We are All Immunologists Now

Week 2

Ed Roy, OLLI, Fall, 2021 Assisted by Marie Roy and Mary Kuetemeyer, Class Moderator

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1

Unresolved Questions

from Week 1

- How fast do lymphocytes migrate?
- What was responsible for the late 1800's peak in smallpox cases in London?
- How does immunity change over the lifespan?
- What did Lady Montague bring back from Turkey?

2

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Lymphocyte Migration Speed

- Dupre et al, 2015: migrates toward chemokine gradient at 10 microns/min, or approximately 1 cell diameter per minute

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What was responsible for the smallpox peak in 1871?

- Krylova and Earn, 2020:
- The 1871 peak in smallpox cases was related to the Franco-Prussian War, 1870-1871. This pandemic was last major smallpox pandemic in Europe, and prompted mandatory vaccination in England, Sweden and other countries.

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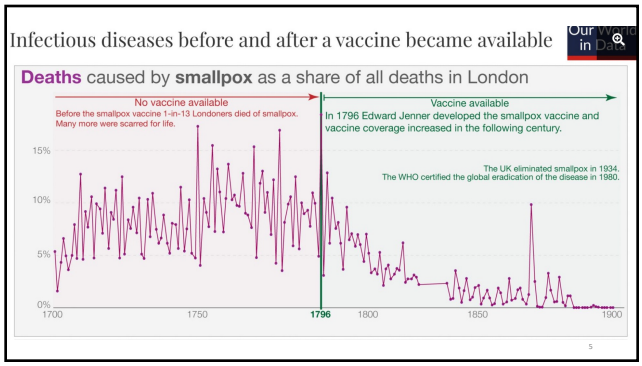

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How does immunity change over the lifespan?

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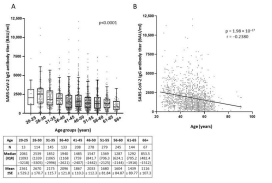

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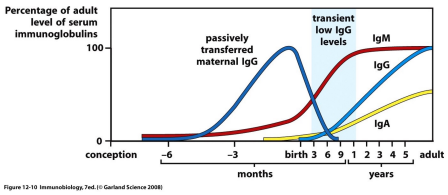

Figure 12-10 Immunobiology, 7ed. (© Garland Science 2008)

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What is donated/sold plasma used for?

Fluid portion of blood with proteins (antibodies) and small molecules.

- To replace fluid volume in burn victims.
- To treat people with clotting factor deficiencies.
- To provide immunoglobulin (antibodies) in people with immunodeficiencies.
- To treat COVID-19 if provider of plasma is survivor of SARS-CoV-2
- Plasma can be given every two weeks, at $30-$80 per time.

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What about role of Lady Montague in variolation?

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Review from Week 1

- Powerpoint from Week 1 and Glossary are on OLLI website
- Immune system protects us from pathogens (bacteria, viruses, fungi)
- Protection is from physical barriers, innate immune system, and adaptive immune system
- Innate system recognizes conserved features of pathogens and responds rapidly
- Neutrophils, macrophages, and dendritic cells are main innate cells that respond and phagocytose pathogens
- cooperation of innate and adaptive immunity: Antibodies coating surface of bacteria make them more easily eaten by macrophages and neutrophils

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Learning Objectives for Week 2

- Background on DNA and Evolution
- Structure of Antibodies and T cell receptors
- Generation of Diversity of B cell receptors (antibodies) and T cell receptors

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Biology Background

A couple of important concepts

- Evolution: Generate Diversity, Selective Pressure, Survival of the Survivors
  - Pathogens Evolve, Immune Responses evolve
  - For viruses, bacteria, and Adaptive Immune cells, nucleic acids are key to changes
- The Central Dogma: DNA to RNA to protein

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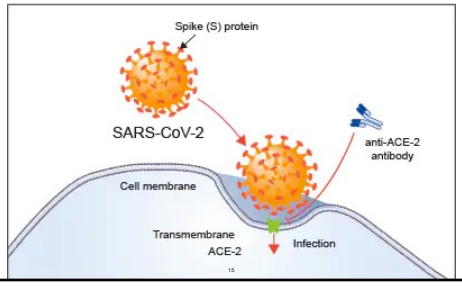
Spike Protein: entry of SARS-CoV into cells



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Spike Protein binds to Angiotensin-Coverting Enzyme -2 (ACE2)

- Region of the spike protein that interacts with ACE-2 is called the Receptor Binding Domain
- The tighter the spike protein binds to ACE-2, the more transmissable the virus is

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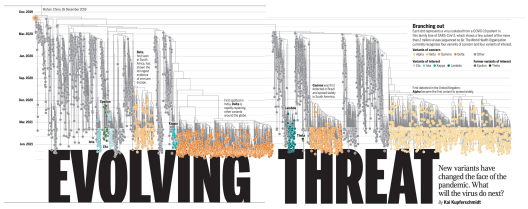
Science, August 20, 2021



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Evolution of Pathogens and Defenses

Viral Variants (mutants) and adaptive immunity

- Generation of Diversity
- Selection pressure, competition (e.g., which viral sequence makes it easier to enter cells?)
- Successful variants increase in the population (for viruses, e.g., through increased transmissability or immune evasion),
- E.g., alpha variant, delta variant have become more common
  - Viral spike protein allows entry into cells via binding to surface ACE2 receptor
  - Alpha was about 50% more transmissable than earlier virus, spike binds more tightly to ACE2 receptor (how it gets into cells), lowers infectious dose; delta more transmissable than alpha
- More vaccinated people increases selective pressure for immune evasion
  - Fewer people infected, fewer variants, but an immune escape variant now has huge advantage over other variants

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Pathogen Evolution

- Pathogens evolve on a time-scale related to how often they replicate, can be hours
- Animals evolve on a generation time-scale, years
- The immune system has evolved a way to evolve during a single life-span, the adaptive immune system

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Evolution takes place by changes in DNA sequences

- DNA sequence is the blueprint for proteins

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Information flow in cells

- DNA contains the blueprint for cell activities and identity, by carrying code for what proteins to make; proteins carry out those instructions of DNA
- True for both body cells and pathogen cells, including viruses
- Viruses contain nucleic acid core, and surface proteins, including the proteins that allow entry into your cells (in infamous "spike protein" of SARS-Cov-2)

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The Central Dogma of Biology

Francis Crick

- DNA contains the instructions for all cell activity
- Replication of DNA transfers that information when cells divide
- DNA is transcribed into RNA, which is translated into protein, which carries out functions (enzymes to allow chemical metabolic reactions, structures of cells)

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Pathogen evolution and adaptive immunity both require understanding DNA-RNA-protein, the Central Dogma of biology

- Viruses contain DNA or RNA, sequences of nucleic acids which encode information
- Central Dogma of Molecular Biology is that the blueprint for an organism is encoded in DNA, which is transcribed into RNA, which is translated into protein; viruses can be either DNA or RNA, and they use your cellular machinery to replicate their nucleic acids and make viral proteins

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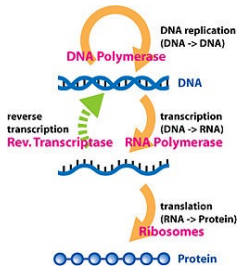

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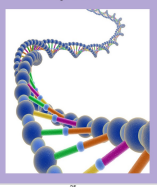
DNA Structure

Teachable tidbit developed for the
SMI
Scientific Teaching Institute
July 2011



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- Participants:
  - Allison Keller, Lisa Snuffin, Andrea Biggs, Danielle Moya
- Learning Goals:
  - **Understand** the structure of DNA incorporating the three components of a nucleotide
- Learning Outcomes:
  - **Construct** a DNA molecule
  - **Show** complementary base pairing
  - **Label** the different parts of the DNA molecule
  - **Predict** how DNA replicates
- Materials:
  - Colored paper (6 different colors), scissors, and tape.

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- Prior Knowledge:
  - The students now are in the beginning genetic unit for their high school Introductory Biology class and have already completed some units previous to today.
  - The following information would have been taught in those previous lessons and will be reinforced in today's lesson as well:
    - DNA is made up of nucleotides which are made up of a 5 carbon sugar, phosphate, and a base.
    - There are 4 different bases in a DNA molecule:
    - adenine (a purine)
    - cytosine (a pyrimidine)
    - guanine (a purine)
    - thymine (a pyrimidine)
    - Experiments: Griffith, Hershey and Chase, and Rosalind Franklin.
    - DNA is the molecule which holds genetic information.
    - DNA is a polymer, which is made up of monomers.
    - Hydrogen bonding

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Dear Colleague;

Now that we know DNA is the molecule of heredity, we need to understand the structure of the molecule. How is it that a string of nucleotides could carry out the three processes that are critical for the molecule to perform? One, it must carry information from one generation to the next. Two, it has to put the information to work by determining the inheritable characteristics of organisms. And thirdly, it has to be easily copied in order for all of a cell's genetic information to be replicated every time a cell divides.

We know that DNA is a very long molecule of nucleotides. A nucleotide has three basic parts, a 5-carbon sugar, phosphate group and a nitrogen base. There are 4 nitrogen bases in DNA, they are Adenine, Guanine, Cytosine, and Thymine. The backbone of the molecule is a phosphate –sugar chain, we believe the bases stick out sideways. We are working to discover the structure of the molecule. It could be a big thing. We could certainly use your help.

Your Friends,
James and Francis

P.S.
Rosalind Franklin's photograph was very helpful to us. Here is a peek.

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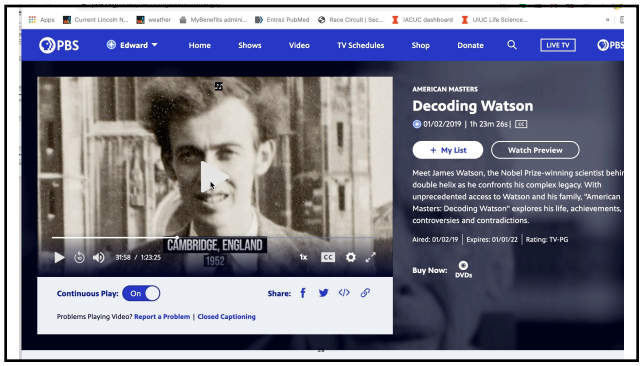

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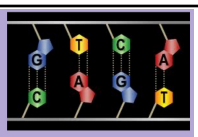
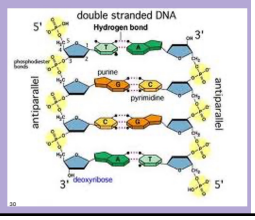
Complementary Pairs:

Hydrogen bonds connect base pairs making opposing DNA strands complementary.



- Adenine and Thymine have 2 Hydrogen Bonds
- Guanine and Cytosine have 3 Hydrogen Bonds



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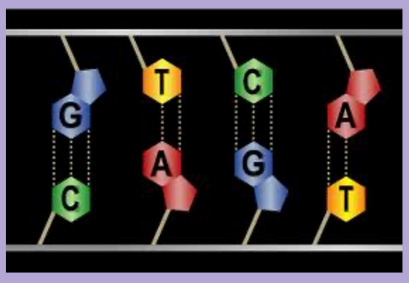
Complements



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Clicker Question:

Given the amount of Adenine in a DNA sample is 37% and the amount of Guanine is 13%, what will be the amount of Thymine?

A. 37%
B. 13%
C. 74%
D. 50%

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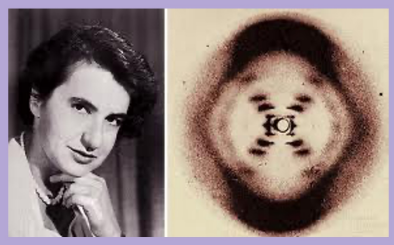

Rosalind Franklin (1920-1958) and her x-ray diffraction pattern of DNA.

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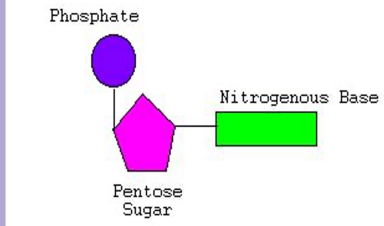

nucleotide

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Teacher Instruction for the Activity:

- Students are given the 3 parts of a nucleotide (base, phosphate, and sugar cut outs) and are told to tape them together.
- Then have them find other students that have complementary bases to theirs.
- Finally, students tape the DNA structure parts together on the wall for a giant visual aid.
- Then a discussion will be held on what they learned.
- Hopefully they noted: that only A and T goes together as well as only G and C, and DNA is double stranded with a phosphate, sugar, and a base keeping it all together.

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Translation

mRNA to protein

- https://www.youtube.com/watch?v=oefAl2x2CQM
- https://www.youtube.com/watch?v=itsb2SqR-R0

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What does this have to do with immunology?

- Antibodies and T cell receptors have huge amount of diversity in amino acid sequences, to make sure they will recognize any novel pathogen
- B cells and T cells must create diversity in the DNA that encodes antibodies and T cell receptors
- This diversity in DNA sequence is created early in the development of B cells in the bone marrow and T cells in the Thymus
- The diversity is created only in the portion of the antibody that binds antigen

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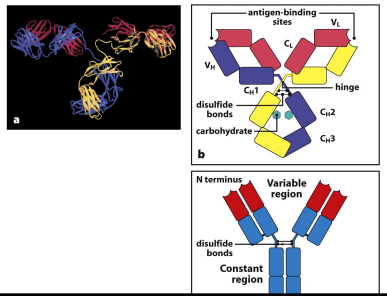
Antibodies have Variable Regions and Constant Regions



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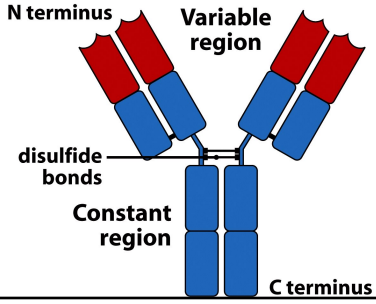
Antibody Structure (Immunoglobulin)



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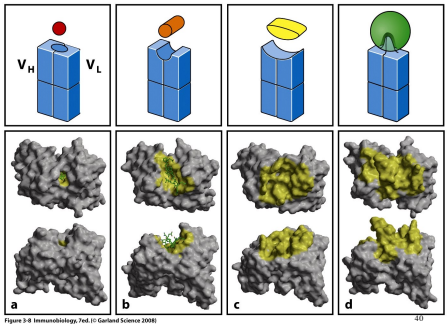

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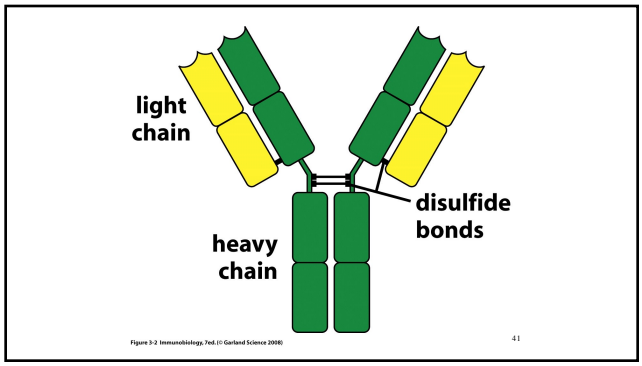
light chain
disulfide bonds
heavy chain
Figure 3-2 Immunobiology, 7ed. (© Garland Science 2008)
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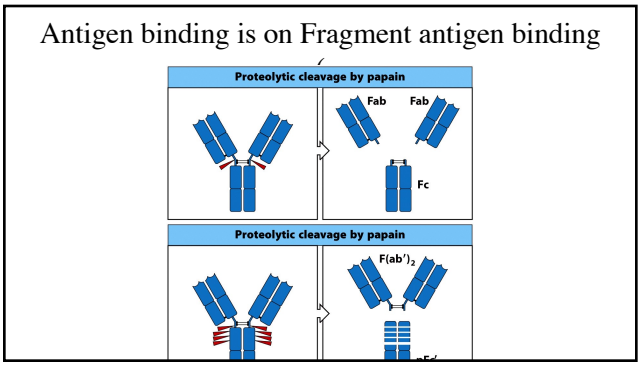
Antigen binding is on Fragment antigen binding
Proteolytic cleavage by papain
Fab
Fab
Fc
Proteolytic cleavage by papain
F(ab')2

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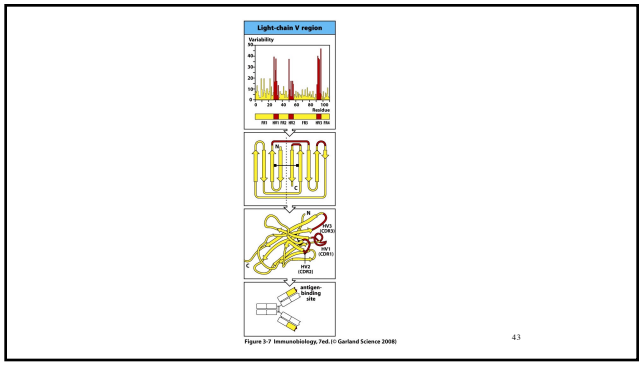
Light-chain V region
Variability
Figure 3-7 Immunobiology, 7ed. (© Garland Science 2008)
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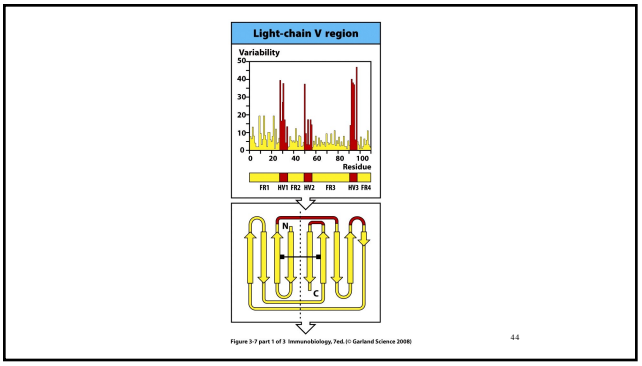
Light-chain V region
Variability
Residue
FR1 HV1 FR2 HV2 FR3 HV3 FR4
Figure 3-7 part 1 of 3 Immunobiology, 7ed. (© Garland Science 2008)
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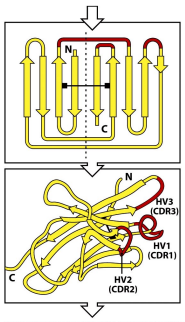

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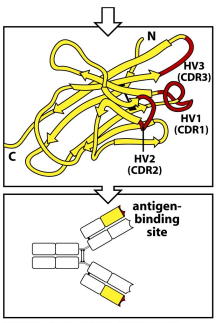

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Antibody Diversity

DNA diversity

As B cells develop in bone marrow, diversity is created in the DNA sequences encoding the Light Chain Hypervariable domains

Some variability is created by combining already present sequences (e.g., there are about 40 V region sequences to choose one from)

Some variability is created by random mutations in sequences at the junctions of the already present segments

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Number of functional gene segments in human immunoglobulin loci

Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

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Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Variable segments (V)	40	70	52	~70
Diversity segments (D)	25	0	2	0
D segments read in three frames	rarely	–	often	–
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	1.9×10^6		5.8×10^6	
Junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

Figure 4-12 Immunobiology, 7ed. (© Garland Science 2008)

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37 x 10E12 cells in whole body

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Different Constant Regions create Different Isotypes

These are called Isotypes, IgM, IgG, IgE, IgA, have different functions

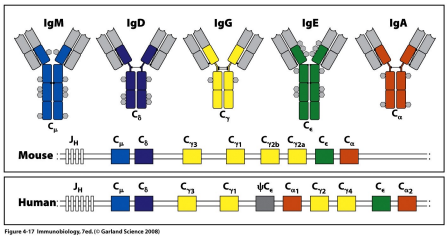

Figure 4-17 Immunobiology, 7ed. (© Garland Science 2008)

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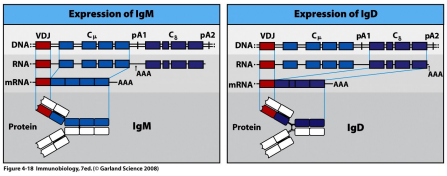

Figure 4-18 Immunobiology, 7ed. (© Garland Science 2008)

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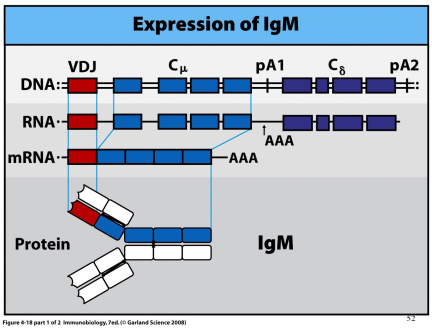

Figure 4-18 part 1 of 2 Immunobiology, 7ed. (© Garland Science 2008)

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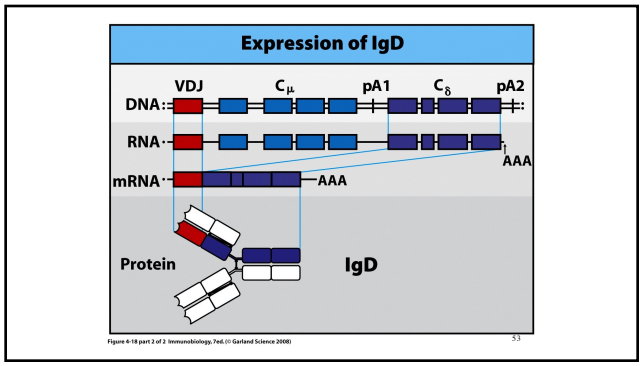
Expression of IgD
VDJ
C_μ
pA1
C_δ
pA2
DNA
RNA
AAA
mRNA
AAA
Protein
IgD
Figure 4-18 part 2 of 2 Immunobiology, 7ed, (© Garland Science 2008)
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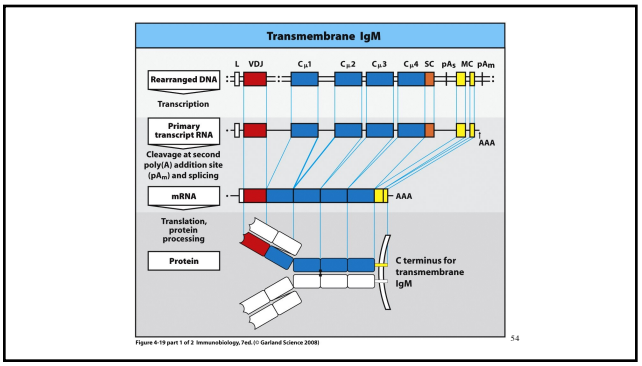
Transmembrane IgM
L VDJ $C_\mu1$ $C_\mu2$ $C_\mu3$ $C_\mu4$ SC pA_S MC pA_m
Rearranged DNA
Transcription
Primary transcript RNA
Cleavage at second poly(A) addition site (pA_m) and splicing
AAA
mRNA
AAA
Translation, protein processing
Protein
C terminus for transmembrane IgM
Figure 4-19 part 1 of 2 Immunobiology, 7ed, (© Garland Science 2008)
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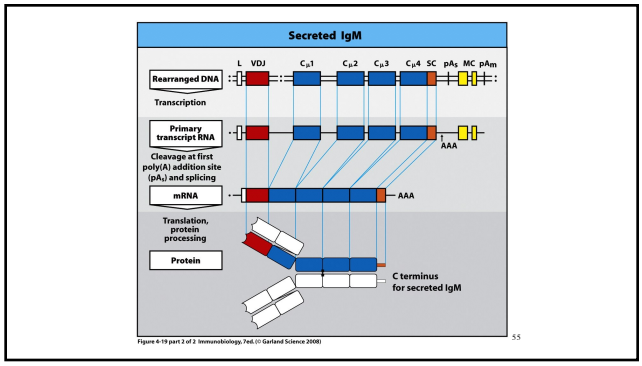
Secreted IgM
L VDJ $C_\mu1$ $C_\mu2$ $C_\mu3$ $C_\mu4$ SC pA_S MC pA_m
Rearranged DNA
Transcription
Primary transcript RNA
Cleavage at first poly(A) addition site (pA_S) and splicing
AAA
mRNA
AAA
Translation, protein processing
Protein
C terminus for secreted IgM
Figure 4-19 part 2 of 2 Immunobiology, 7ed, (© Garland Science 2008)
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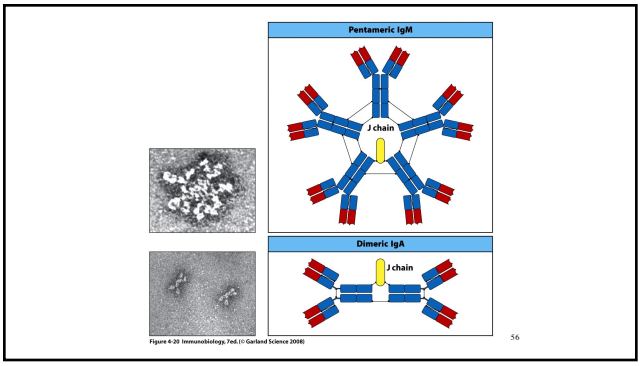
Pentameric IgM
J chain
Dimeric IgA
J chain
Figure 4-20 Immunobiology, 7ed, (© Garland Science 2008)
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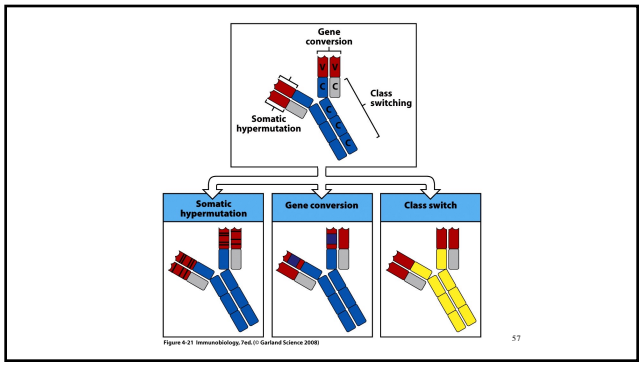
Gene conversion
Somatic hypermutation
Class switching
Somatic hypermutation
Gene conversion
Class switch
Figure 6-21 Immunobiology, 7ed.(© Garland Science 2008)
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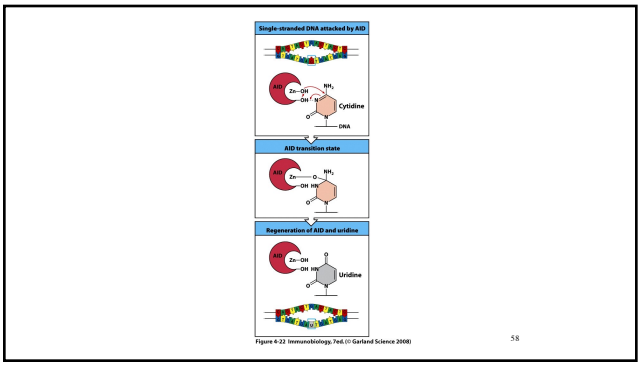
Single-stranded DNA attacked by AID
AID
Zn
OH
NH2
Cytidine
DNA
AID transition state
Regeneration of AID and uridine
Uridine
Figure 6-22 Immunobiology, 7ed.(© Garland Science 2008)
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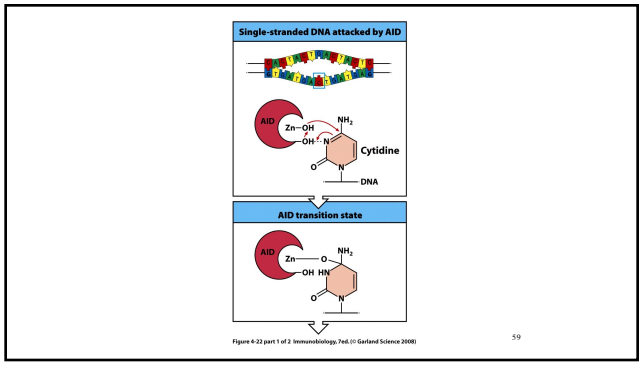
Single-stranded DNA attacked by AID
AID
Zn
OH
NH2
Cytidine
DNA
AID transition state
Figure 6-22 part 1 of 2 Immunobiology, 7ed.(© Garland Science 2008)
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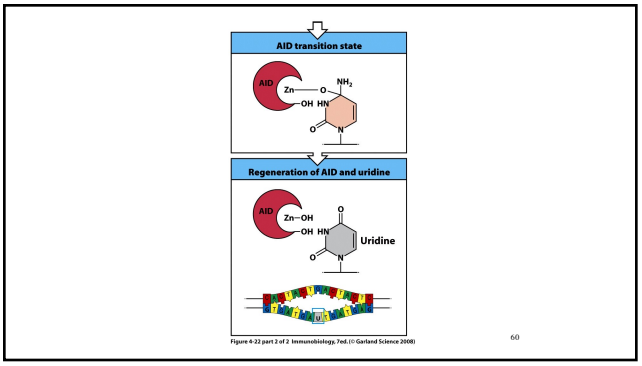
AID transition state
AID
Zn
OH
NH2
Regeneration of AID and uridine
Uridine
Figure 6-22 part 2 of 2 Immunobiology, 7ed.(© Garland Science 2008)
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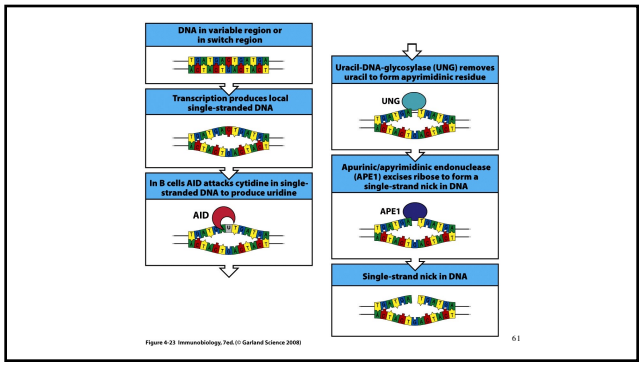
DNA in variable region or in switch region
Transcription produces local single-stranded DNA
In B cells AID attacks cytidine in single-stranded DNA to produce uridine
AID
Uracil-DNA-glycosylase (UNG) removes uracil to form apyrimidinic residue
UNG
Apurinic/apyrimidinic endonuclease (APE1) excises ribose to form a single-strand nick in DNA
APE1
Single-strand nick in DNA
Figure 4-23 Immunobiology, 7ed. (© Garland Science 2008)
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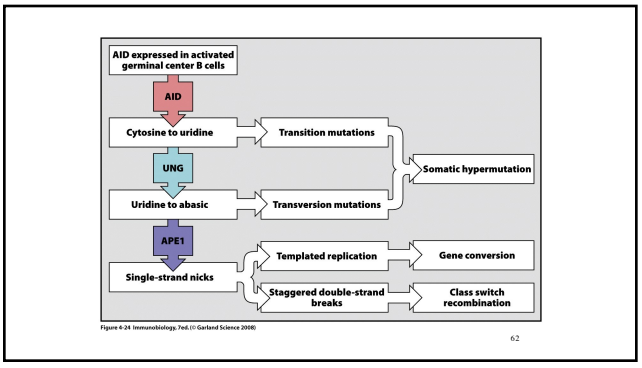
AID expressed in activated germinal center B cells
AID
Cytosine to uridine
Transition mutations
UNG
Uridine to abasic
Transversion mutations
Somatic hypermutation
APE1
Single-strand nicks
Templated replication
Gene conversion
Staggered double-strand breaks
Class switch recombination
Figure 4-24 Immunobiology, 7ed. (© Garland Science 2008)
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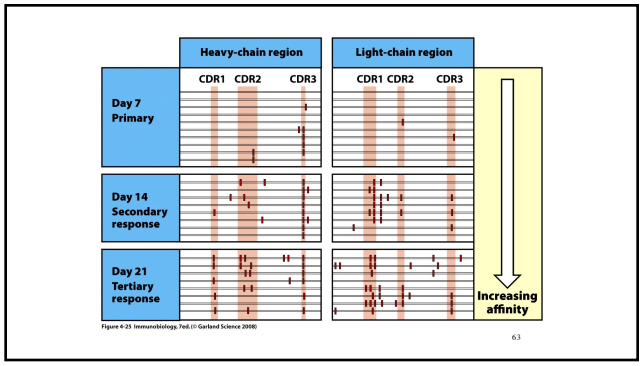
Heavy-chain region
Light-chain region
CDR1 CDR2 CDR3
CDR1 CDR2 CDR3
Day 7 Primary
Day 14 Secondary response
Day 21 Tertiary response
Increasing affinity
Figure 4-25 Immunobiology, 7ed. (© Garland Science 2008)
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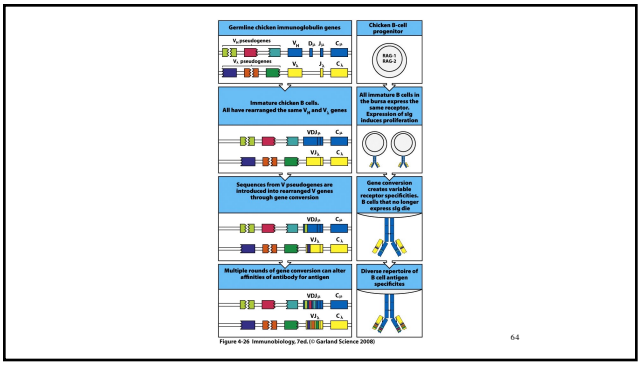
Germline chicken immunoglobulin genes
Chicken B-cell progenitor
RAG-1
RAG-2
Immature chicken B cells. All have rearranged the same V_H and V_L genes
All immature B cells in the bursa express the same receptor. Expression of sIg induces proliferation
Sequences from V pseudogenes are introduced into rearranged V genes through gene conversion
Gene conversion creates variable receptor specificities. B cells that no longer express sIg die
Multiple rounds of gene conversion can alter affinities of antibody for antigen
Diverse repertoire of B cell antigen specificities
Figure 4-26 Immunobiology, 7ed. (© Garland Science 2008)
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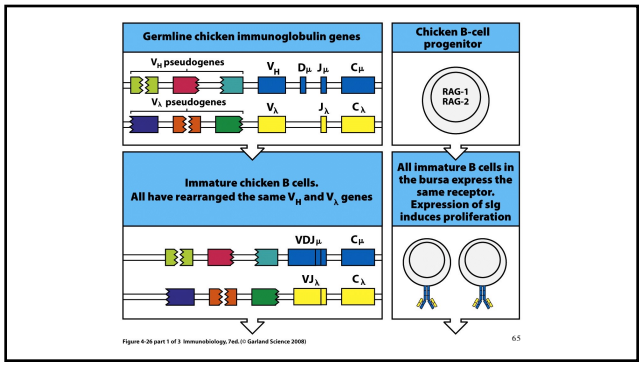
Germline chicken immunoglobulin genes
Chicken B-cell progenitor
V_H pseudogenes
V_H D_μ J_μ C_μ
V_λ pseudogenes
V_λ J_λ C_λ
RAG-1
RAG-2
Immature chicken B cells.
All have rearranged the same V_H and V_λ genes
All immature B cells in the bursa express the same receptor. Expression of sIg induces proliferation
VDJ_μ C_μ
VJ_λ C_λ
Figure 4-26 part 1 of 3 Immunobiology, 7ed.(© Garland Science 2008)
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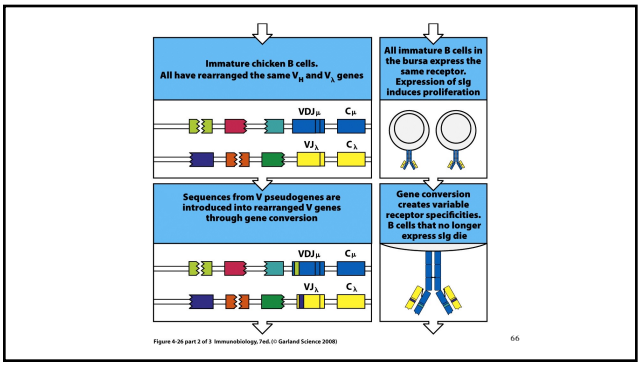
Immature chicken B cells.
All have rearranged the same V_H and V_λ genes
All immature B cells in the bursa express the same receptor. Expression of sIg induces proliferation
VDJ_μ C_μ
VJ_λ C_λ
Sequences from V pseudogenes are introduced into rearranged V genes through gene conversion
Gene conversion creates variable receptor specificities. B cells that no longer express sIg die
Figure 4-26 part 2 of 3 Immunobiology, 7ed.(© Garland Science 2008)
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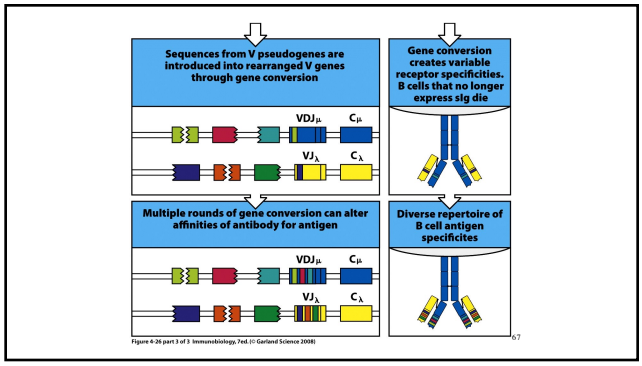
Sequences from V pseudogenes are introduced into rearranged V genes through gene conversion
Gene conversion creates variable receptor specificities. B cells that no longer express sIg die
VDJ_μ C_μ
VJ_λ C_λ
Multiple rounds of gene conversion can alter affinities of antibody for antigen
Diverse repertoire of B cell antigen specificites
Figure 4-26 part 3 of 3 Immunobiology, 7ed.(© Garland Science 2008)
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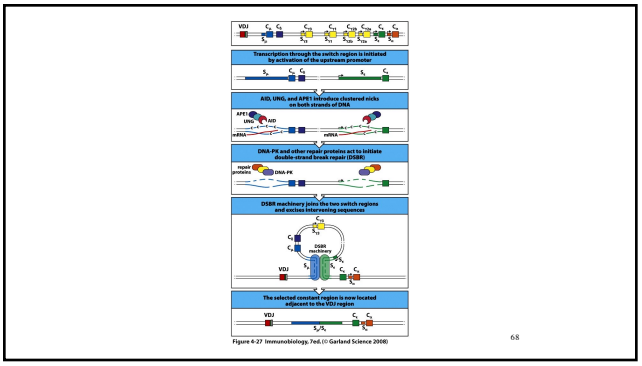
Transcription through the switch region is initiated by activation of the upstream promoter
AID, UNG, and APE1 introduce clustered nicks on both strands of DNA
DNA-PK and other repair proteins act to initiate double-strand break repair (DSBR)
DSBR machinery joins the two switch regions and excises intervening sequences
The selected constant region is now located adjacent to the VDJ region
Figure 4-27 Immunobiology, 7ed.(© Garland Science 2008)
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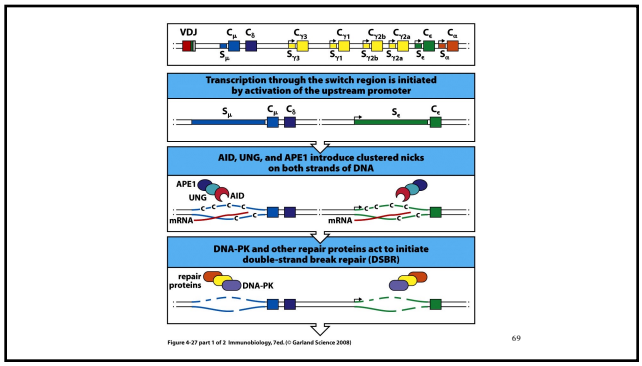

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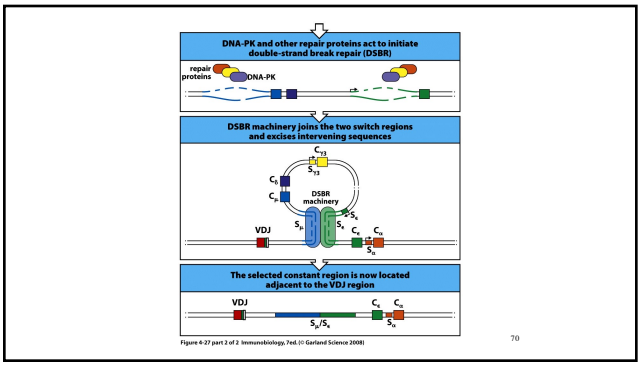

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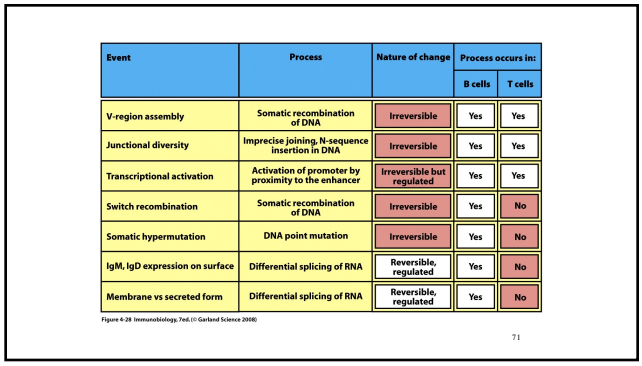

Event	Process	Nature of change	Process occurs in:	
			B cells	T cells
V-region assembly	Somatic recombination of DNA	Irreversible	Yes	Yes
Junctional diversity	Imprecise joining, N-sequence insertion in DNA	Irreversible	Yes	Yes
Transcriptional activation	Activation of promoter by proximity to the enhancer	Irreversible but regulated	Yes	Yes
Switch recombination	Somatic recombination of DNA	Irreversible	Yes	No
Somatic hypermutation	DNA point mutation	Irreversible	Yes	No
IgM, IgD expression on surface	Differential splicing of RNA	Reversible, regulated	Yes	No
Membrane vs secreted form	Differential splicing of RNA	Reversible, regulated	Yes	No

Figure 4-28 Immunobiology, 7ed.(© Garland Science 2008)

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