LESSON 9

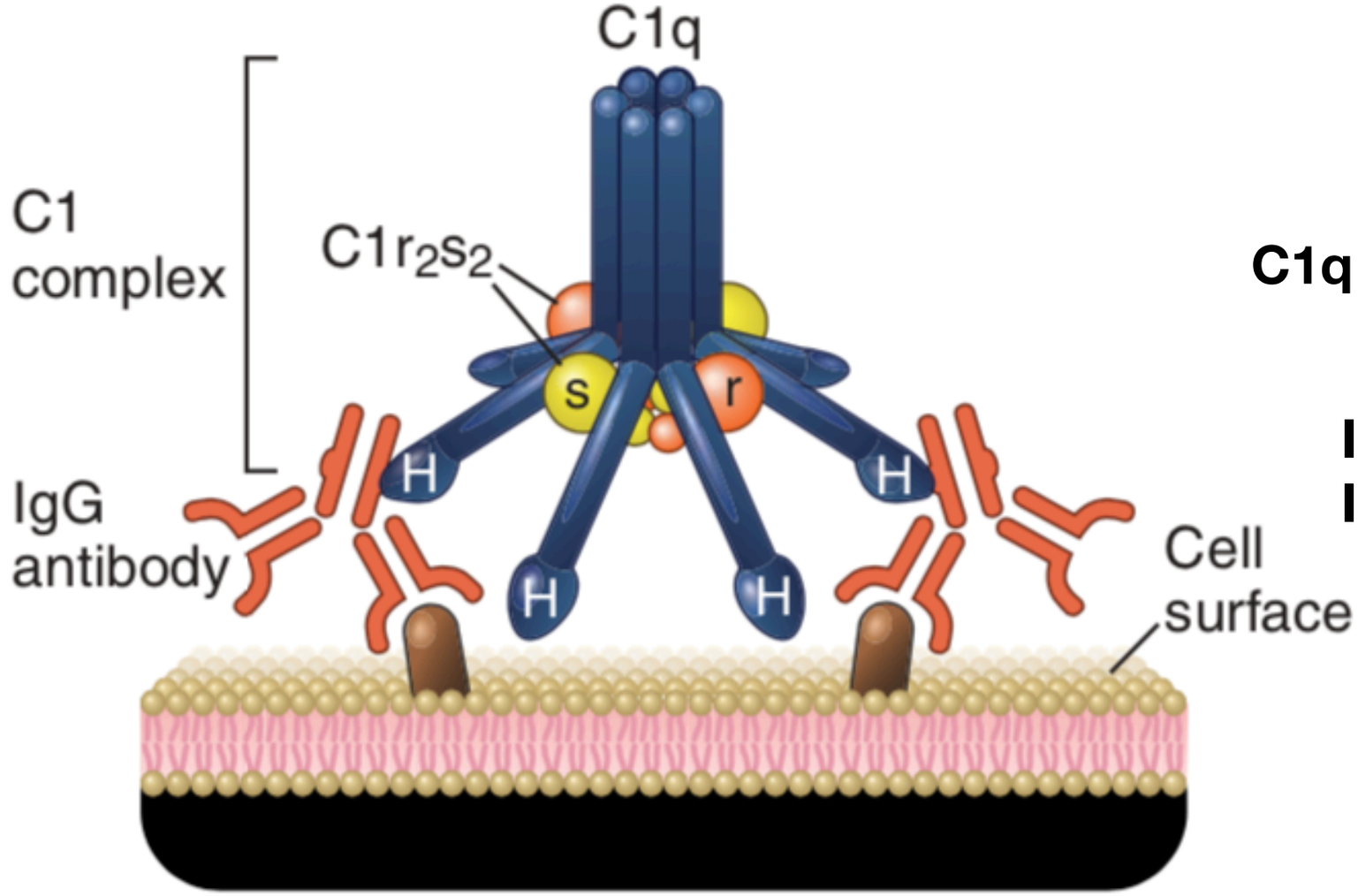
Classical pathway of complement activation

Lectin pathway of complement activation

Regulation of complement cascade

Diseases

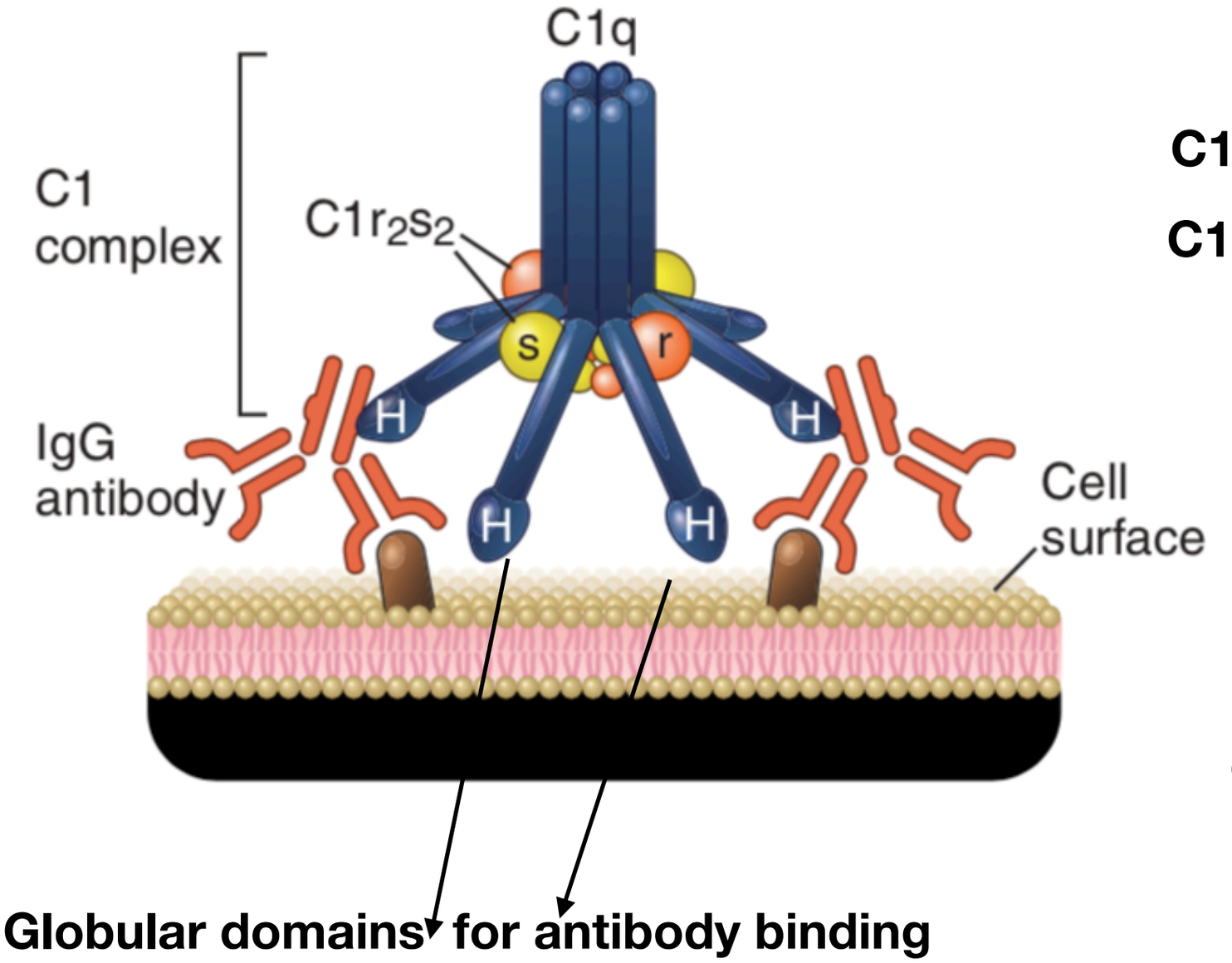
Classical pathway of complement activation start with the binding by C1complex to antibodies linked to a multivalent antigen



C1q fraction bind Fcs of:

IgG (IgG1 and IgG3)
IgM

C1q recognizes antigen-antibody complexes



Globular domains for antibody binding

C1 is constituted by:

C1q (6 subunits) > recognition

C1r (2 subunits) > enzymatic activity

C1s (2 subunits) > enzymatic activity

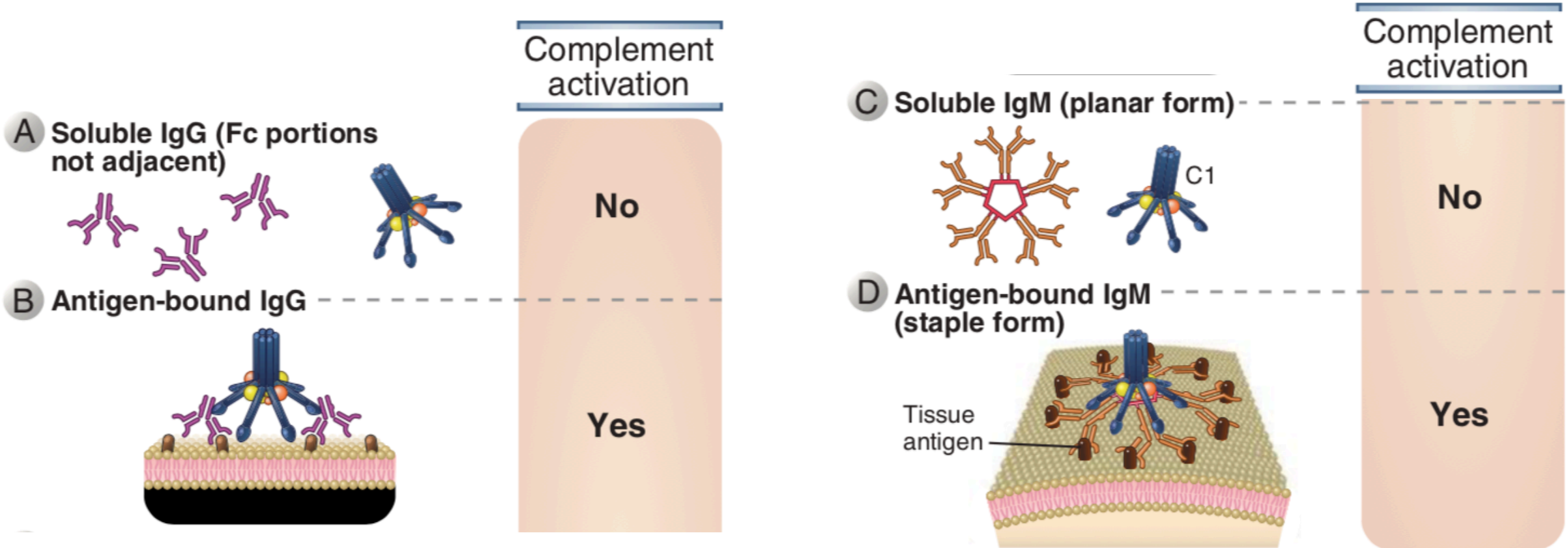
$C1r_2$-$C1s_2$ tetramer

C1r and C1s are **inactive** when the complex is not bound to Ig

C1r and C1s are **activated** when the complex is bound to Ig

C1q recognizes antigen bound IgG and IgM

(IgG1 and IgG3)



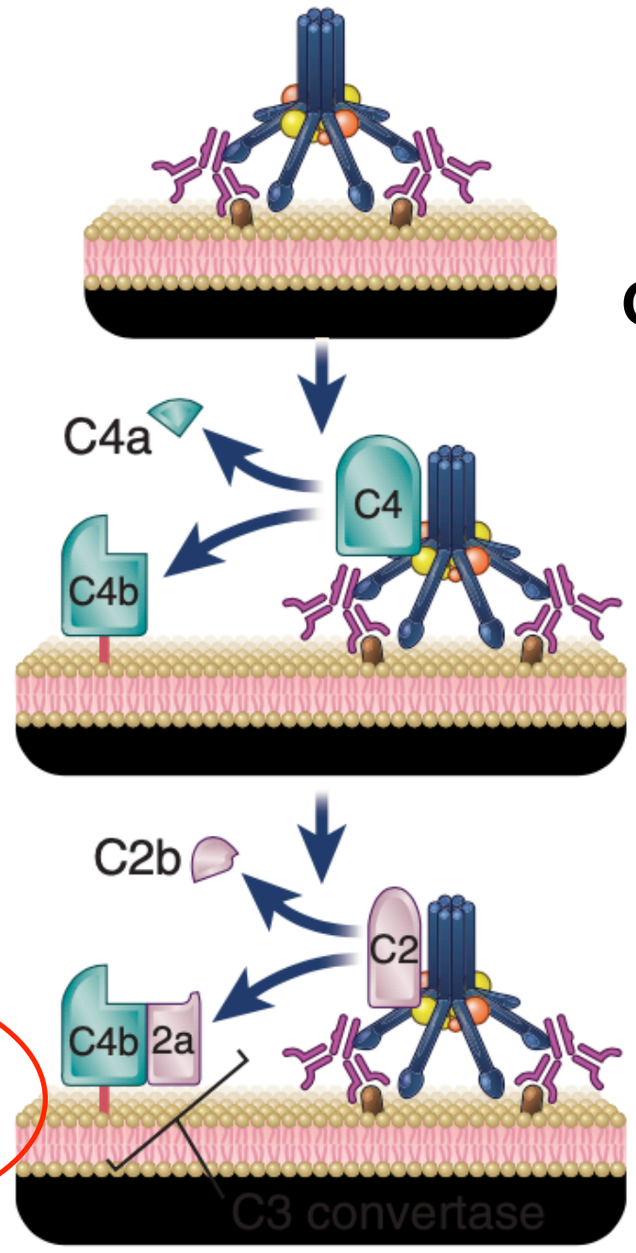
IgM activate C1 more efficiently due to their pentameric structure (each IgM can bind 2 C1q)

Activated C1 mediate the proteolysis of C4 and C2

Binding of antibodies to multivalent antigen; binding of C1 to antibodies

Cleavage of C4 by $C1r_2S_2$ enzyme; covalent attachment of C4b to antigenic surface and to antibodies

Cleavage of C2; binding of C2a to C4b to form C4b2a complex (C3 convertase)



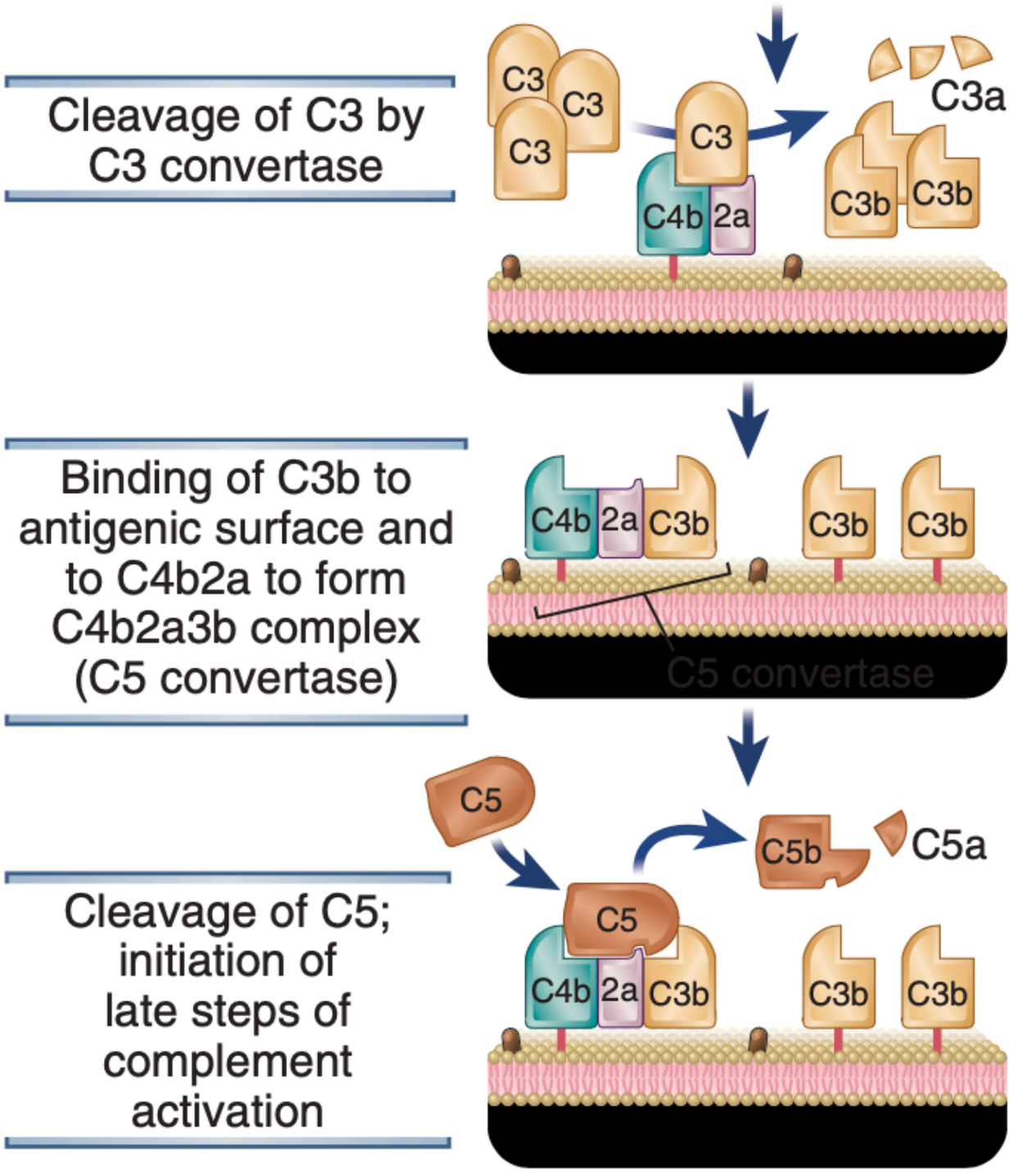
C1q → C1r → C1s

Alternative pathway C3bBb

Assembly of C3 convertase

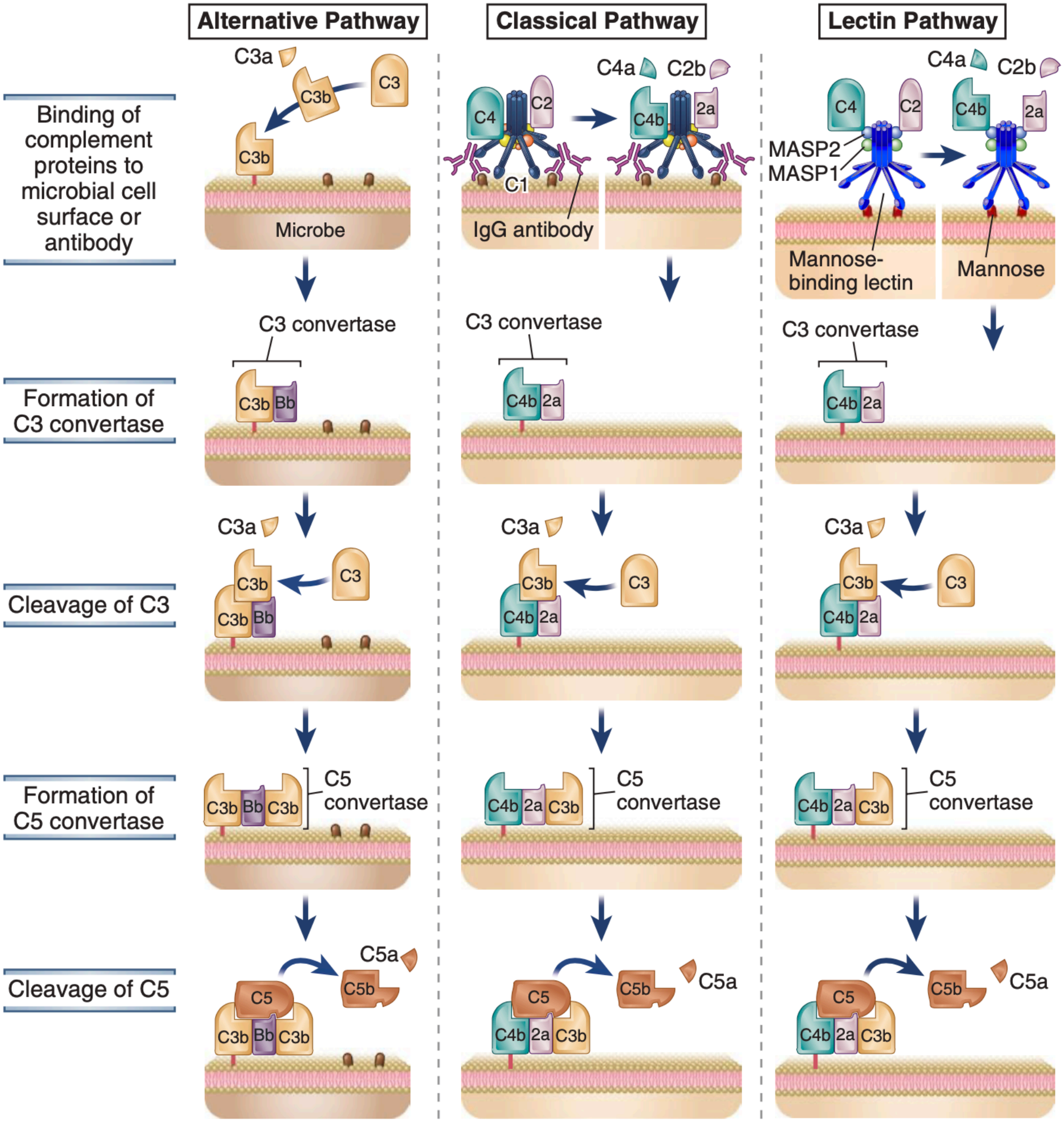
Different nomenclature!!!

Assembly of the C5 convertase

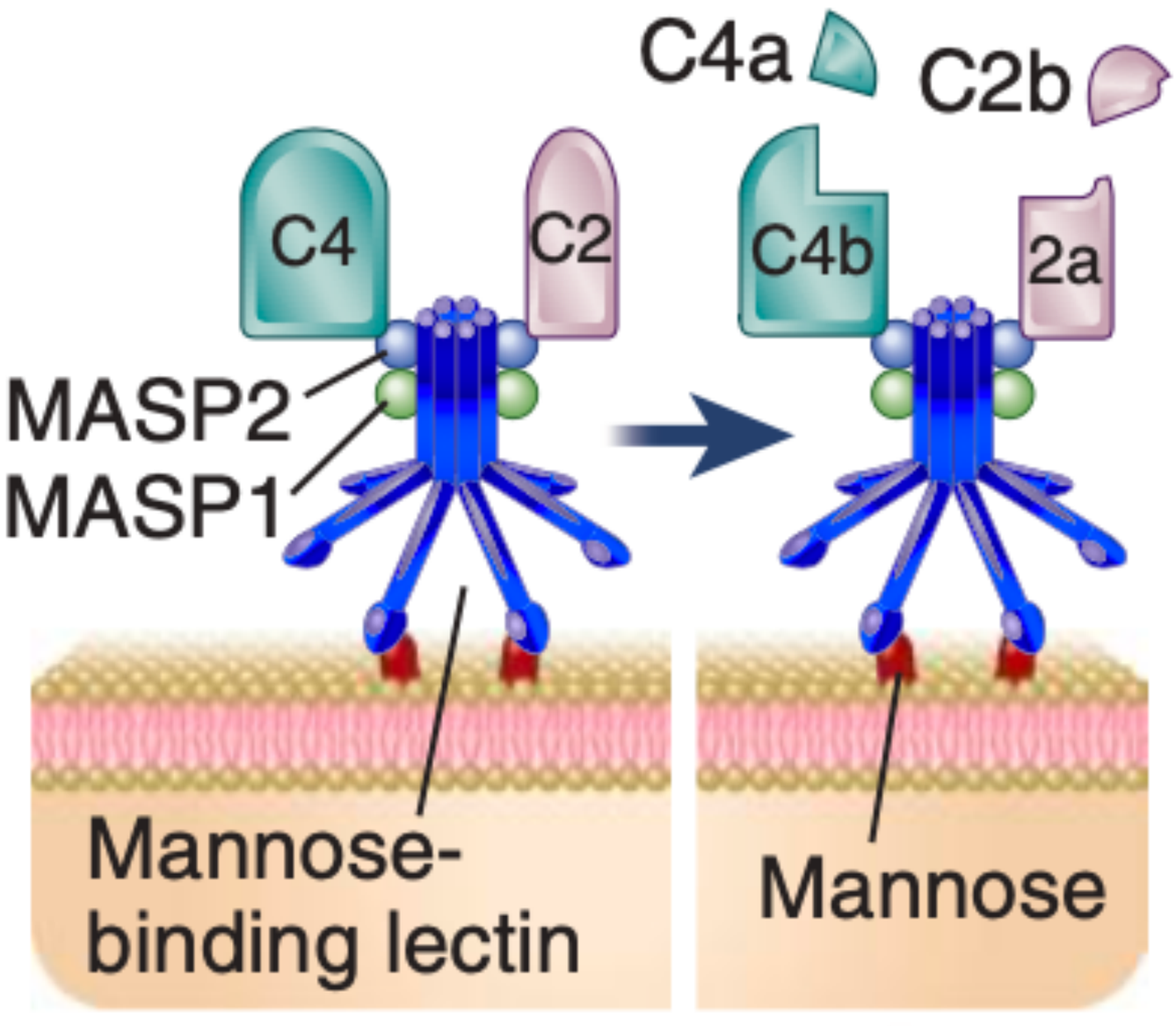


Assembly of C5 convertase

Alternative pathway C3bBbC3b


Alternative Pathway
Classical Pathway
Lectin Pathway
Binding of complement proteins to microbial cell surface or antibody
Formation of C3 convertase
Cleavage of C3
Formation of C5 convertase
Cleavage of C5
C3a
C3b
C3
Microbe
C4a
C2b
C4
C2
C4b
2a
C1
IgG antibody
MASP2
MASP1
Mannose-binding lectin
Mannose
C3 convertase
Bb
C5
C5 convertase
C5a
C5b

Lectin pathway of complement activation



Triggered by collectins and ficolins

Similar in structure to C1

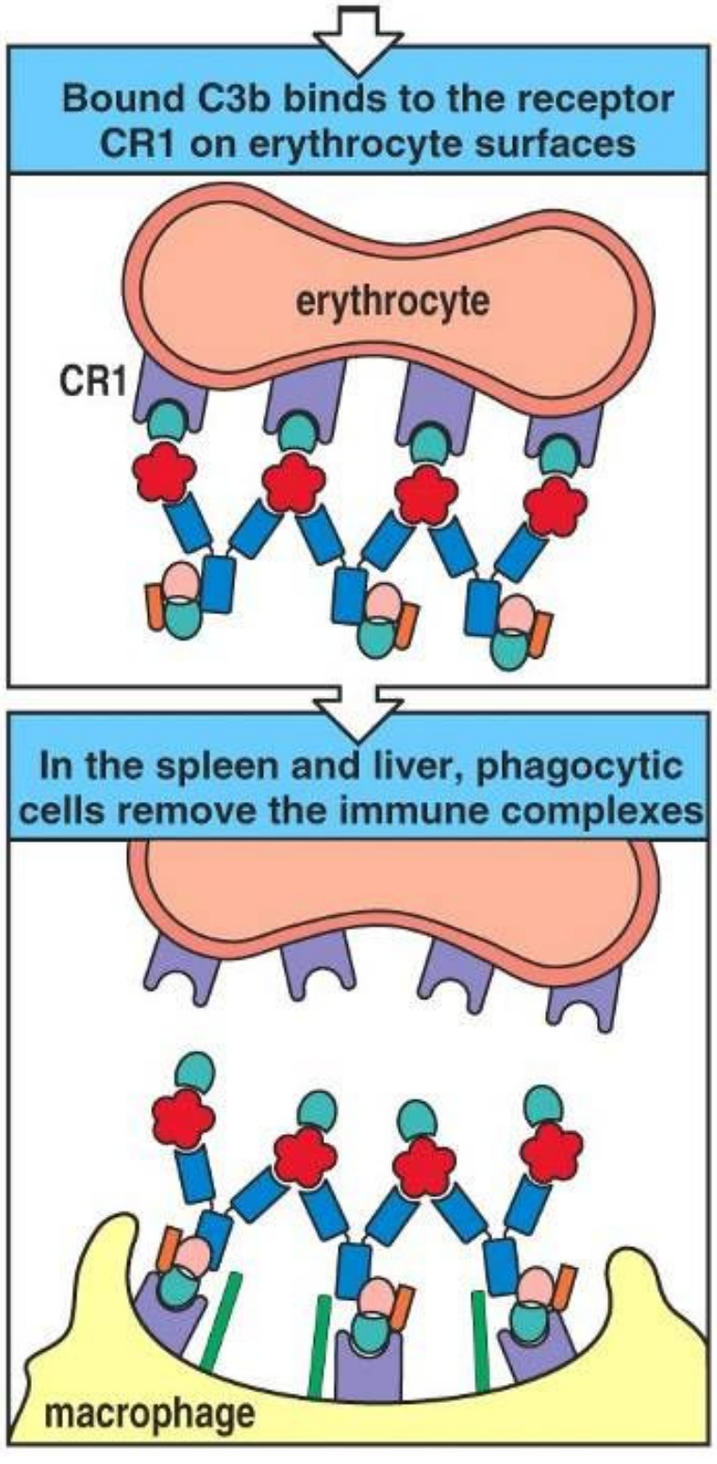
MBL recognising mannose residues

Upon recognition, **MASP1** and **MASP2** get activated by proteolysis, and can cleave C4 and C2 (as in the classical pathway)

Regardless of the activation pathway…

1. Lysis (MAC)
2. Opsonisation - complement recognition by phagocytes (C3b/C4b CR1, CR3 and CR4)
3. Support humoral response of B lymphocytes to respond to antigen (C3d, iC3b through CR2)
4. Inflammation (C3a and C5a lead to release of histamine by mast cells and/or basophils and act on vascular endothelium)
5. Destruction of immune complexes in the liver and spleen (CR1 on erythrocytes)

CR1 on erythrocytes allows the removal of immune complexes

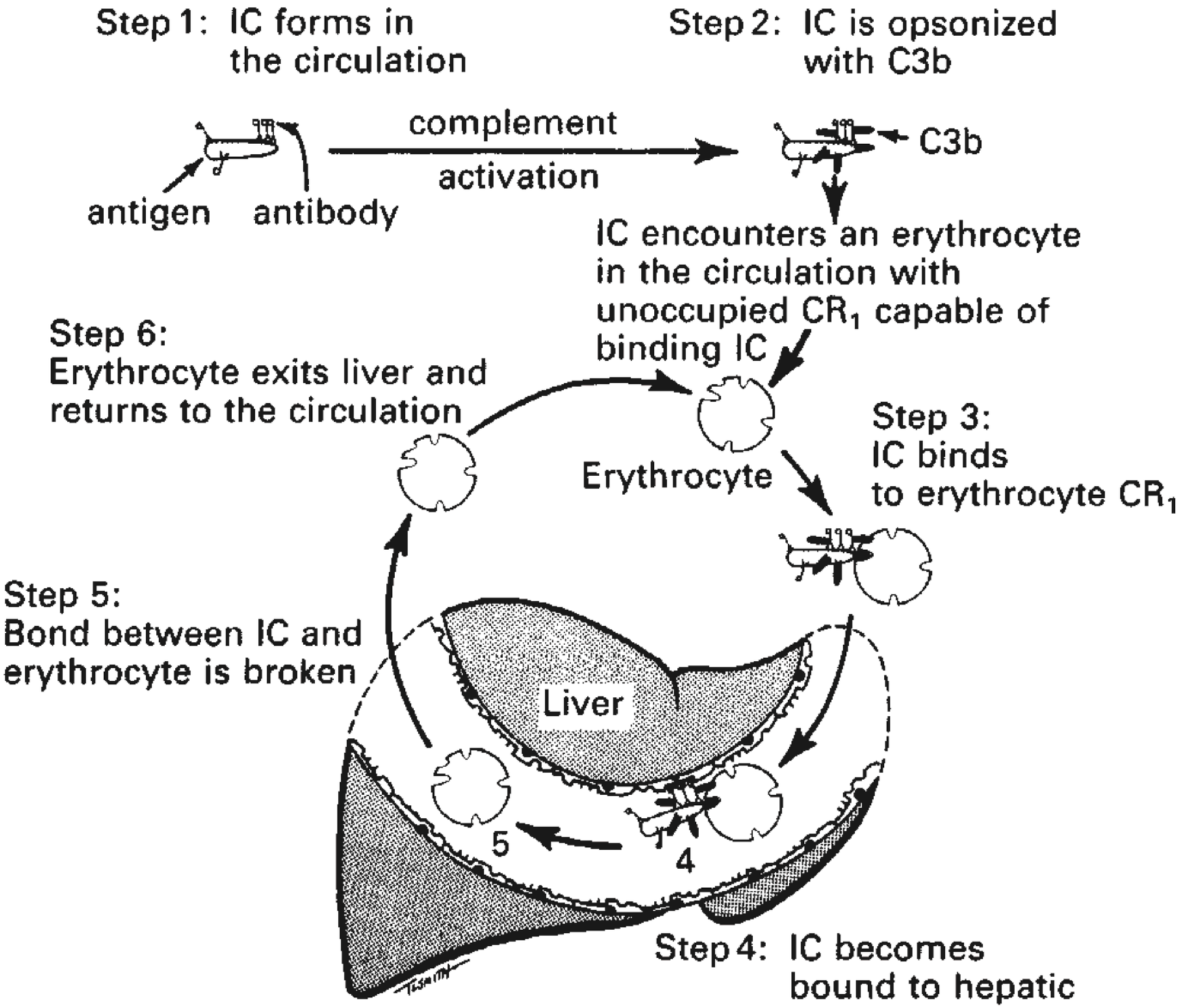


Immune complex = antigen antibody complex

Can have deposition of complement

In the liver specialized macrophages called Kupffer cells express CR1, CR3, CR4 and CRIg, which capture immune complexes transported by erythrocytes

Vintage version



Regulation of complement activity

1. Protect autologous cells

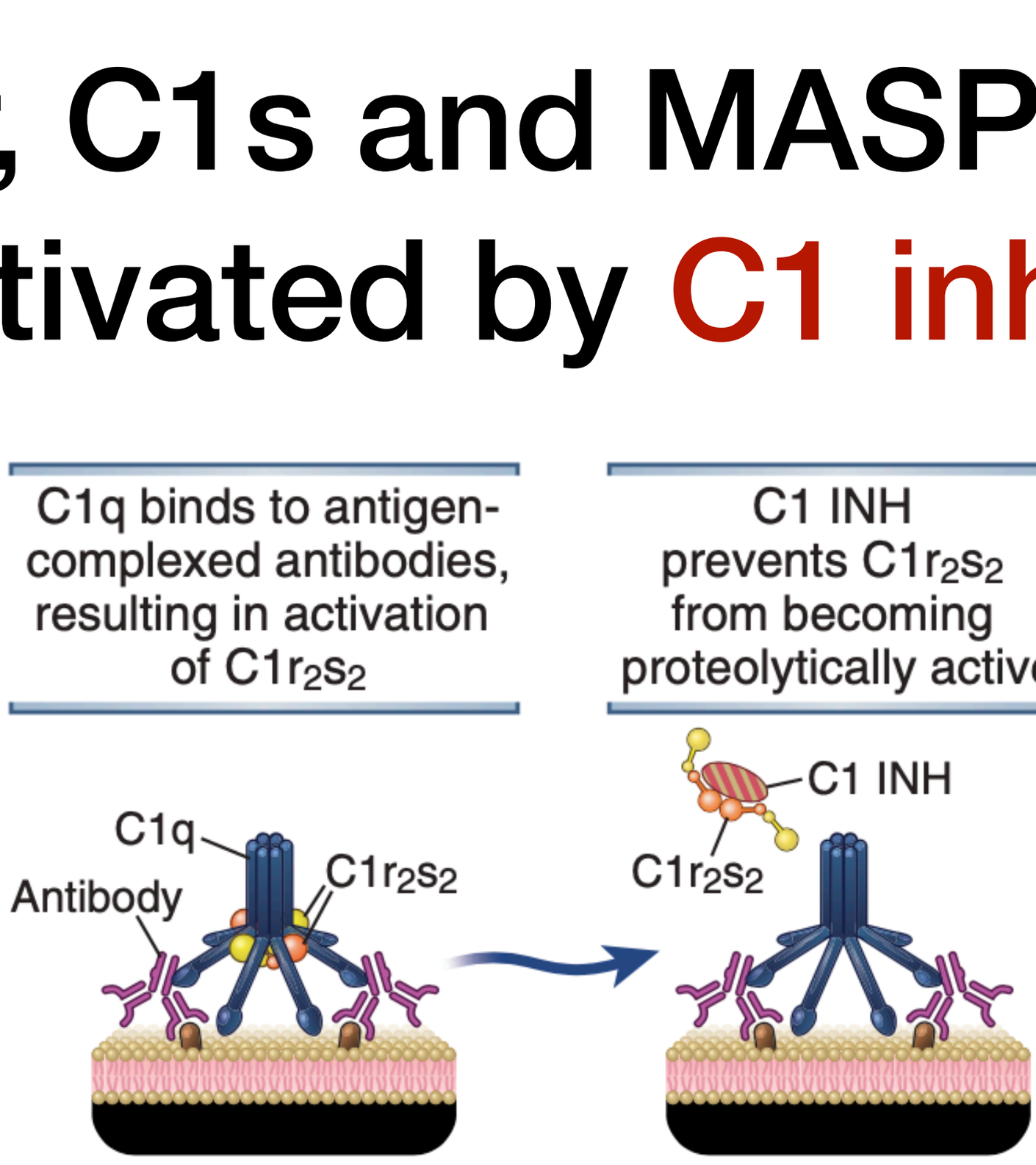
2. Limit in time complement activation

Receptor	Structure	Distribution	Interacts With	Function
C1 inhibitor (C1 INH)	104 kD	Plasma protein; conc. 200 μg/mL	C1r, C1s	Serine protease inhibitor; binds to C1r and C1s and dissociates them from C1q
Factor I	88-kD dimer of 50- and 38-kD subunits	Plasma protein; conc. 35 μg/mL	C4b, C3b	Serine protease; cleaves C3b and C4b by using factor H, MCP, C4BP, or CR1 as cofactors
Factor H	150 kD; multiple CCPRs	Plasma protein; conc. 480 μg/mL	C3b	Binds C3b and displaces Bb Cofactor for factor I–mediated cleavage of C3b
C4-binding protein (C4BP)	570 kD; multiple CCPRs	Plasma protein; conc. 300 μg/mL	C4b	Binds C4b and displaces C2 Cofactor for factor I–mediated cleavage of C4b
Membrane cofactor protein (MCP, CD46)	45–70 kD; four CCPRs	Leukocytes, epithelial cells, endothelial cells	C3b, C4b	Cofactor for factor I–mediated cleavage of C3b and C4b
Decay-accelerating factor (DAF)	70 kD; GPI linked, four CCPRs	Blood cells, endothelial cells, epithelial cells	C4b2a, C3bBb	Displaces C2a from C4b and Bb from C3b (dissociation of C3 convertases)
CD59	18 kD; GPI linked	Blood cells, endothelial cells, epithelial cells	C7, C8	Blocks C9 binding and prevents formation of the MAC

C1r, C1s and MASP2 are inactivated by C1 inhibitor

C1q binds to antigen-complexed antibodies, resulting in activation of $C1r_2s_2$

C1 INH prevents $C1r_2s_2$ from becoming proteolytically active



C1 INH deficiency cause a genetic disease called hereditary angioedema

C3b attached to the cell membrane can be degradated

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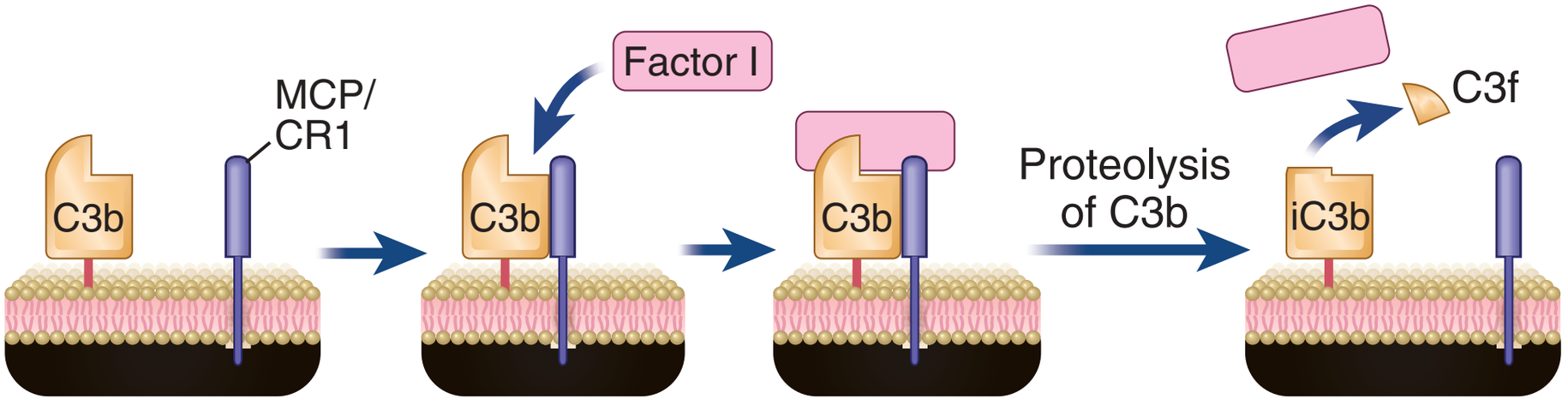
Factor I–mediated cleavage of C3b

Covalent attachment of C3b (or C4b) to cells	MCP (and CR1) act as cofactors for Factor I–mediated proteolytic cleavage of C3b, producing iC3b



MCP, CR1,DAF,C4BP and factor F

Generated fragments are recognized by phagocytes and B Lymphocytes

C3 and C5 convertases assembly are blocked by several proteins

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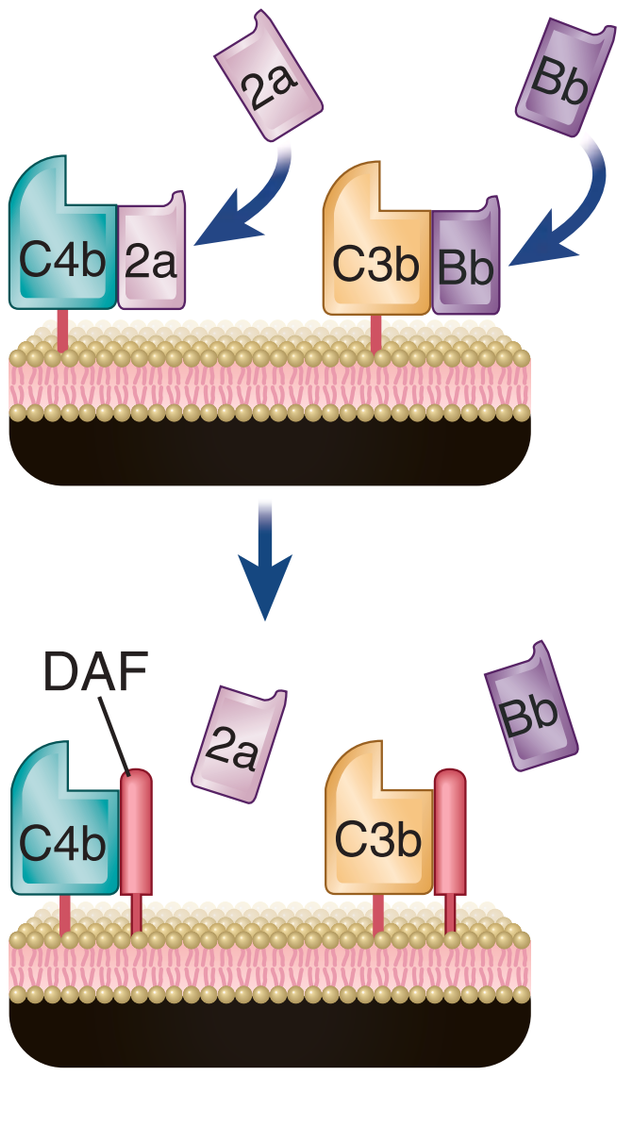
C3 and C5 convertases assembly are blocked by RCA proteins

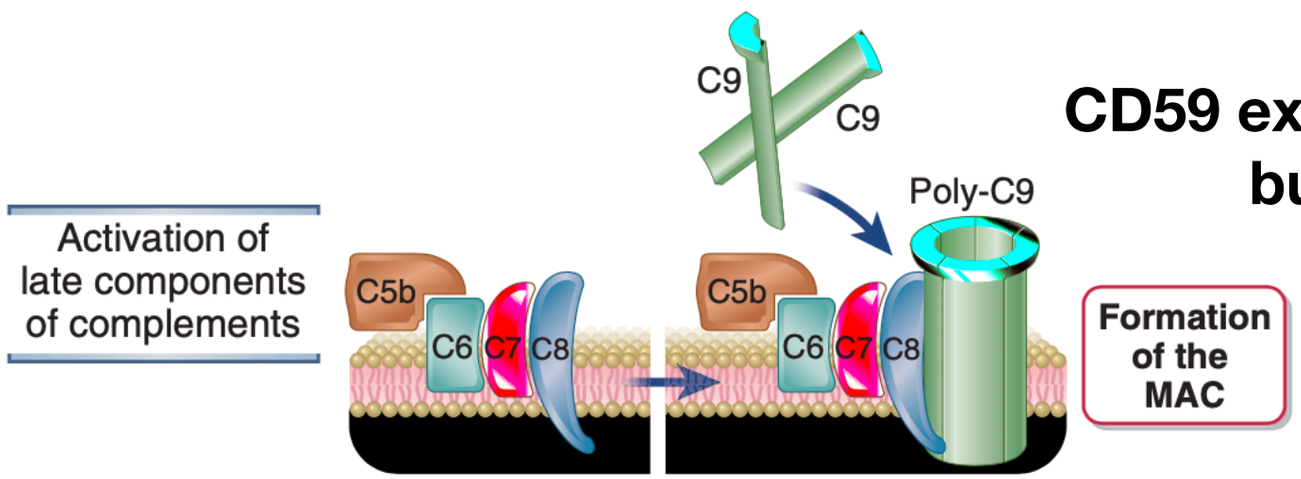
MCP, CRI,DAF,C4BP and factor H

Formation of C3 convertases

Dissociation of C3 convertases by DAF

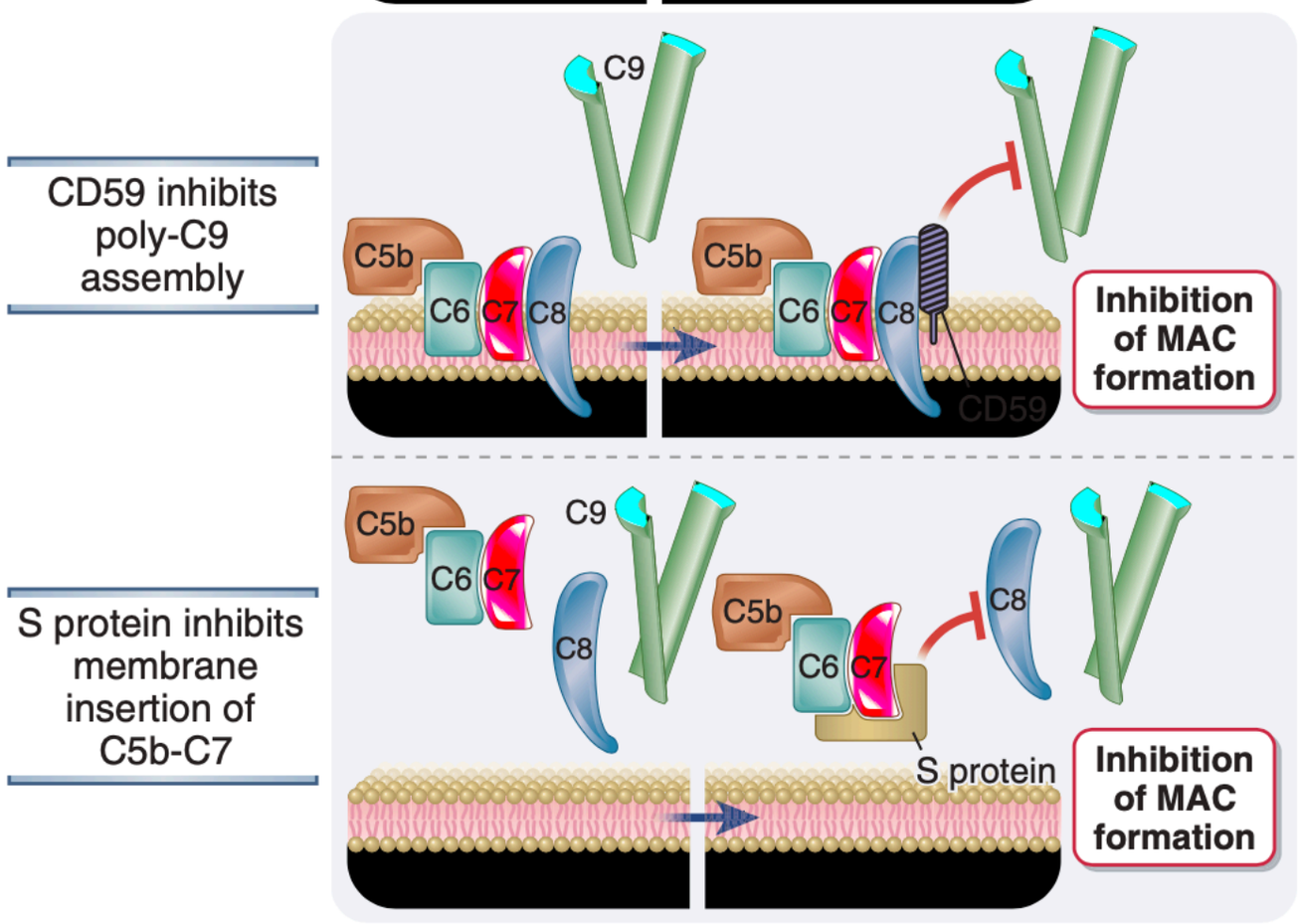


CD59 blocks the binding of C9 and MAC assembly



CD59 expressed on host cell membrane but not on microorganisms

CD59 is expressed on membrane



To avoid MAC insertion in not infected cells

S protein is expressed on cytosol

Paroxysmal nocturnal hemoglobinuria (PNH)

DAF and CD59 are GPI (glycosylphosphatidylinositol) anchored proteins.

Acquired (somatic) mutations in hematopoietic stem cells in the PIG-A gene (Phosphatidylinositol N-acetylglucosaminyltransferase subunit A) lead to the loss of GPI anchored proteins. The gene is in the X chromosome.

COMPLEMENT INDUCED LYSIS OF ERYTHROCYTES

Unregulated complement activation on the surface of erythrocytes. Recurrent intravascular hemolysis that in turn leads to chronic hemolytic anemia and venous thrombosis.

Treatment: Eculizumab inhibits the cleavage of C5 by the C5 convertase (warning: meningococcal infections, as deficit in alternative pathway)

Pentraxins

A group of structurally homologous pentameric plasma proteins

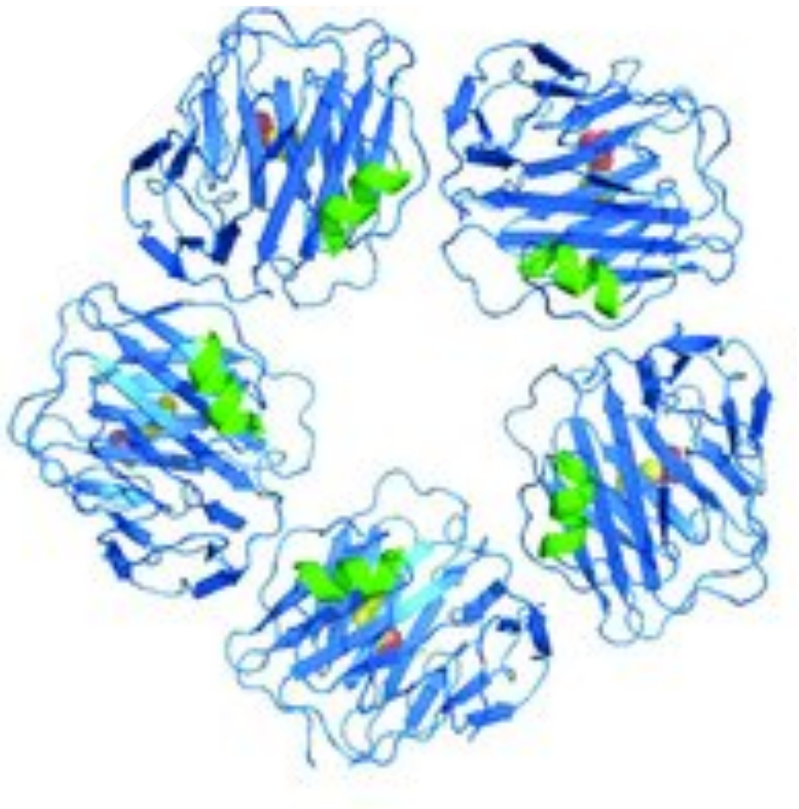
Famous examples:

- C Reactive Protein (CRP)

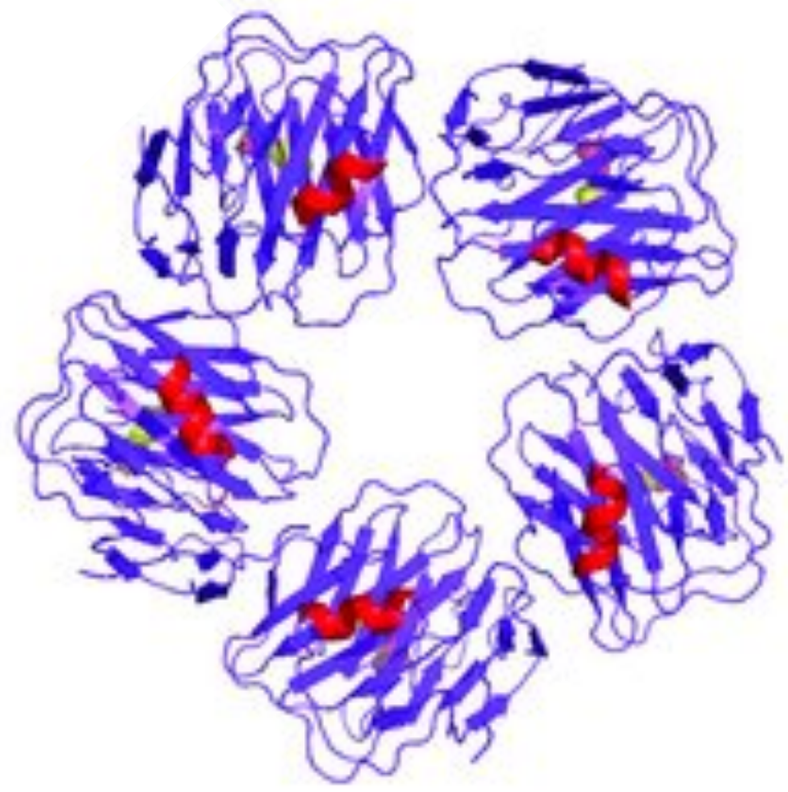
- Serum amyloid P (SAP)

- Pentraxin 3 (PTX3)

CRP



SAP



LIGANDS

Phosphorylcholine

Phosphatidylethanolamine

Bacterial membranes but also human apoptotic cells

C Reactive Protein (CRP) and SAP

Marker of inflammation!

Low levels in healthy individuals

High levels in response to inflammation (**IL-6** and **IL-1**) by Phagocytes and DCs

Produced by the **liver**

Extremely common blood test

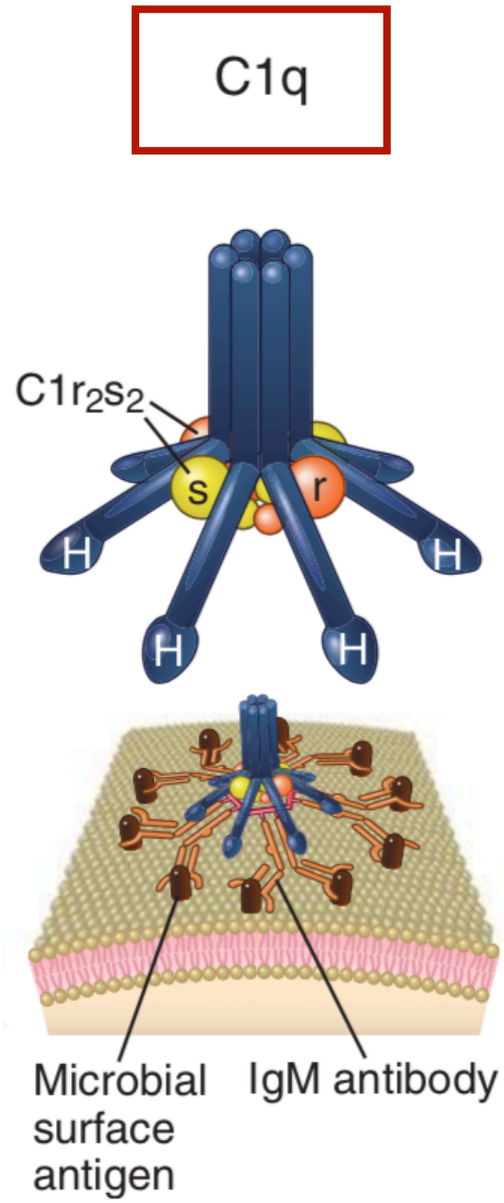
CRP, SAP (and others) are acute phase proteins

PTX3 respond to TLR activation and TNF accumulated also in granules of neutrophils

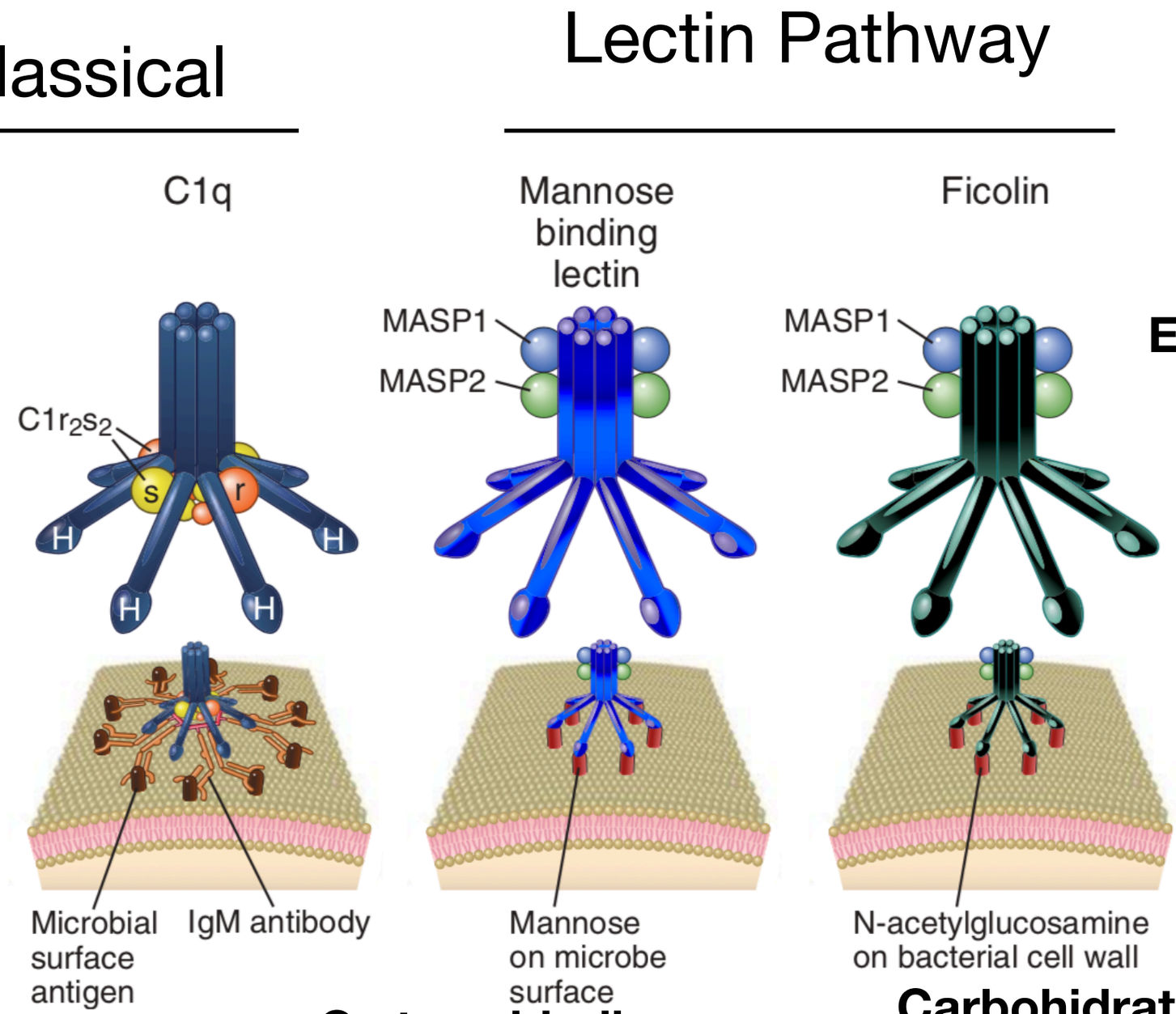
Pentraxin can initiate complement cascade

CRP, SAP and PTX3 bind to complement subunit **C1q**

C1q initiates the “**classical pathway**” of complement activation



Collectins (MBL, SP-A,SP-D) and Ficolins are structurally similar to C1q

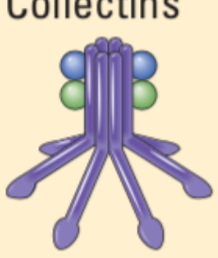
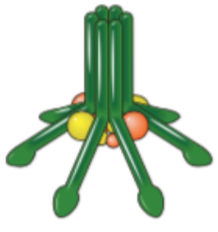


Hexameric proteins
Each subunit with collagen simil domain

Ca type binding

Carbohidrates fibrinogen like

In summary

Soluble			
Pentraxins 	Plasma	C-reactive protein	Microbial phosphorylcholine and phosphatidylethanolamine
Collectins 	Plasma	Mannose-binding lectin	Carbohydrates with terminal mannose and fructose
	Alveoli	Surfactant proteins SP-A and SP-D	Various microbial structures
Ficolins 	Plasma	Ficolin	*N*-acetylglucosamine and lipoteichoic acid components of the cell walls of gram-positive bacteria
Complement 	Plasma	Various complement proteins	Microbial surfaces