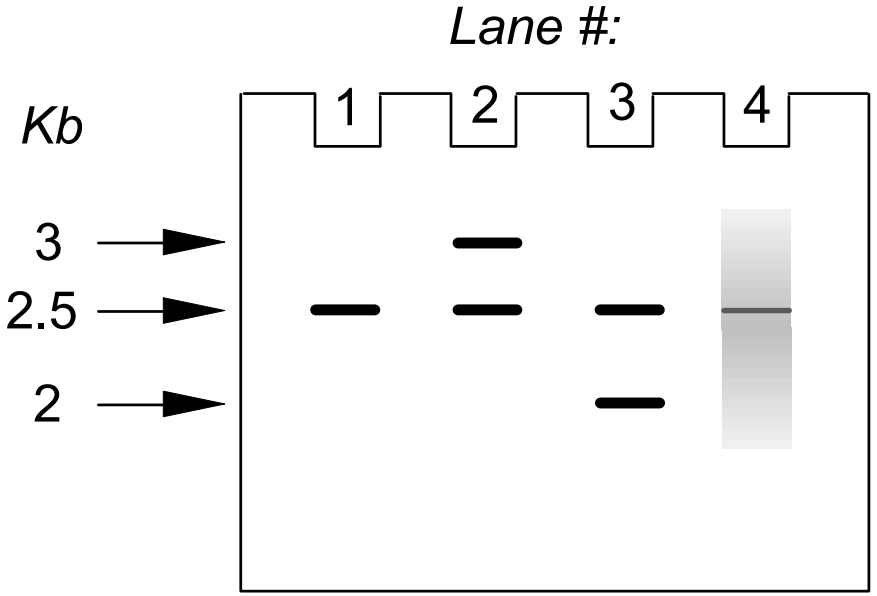
DNA markers – Southern Blotting

Analysis of Kappa Gene Rearrangements by Southern Blotting



- *Cut DNA with EcoRI*
- *Separate fragments by agarose gel electrophoresis (size)*
- *Blot & hybridize with C-kappa specific probe*

Autoradiograph of Southern blot hybridized with Ck probe



#1: *germline* band

#2,3: single *monoclonal* rearrangements (+ unrearranged allele)

#4: *polyclonal* rearrangements (+ unrearranged allele)

MONDAY

Immunoglobulin Biosynthesis,
Chapter 9

TUESDAY

ABO & Rh Blood Groups,
Chapter 10