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Molecular Mechanisms of Multimeric Assembly of IgM and IgA

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Abstract

As central effectors of the adaptive immune response, immunoglobulins, or antibodies, provide essential protection from pathogens through their ability to recognize foreign antigens, aid in neutralization, and facilitate elimination from the host. Mammalian immunoglobulins can be classified into five isotypes—IgA, IgD, IgE, IgG, and IgM—each with distinct roles in mediating various aspects of the immune response. Of these isotypes, IgA and IgM are the only ones capable of multimerization, arming them with unique biological functions. Increased valency of polymeric IgA and IgM provides high avidity for binding low-affinity antigens, and their ability to be transported across the mucosal epithelium into secretions by the polymeric immunoglobulin receptor allows them to play critical roles in mucosal immunity. Here we discuss the molecular assembly, structure, and function of these multimeric antibodies.

1. INTRODUCTION

Immunoglobulin: multichain glycoprotein that specifically binds antigen and mediates adaptive immunity

Antigen: a protein, peptide, polysaccharide, or hapten molecule, often derived from a pathogen, that is recognized by an antibody

Antibody-dependent cellular cytotoxicity (ADCC): immune cell–mediated killing that requires binding and activation of an Fc receptor by an antibody-antigen immune complex

Polymeric immunoglobulin receptor (pIgR): a type I transmembrane protein that transports J chain–containing polymeric IgA and IgM across the epithelium to mucosal secretions

Transcytosis: receptor-mediated vesicular transport of macromolecules from one side of a cell to the other to cross a tissue barrier

Immunoglobulins, or antibodies, are macromolecular glycoproteins that mediate the adaptive immune response by recognizing pathogens, preventing entry into the host, facilitating neutralization, and enabling clearance and destruction. The protective nature of antibodies is due to their ability to bind both foreign antigens and cellular receptors or signaling proteins via their variable and constant regions, respectively. The immune system creates large antibody repertoires, each with unique variable region sequences, allowing specific recognition of diverse antigens, including proteins, peptides, polysaccharides, and haptens. The immunoglobulin constant regions provide binding sites for common cellular receptors and other signaling proteins, enabling them to trigger complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis, and other effector functions. Through this unique ability to bridge antigen recognition and effector function, immunoglobulins couple detection of pathogens, foreign matter, or other harmful factors with activation of signaling cascades to facilitate their removal.

Mammalian immunoglobulins can be divided into five classes, or isotypes, based on their constant domain sequences. These isotypes, named IgA, IgD, IgE, IgG, and IgM, each have a distinct structure and function (1). Depending on the isotype, immunoglobulins have membrane-bound forms that are part of the B cell receptor (BCR) and/or soluble forms found in plasma, connective tissue, and/or mucosal secretions. Moreover, the oligomeric state of the soluble forms is also isotype dependent, with some being solely monomeric while others can form higher-order oligomers.

The monomeric immunoglobulin isotypes are IgD, IgE, and IgG. IgD is found primarily on mature naive B cells prior to antigen exposure and mediates B cell activation upon encountering antigen during the primary immune response (2). IgE plays a primary role in the allergic response, recognizing allergens and triggering histamine and cytokine release from basophils and mast cells (3). It enables delivery of leukocytes, complement factors, and antibodies to sites of infection by inducing vasodilation and vascular leakage. Furthermore, IgE aids in detection and killing of parasites. IgG is the major isotype produced during the secondary immune response and is the most abundant isotype found in serum (4). It binds antigens with high affinity, activates the complement cascade, and can mediate ADCC. As the only isotype capable of crossing the placenta, IgG also provides passive immunity to the fetus.

Of the five mammalian isotypes, only IgA and IgM are able to form covalently linked oligomers, providing them with unique functions (5, 6). First, the increased valency of the oligomers allows robust binding to lower-affinity antigens through strong avidity. Second, only polymeric antibodies can be recognized and transported across cells by the polymeric immunoglobulin receptor (pIgR), a process known as transcytosis (7). This allows delivery of IgA and IgM to the mucosal epithelium, which is largely inaccessible to IgD, IgE, and IgG. Third, in the case of IgM, the oligomeric state allows robust activation of complement.

IgM is expressed on immature naive B cells and also found at significant concentrations within the blood (8). It functions predominantly in the primary immune response as the first isotype secreted by B cells in response to antigen, before significant amounts of IgG can be made. IgM binds antigens with low affinity but high avidity and potently activates complement. IgM can also be secreted to mucosal tissues, although less efficiently than IgA, where it can help mediate neutralization and exclusion of pathogens.

IgA is the predominant antibody present in tears, saliva, and mucosal secretions (9). With access to mucosal tissues lining the oral cavity, airway, digestive system, and urogenital tract, secreted polymeric IgA mediates immune exclusion of external invaders from the host. It is also secreted into colostrum, providing passivity immunity from mothers to newborns prior to development

and maturation of the neonatal immune system (10). In addition, IgA is present predominantly in the monomeric state in circulation, where it can trigger phagocytosis, respiratory burst, cytokine release, and ADCC. In humans there are two IgA isotypes, IgA1 and IgA2, with IgA1 being the predominant IgA isotype in circulation and IgA2 being the most abundant IgA isotype in secretions.

Long before antibodies could be produced recombinantly, multimeric IgA and IgM were studied due to ease of access to the naturally occurring immunoglobulins. Over the years extensive biochemical, biophysical, genetic, and structural characterizations have been performed, but high-resolution structures remained elusive. Recent breakthroughs in single-particle cryo–electron microscopy (cryo-EM) enabled structural elucidation of secretory IgA and IgM at the molecular level, shedding light on the architectural details of these multimeric immunoglobulins. Here we discuss the molecular assembly and structure and function of the multimeric immunoglobulins, IgA and IgM.

Immunoglobulin domain: globular protein fold consisting of approximately 110 amino acids forming two antiparallel β sheets; tethered by an intersheet disulfide bond

Complementarity-determining regions (CDRs): three loops within each antibody variable domain that are hypervariable in amino acid sequence and mediate antigen binding

Antigen-binding fragment (Fab): immunoglobulin fragment that binds antigen; comprises a variable domain and first constant domain of a light chain and heavy chain

2. ARCHITECTURE OF IMMUNOGLOBULIN MONOMERS

2.1. Chains

Immunoglobulin monomers are composed of four polypeptides: two identical light chains and two identical heavy chains that assemble together into canonical Y-shaped macromolecules (**Figure 1*a***). Each heavy chain heterodimerizes with a light chain to form a half-antibody, and two half-antibodies homodimerize to form the immunoglobulin monomer. The light chain paired with its cognate heavy chain creates the antigen-combining region, or antigen-binding site, of the immunoglobulin. The light chains and heavy chains were originally named according to their apparent molecular masses, with the average light chain just under 25 kDa and the average heavy chain at least twice as large, approximately 50 kDa or more, depending on the isotype. Generally, the light chain and its heavy chain are covalently linked by an interchain disulfide bond, and the two heavy chains of an immunoglobulin are linked via one or more disulfides between their hinge regions.

2.2. Domains

The basic building block of the light chains and heavy chains is the immunoglobulin domain, a globular protein domain composed of approximately 110 amino acids. It forms two antiparallel β sheets, each composed of three to five strands that fold together into a β sandwich tethered by an intersheet disulfide bond. Immunoglobulin domains come in two flavors, variable and constant, and as their names suggest, these have either unique or fixed amino acid sequences. Variable domains are found at the amino terminus of both the light chain and the heavy chain and contain three hypervariable loops referred to as the complementarity-determining regions (CDRs), which are responsible for antigen recognition (**Figure 1*a***). Human light chains are composed of two immunoglobulin domains, one variable and one constant, and can be classified as either κ or λ based on their sequence. Human heavy chains contain one variable and multiple constant domains, with the number and sequences of the constant domains differing with the immunoglobulin isotype. IgA, IgD, and IgG heavy chains contain three constant domains, whereas IgE and IgM contain four (**Figure 1*b***).

2.3. Functional Fragments

The immunoglobulin monomer can be further subdivided into functional fragments, originally identified via protease cleavage of full-length immunoglobulins prior to structural characterization. The antigen-binding fragment (Fab) is composed of the variable domain plus the first constant domain from both the light chain and the heavy chain (**Figure 1*a***). Fab fragments can be

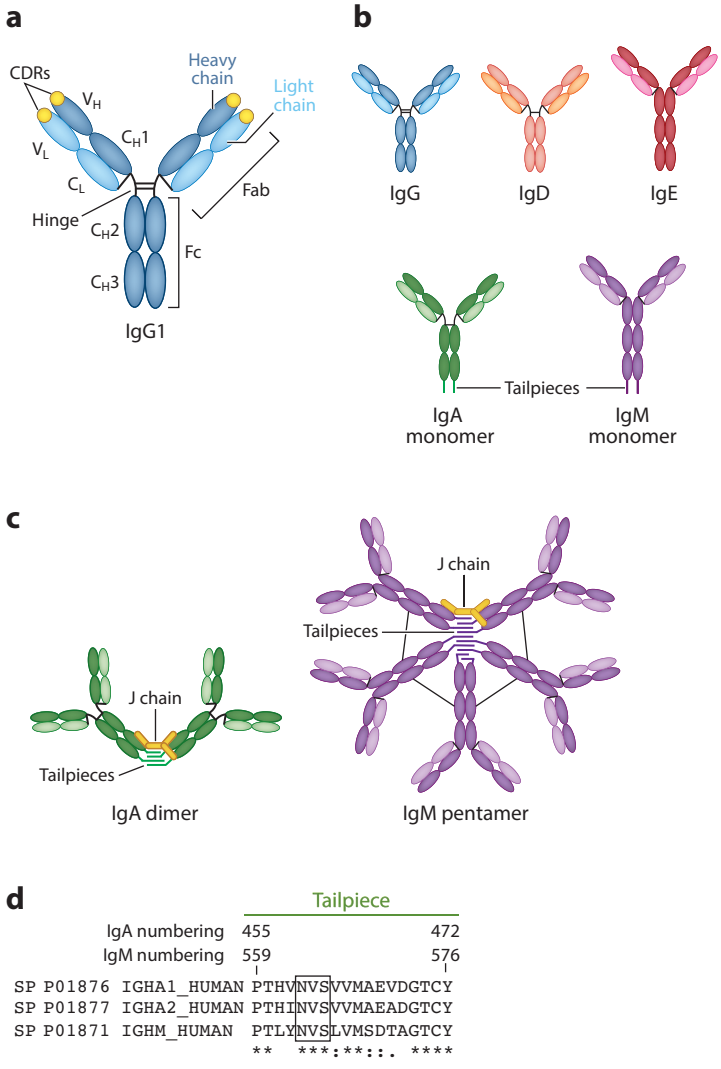


Figure 1

Architecture of the five human immunoglobulin isotypes. (*a*) Chains, domain architecture, and functional fragments of human IgG1. (*b*) Architecture of human monomeric immunoglobulins. (*c*) Architecture of human polymeric immunoglobulins containing the J chain [IgA dimer (*left*) and IgM pentamer (*right*)]. (*d*) Clustal Omega sequence alignment of human IgA1 (Uniprot P01876), IgA2m2 (Uniprot P01877), and IgM (Uniprot P01871) tailpieces. Residue numbers for both IgA and IgM are given above the sequences. Fully conserved residues (*asterisk*), highly similar residues (*colon*), and weakly similar residues (*period*) are indicated, and an N-linked glycosylation site is boxed. Abbreviations: CDR, complementarity-determining region; C_H, constant region heavy chain; C_L, constant region light chain; Fab, antigen-binding fragment; Fc, crystallizable fragment; V_H, variable region heavy chain; V_L, variable region light chain. Panel *d* adapted with permission from Reference 84; copyright 2020 AAAS.

produced by protease cleavage of immunoglobulins or recombinantly generated. It is the minimal self-assembling, stable fragment capable of antigen binding. The crystallizable fragment (Fc) is composed of the constant domains (two or three, depending on the immunoglobulin isotype) located carboxy-terminal to the hinge region (**Figure 1*a***). Originally named for its ability to

readily crystallize, the Fc fragment contains the binding sites for complement factor and the Fc receptors, which are key interacting proteins required for immunoglobulin effector function. Like the Fab fragment, the Fc can be produced by proteolytic cleavage of immunoglobulins or expressed recombinantly.

Crystallizable fragment (Fc): immunoglobulin heavy chain fragment that readily crystallizes; comprises constant domains below the hinge that bind and activate complement and/or receptors

3. POLYMERIC IMMUNOGLOBULIN STRUCTURE AND FUNCTION

Before proteins could be produced recombinantly, immunoglobulins were extensively studied due to their abundance and relative ease of isolation from naturally occurring sources. IgA and IgM in particular have undergone extensive biochemical and biophysical characterization, as they can be readily purified from serum, mucosal secretions, colostrum, and myeloma cell lines. Early characterization of these isotypes helped identify their individual components and built the foundation on which later advances in structural characterization were made.

Of the five human immunoglobulin isotypes, only IgA and IgM are able to form higher-order oligomers (**Figure 1*c***). This ability to multimerize stems from two common structural features, the tailpiece segments and the joining chain (J chain), described below, which are unique to these two isotypes. The predominant oligomeric state of IgA is a dimer; however, higher-order oligomers, including tetramers and pentamers, have been described (11, 12). Although less abundant, the higher-order oligomers provide an avidity advantage and can thereby more potently neutralize pathogens (13). The ability of IgA to efficiently multimerize is dependent on the presence and incorporation of the J chain into the oligomer. In contrast, IgM monomers are capable of multimerizing in the presence or absence of the J chain, resulting in the formation of J chain–containing pentamers or J chain–independent hexamers (14–17). The J chain–containing multimeric immunoglobulins also incorporate an additional polypeptide known as the secretory component (SC), as described below.

Tailpiece: 18 residues at the carboxy terminus of the IgA and IgM heavy chains required for multimerization and mucosal secretion

Joining chain (J chain): a protein that nucleates IgA and IgM multimerization, links immunoglobulin monomers via disulfide bonds, and is required for mucosal secretion

Secretory component (SC): a proteolytic fragment of the pIgR that is incorporated covalently into secreted polymeric IgA and IgM, providing protection from proteases

3.1. Tailpiece Segments

Tailpiece segments are 18–amino acid carboxy-terminal extensions of the IgA and IgM heavy chains (**Figure 1*d***). Their sequence is highly conserved between these two isotypes (18) and is required for oligomerization (15, 19). The tailpieces also contain a critical penultimate cysteine that is important for covalent multimerization of IgA and IgM monomers (19).

3.2. Joining Chain

The J chain is a 137–amino acid polypeptide, first discovered as a component of secreted IgA and IgM molecules (20, 21). Originally hypothesized to be either a subunit or fragment of the SC or a light chain based on its gel filtration elution profile and fast electrophoretic mobility, the J chain was found through careful amino acid composition analysis to be a novel immunoglobulin chain containing an atypically high frequency of cysteines and acidic residues (20–22). Despite being a component of secreted IgA and IgM, the J chain is not composed of immunoglobulin domains. It shows no sequence homology to other immunoglobulin chains or any other proteins of known structure. The unique amino acid composition of the J chain includes eight cysteine residues important for its function in mediating IgA and IgM multimeric assembly (20, 21).

The J chain was named for its ability to join IgA and IgM monomers into higher-order oligomers (20, 21). Early biochemical studies demonstrated that a single copy is covalently linked to multiple IgA or IgM monomers via disulfide bonds with the penultimate cysteines of the tailpiece segments (12, 23–27). While mild reducing conditions were sufficient to free the light chain from secreted IgA and IgM, much more stringent reducing and denaturing conditions were

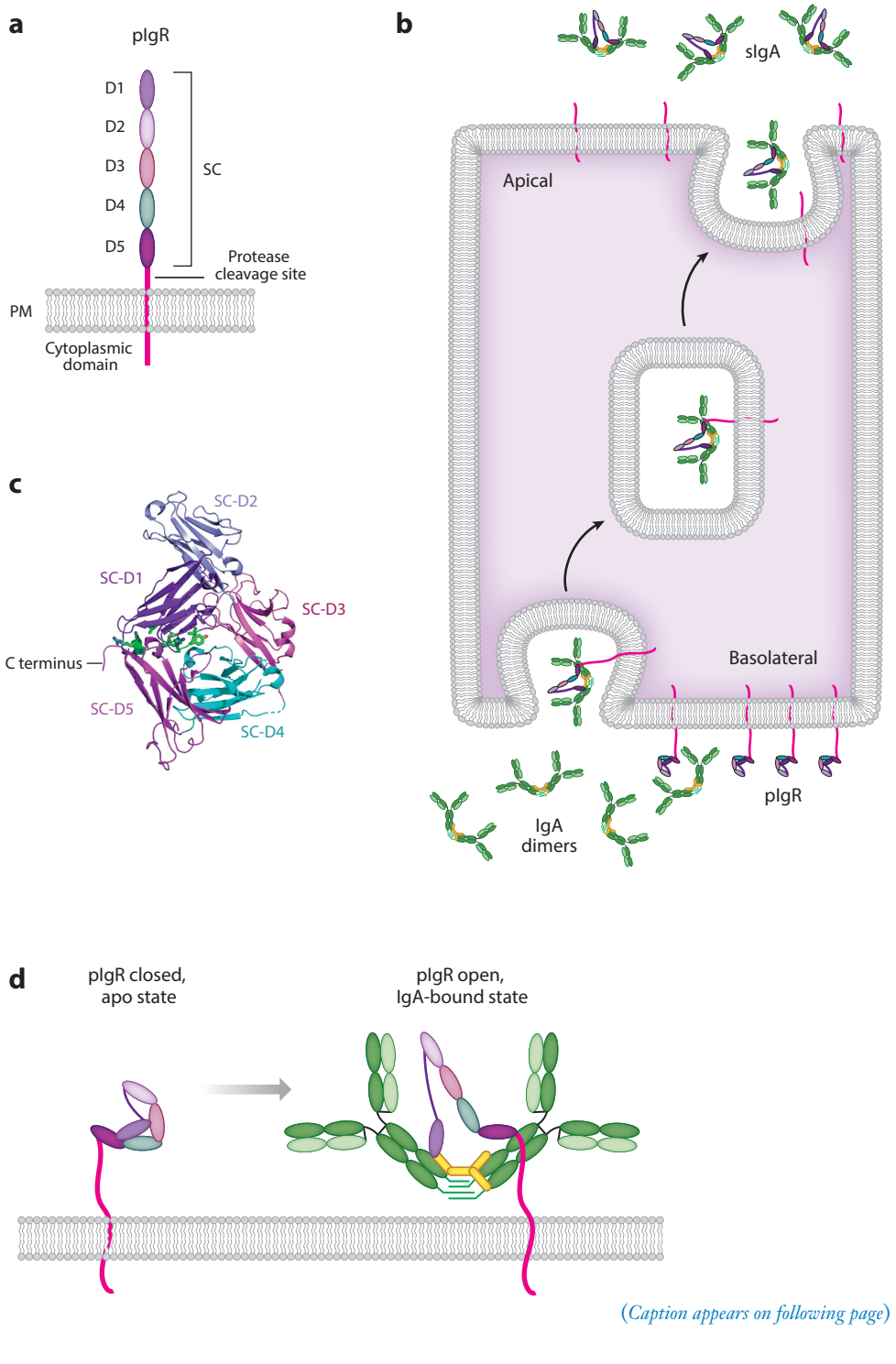
required to release the J chain, suggesting it to be an integral component of these immunoglobulins (20). Furthermore, biochemical analysis of IgA tetramers demonstrated dissociation into two monomers plus a J chain–containing dimer core, suggesting stable association of the J chain with just two IgA monomers (25, 28). Similarly, IgM pentamers could be dissociated into a J chain–containing dimer core plus monomers, suggesting a common oligomerization mechanism (29). Mapping of the J chain disulfides by mass spectrometry later revealed that six of the cysteines form intramolecular disulfide bonds (C13-C101, C72-C92, and C109-C134) to stabilize the J chain fold while the remaining two cysteines (C15 and C69) form intermolecular disulfide bonds with the penultimate cysteines of one of the tailpieces from two IgA (C471) or IgM (C575) monomers (30–32). Mutational analysis demonstrated the importance of the intermolecular disulfides in stable IgA oligomer formation, as mutation of either C15 or C69 in the J chain or C471 in the tailpiece results in formation of predominantly monomeric IgA (19, 33). While the J chain forms disulfides with only one of two tailpieces of two monomers, the remaining tailpieces have been observed to form inter-tailpiece disulfides to further stabilize the higher-order oligomeric structure (32).

3.3. Secretory Component

Along with the J chain, IgA and IgM contain an additional polypeptide unique to the secreted forms of these polymeric immunoglobulins known as the SC. The unique composition of secreted immunoglobulins was first described in a study comparing the biochemical and biophysical characteristics of secreted human IgA isolated from saliva and colostrum to those of systemic IgA isolated from serum (11). Analytical ultracentrifugation experiments demonstrated that secreted IgAs exist largely as polymers whereas serum IgA is predominantly monomeric (11). Furthermore, immunological reactivity differences between secreted and serum IgA suggested the presence of a unique component of secreted IgA that was absent in both monomeric and polymeric serum IgA (11). The authors hypothesized that the glandular epithelium may selectively alter locally produced IgA by adding a unique component during transport. In this landmark paper, Tomasi and colleagues (11, pp. 101–102) concluded, "These studies indicate that the immunoglobulins present in these external secretions differ in chemical and immunological properties from those of serum. The local production of a unique type of antibody suggests the existence of an immune system characteristic of certain external secretions whose properties differ, at least in part, from those of the immunological system responsible for the production of classical circulating antibody." This unique component of secreted immunoglobulins was initially termed the transport piece (34) and later named the SC (35). Subsequent studies revealed the SC to be a proteolytic fragment of the extracellular domain of the pIgR (36–38). In addition to providing transport of polymeric immunoglobulins to mucosal secretions, the SC also protects immunoglobulins in harsh external environments from proteolytic cleavage (39, 40).

3.4. Transcytosis of Polymeric IgA and IgM

Only polymeric IgA and IgM are able to undergo transcytosis across the mucosal epithelium through their unique ability to bind to the pIgR, an interaction that requires the presence of the Cα2/Cα3 and Cμ3/Cμ4 domains, the Fc tailpieces, and the J chain for oligomerization and binding (41–48). pIgR is a type I single-pass transmembrane protein expressed on the basolateral side of epithelial cells (**Figure 2*a***). The pIgR contains a large extracellular domain composed of five immunoglobulin-like domains followed by an unstructured stalk region (7). Polymeric IgA and IgM are locally produced by plasma cells in the lamina propria (**Figure 2*b***). Upon secretion from the plasma cells, the polymeric immunoglobulins bind to the extracellular portion of the pIgR and undergo clathrin-mediated endocytosis. The pIgR-bound polymeric immunoglobulins



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Transcytosis via the pIgR. (*a*) Domain architecture of the pIgR and the SC. (*b*) Transcytosis and formation of secreted polymeric immunoglobulins at the mucosal epithelium. Polymeric immunoglobulins, such as the IgA dimer depicted, are secreted by plasma cells in the lamina propria, where they come into contact with the pIgR expressed on the basolateral surface of mucosal epithelial cells. Once the polymeric immunoglobulin binds to the pIgR, the complex undergoes transcytosis to cross to the apical side of the cell. Prior to secretion, a cleavage event occurs in the unstructured portion of the extracellular domain of the pIgR, releasing the SC, which remains covalently linked to the polymeric IgA via a disulfide bond. The covalent complex, now known as sIgA, is released into the mucosal secretions on the apical side of the epithelium. (*c*) Crystal structure of the human SC in the apo state (PDB 5D4K) in a closed conformation stabilized by a D1-D4-D5 interface. CDR-like residues of D1 involved in polymeric IgA and IgM binding are green, and their side chains are shown as sticks. (*d*) Model of the pIgR in the apo state (*left*) and the IgA-bound state (*right*). The five immunoglobulin-like domains of the pIgR adopt a closed conformation in the apo state, stabilized by the D1-D4-D5 interface, but upon polymeric IgA binding a large conformation change occurs, separating D1 and D5 by >70 Å and allowing formation of a D1-, D5-mediated bipartite interaction with the polymeric IgA. Abbreviations: CDR, complementarity-determining region; D1, domain 1; PDB, Protein Data Bank; pIgR, polymeric immunoglobulin receptor; PM, plasma membrane; SC, secretory component; sIgA, secretory IgA. Panel *c* adapted with permission from Reference 84; copyright 2020 AAAS.

transcytose across the epithelial cell layer and are subsequently released at the apical surface on the luminal side. During transcytosis, a disulfide bond forms between the polymeric immunoglobulin and pIgR, covalently linking the two molecules (49, 50). Proteolytic cleavage of the extracellular stalk region of pIgR then releases the SC of the receptor, which remains covalently associated with the polymeric immunoglobulin. These secreted forms of IgA and IgM are thus referred to as secretory IgA (sIgA) and secretory IgM (sIgM).

Secretory IgA (sIgA): J chain–containing IgA dimer, tetramer, or pentamer that has incorporated the SC and has undergone transcytosis to mucosal secretions

Secretory IgM (sIgM): J chain–containing IgM pentamer that has incorporated the SC and has undergone transcytosis to mucosal secretions

Negative-stain EM: technique where proteins stained with heavy metal salts are subjected to an electron beam for low-resolution (~15 Å) structural analysis

4. EARLY STRUCTURAL CHARACTERIZATION OF MULTIMERIC IMMUNOGLOBULINS

4.1. Low-Resolution Structures of Native Polymeric IgA and IgM

With their natural abundance and relative ease of isolation, polymeric IgA and IgM were extensively used in early structural studies of immunoglobulins. Because they are large, polymeric IgA and IgM have been particularly amenable to structural analysis by electron microscopy (EM).

Early negative-stain EM images of polymeric IgM revealed spider-like structures with five legs around a central ring, demonstrating the pentameric nature of the secreted form of IgM (51, 52). Additional studies revealed the legs to be Y-shaped molecules linked together at their base, with the antigen-binding regions at the tips of the two arms of each Y (53). Upon antigen binding, these arms were capable of adopting bent conformations to grasp the antigen, resulting in a staple-like structure (53). Solution studies on IgM in the absence of antigen confirmed a stellate structure (54), suggesting a large conformational change occurring upon antigen binding to form the staple-like structure described by Feinstein & Munn (53). Cryo–atomic force microscopy (cryo-AFM) has also been used to study IgM, confirming its pentameric assembly (55). More recently, cryo–electron tomography (cryo-ET) studies on IgM bound to antigen further confirmed the staple- or dome-shaped conformation of both pentameric and hexameric IgM and provided new insight into the binding and activation of complement factors C1 and C4b (56).

Early EM images of IgA dimers revealed their double-Y architecture. Similar to IgM, the Y-shaped IgA monomers are linked at their base, tail-to-tail, with the two arms of each monomer capable of binding antigen (57–59). Later studies using high-speed AFM further revealed the architecture of higher-order sIgA oligomers, such as tetramers (13). X-ray and neutron scattering along with analytical ultracentrifugation have also been used to study the solution structures of

human SC, monomeric IgA1, monomeric IgA2, dimeric IgA1, dimeric sIgA1, and dimeric sIgA2 at low to medium resolution (60–66). These studies led to models of end-to-end dimers for both IgA and sIgA, with the two Fcs bent slightly toward each other, independent of SC binding, and suggested that the SC binds in an extended conformation along the convex edge of the Fcs.

4.2. High-Resolution Structures of Individual Components of Secretory IgA and IgM

Over the years as recombinant protein production became feasible and technical advances in structural biology methods such as X-ray crystallography and nuclear magnetic resonance (NMR) were achieved, atomic-level resolution structures of individual domains or fragments of immunoglobulins were obtained, providing insight into the architecture of immunoglobulin substructures. A crystal structure of the IgA1 Fc fragment in complex with the Fcα receptor I (FcαRI or CD89) demonstrated the structural organization of the constant domains of the IgA Fc along with the interface involved in receptor binding (67). Similarly, a second crystal structure of the IgA1 Fc in complex with the *Staphylococcus aureus* superantigen-like protein SSL7 demonstrated how this pathogen can facilitate immune evasion by competing for FcαRI binding to IgA (68). For IgM, X-ray crystal structures and NMR solution structures of the individual constant domains of the Fc were also obtained (69). However, none of these Fc fragment structures contained the tailpieces, leaving their structures unresolved.

In contrast to the immunoglobulin domains of the Fcs, the J chain by itself has been recalcitrant to structural determination, suggesting its fold may be dependent on its interaction with the IgA and IgM monomers. In the absence of structure, many models have been proposed for the J chain based on secondary-structure predictions and disulfide bond mapping (31, 32, 70–72); however, due to the lack of sequence homology to other proteins with known structure it has been difficult to build homology models of the J chain with high confidence.

The SC, on the other hand, has been quite amenable to structure-function analysis by mutagenesis, biochemical and biophysical characterization, and X-ray crystallography. The SC contains five domains (D1–D5) that share sequence homology to the immunoglobulin variable domains (**Figure 2*a***). Indeed, the first crystal structure of D1 revealed its immunoglobulin-like fold (73), and a subsequent structure of the full SC confirmed its five immunoglobulin-like domains (74) (**Figure 2*c***). Truncation, mutagenesis, and antibody competition experiments have shown that D1 is both necessary and sufficient for polymeric IgA and IgM binding (74–76) and that the SC uses two separate regions to bind IgA, making contacts with both the Cα2 and Cα3 domains of the Fc (77, 78). D1 contains three CDR-like loops that are critical for the interaction with the polymeric immunoglobulins (76, 79–81), whereas D5 forms a covalent disulfide bond with Cys311 in the Cα2 domain of the IgA Fc (50).

The crystal structure of the full-length SC revealed an unexpected closed conformation for the apo state of this polypeptide (74) (**Figure 2*c***). The SC folds back on itself, adopting a triangular conformation that creates a significant interface between D1, D4, and D5, with 1,480 Å^2 of total buried surface area. This closed conformation partially buries the CDR-like loops of D1 (**Figure 2*c***) and suggests a functional role for protecting these critical polymeric immunoglobulin-binding loops from proteases. Double electron-electron resonance (DEER) spectroscopy experiments confirmed close association of D1 and D5 of the apo SC in solution, consistent with the closed state observed in the crystal structure, whereas separation of these domains by more than 70 Å was measured upon polymeric IgA and IgM binding. This suggests a large conformational change in pIgR is required for polymeric immunoglobulin binding and formation of sIgA and sIgM (74) (**Figure 2*d***).

5. RECENT ADVANCES IN STRUCTURAL CHARACTERIZATION OF MULTIMERIC IMMUNOGLOBULINS BY CRYO–ELECTRON MICROSCOPY

With the considerable progress made in structural characterization of individual components of the polymeric immunoglobulins, researchers no doubt focused their efforts on characterization of fully assembled IgA and IgM multimers. Despite the best efforts of many labs, these large, highly glycosylated oligomers proved to be recalcitrant to crystallization and X-ray diffraction, with atomic-level resolution structures of assembled polymeric immunoglobulins remaining elusive for many years. It would take significant technical advances, not only in recombinant protein production but also in single-particle cryo-EM (see the sidebar titled Single-Particle Cryo–Electron Microscopy), to reveal the big picture of higher-order multimeric assembly of IgA and IgM.

5.1. Characterization of Recombinant Polymeric IgA and IgM by Negative-Stain Electron Microscopy

With advances in recombinant immunoglobulin production along with improvements in EM, recent negative-stain EM studies provided low-resolution structural information about polymeric IgA (82) and IgM (83). Results of these structural studies with recombinant immunoglobulins were consistent with early EM studies of immunoglobulins isolated from natural sources, confirming the overall oligomeric architecture of the polymeric immunoglobulins. They also provided new insight, revealing details that were previously unappreciated. For IgM, the EM studies revealed an asymmetric pentameric assembly in the presence of the J chain, with a gap of approximately 40° where a sixth monomer would be expected to fit to form a symmetric hexamer (83). Prior to this, the IgM pentamer was thought to form a regular pentagon, as often depicted in traditional drawings of IgM. For IgA, these structural studies revealed that the oligomeric state of J chain–containing IgA could be controlled simply by altering the relative expression levels of the individual chains (82). When the amount of J chain–encoding DNA used in transient transfections was in excess relative to heavy chain–encoding DNA, this resulted in formation of predominantly dimeric IgA, confirmed by negative-stain EM. In contrast, when the relative amount of J chain–encoding DNA was decreased and the heavy chain–encoding DNA

SINGLE-PARTICLE CRYO–ELECTRON MICROSCOPY

Single-particle cryo–electron microscopy (cryo-EM) is a structural biology technique used to determine the high-resolution structure of macromolecular complexes (100, 101). Cryo-EM is a complementary method to X-ray crystallography that does not require formation of an ordered crystalline lattice. Rather, proteins of interest are frozen under cryogenic conditions in a thin layer of vitreous ice in random orientations and subjected to a focused beam of electrons to produce 2D projection images of individual protein particles. To boost signal-to-noise, thousands of projection images of identically oriented particles are computationally averaged together to form a 2D class average for a given view of the protein. By obtaining 2D class averages of the protein in all possible orientations in space, these can then be computationally back-projected to determine the protein's 3D structure. In recent years improvements in both hardware and software have significantly increased the achievable resolution of cryo-EM structures, leading to the so-called resolution revolution in the field of structural biology (102–104). With this has come an explosion in the number of atomic-level resolution cryo-EM structures, providing new insights into the architecture of macromolecular complexes such as the polymeric immunoglobulins, which previously were refractory to structure determination by more traditional methods.

was increased, this resulted in an increased proportion of higher-order oligomers, in particular tetramers, relative to dimers. Furthermore, these studies provided clues as to regions of the immunoglobulins that were too flexible for high-resolution structure determination. In particular, significant flexibility of the Fab arms in both IgM (83) and IgA (82) suggested that limiting the recombinant protein constructs to the Fc, tailpieces, and J chain core region of the polymeric immunoglobulins could be a successful strategy for high-resolution structure determination.

5.2. High-Resolution Structures of Secretory IgA

The first atomic-level resolution structures of sIgA, all determined by cryo-EM, were reported in 2020 by three independent groups (**Figure 3**). Two of these studies focused on human sIgA (84, 85) and one on murine sIgA (86). These structures provided complementary insights into the molecular determinants of IgA multimerization.

Kumar et al. (84) determined the cryo-EM structures of the human sIgA1 dimer, sIgA2m2 tetramer, and sIgA2m2 pentamer to 2.9-, 3.0-, and 3.0-Å resolution, respectively (**Figure 3**). Wang et al. (85) determined the cryo-EM structures of the human sIgA1 dimer alone and in complex with the *Streptococcus pneumoniae* sIgA-binding protein (SpsA) to 3.2- and 3.3-Å resolution, respectively. Both studies used recombinant constructs containing the IgA Fc (Cα2 and Cα3 domains) plus the tailpieces, the J chain, and the SC. The two independently determined structures of the sIgA1 dimer are in excellent agreement, with a global root-mean-square deviation (rmsd) of only 1.6 Å (85). Kumar Bharathkar et al. (86) determined the cryo-EM structures of the murine IgA dimer and sIgA dimer, both to 3.3-Å resolution. The constructs used in these studies encoded full-length murine IgA including the light chain; however, density for the Fab arms was poorly resolved, indicating significant flexibility of these regions relative to the IgA Fc–J chain–SC core. This flexibility of the antigen-binding regions was consistent with previous low-resolution negative-stain EM images of full-length, recombinant human IgA where the Fabs appeared to adopt different orientations in 2D class averages, relative to the Fc core (82). The overall structures of human and murine sIgA dimers are superimposable, despite sequence differences between species of the individual components, with the Fc, tailpieces, J chain, and SC sharing 65–77% sequence identity (84–86).

The IgA structures confirmed the tail-to-tail linking of the IgA monomers, as originally observed nearly 50 years ago in the first EM studies (57–59), and validated the role of the J chain as a clasp linking two monomers together via their tailpieces, as previously suggested from extensive biochemical characterization (28). The structures also confirmed a significant bend between the two Fcs in the dimer (**Figure 3*a***), as suggested by previous solution scattering studies of dimeric IgA and sIgA (63–65). This bend is independent of the SC binding, as illustrated by the structures of the murine IgA dimer where the ∼97–98° bend is maintained in the presence and the absence of the SC (86). This suggests that the J chain, and not the SC, is responsible for holding the two Fcs in this bent conformation. The structures also identified a tilt between the planes of the two Fcs. In the murine IgA dimer the tilt between them is 19°, whereas in sIgA the tilt increases to 30° (86). This suggests that in contrast to having no effect on the bend between the two Fcs, the SC binding to IgA induces a change in the relative tilt of the two Fcs.

Excitingly, for the very first time the fold of the J chain was observed, and in the IgA-bound state it was found to be composed of a small β-sandwich core with three protruding β hairpins or wings (**Figure 4*a*,*b***). Consistent with previous sequence analysis and secondary-structure predictions, the J chain adopts a fold unlike that of any other protein of known structure. The structure of the J chain confirmed the cysteine pairs involved in the three intramolecular disulfides (C13-C101, C72-C92, and C109-C134), as well as the two cysteines (C15 and C69) that form

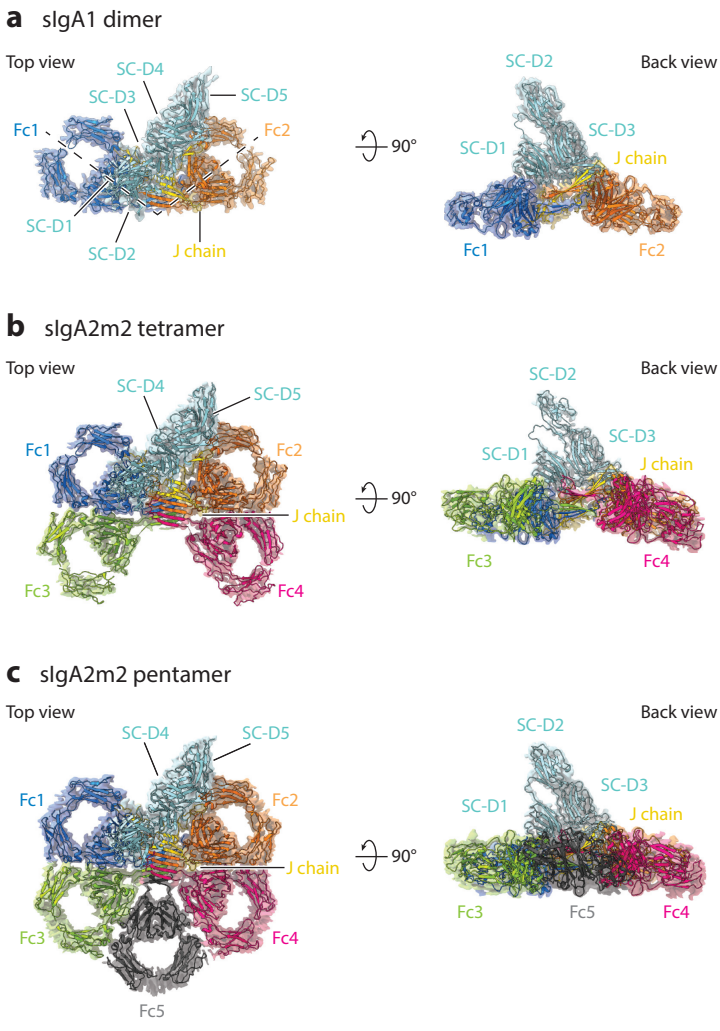


Figure 3

Cryo-EM structures of the human sIgA dimer, tetramer, and pentamer. (*a*) Top and back views of the human sIgA1 dimer (PDB 6UE7). Dashed black lines illustrate the bend between the two Fcs of the dimer. (*b*) Top and back views of the human sIgA2m2 tetramer (PDB 6UE8). (*c*) Top and back views of the human sIgA2m2 pentamer (PDB 6UEA). Transparent cryo-EM maps overlaid with the corresponding models are shown in panels *a*–*c*. Abbreviations: EM, electron microscopy; Fc, crystallizable fragment; PDB, Protein Data Bank; SC-D1, secretory component domain 1; sIgA, secretory IgA. Panels *a*–*c* adapted with permission from Reference 84; copyright 2020 AAAS.

intermolecular disulfide bonds with the penultimate cysteines (C471) of two of the tailpieces (**Figure 4*b*,*c***). The J chain is intricately intertwined with both IgA monomers of the dimer, making extensive contacts with the Cα2 and Cα3 domains as well as the tailpieces. The J chain binds two identical IgA monomers in an asymmetric manner, with β hairpins 1 and 2 binding the top of one Fc and β hairpin 3 binding to the bottom of the second Fc in the dimer, forming a pseudosymmetric dimer (**Figure 4*a***). The J chain β hairpins have predominantly hydrophobic interactions with nonpolar residues on the surface of the Fcs (**Figure 4*d***), while the tailpieces of

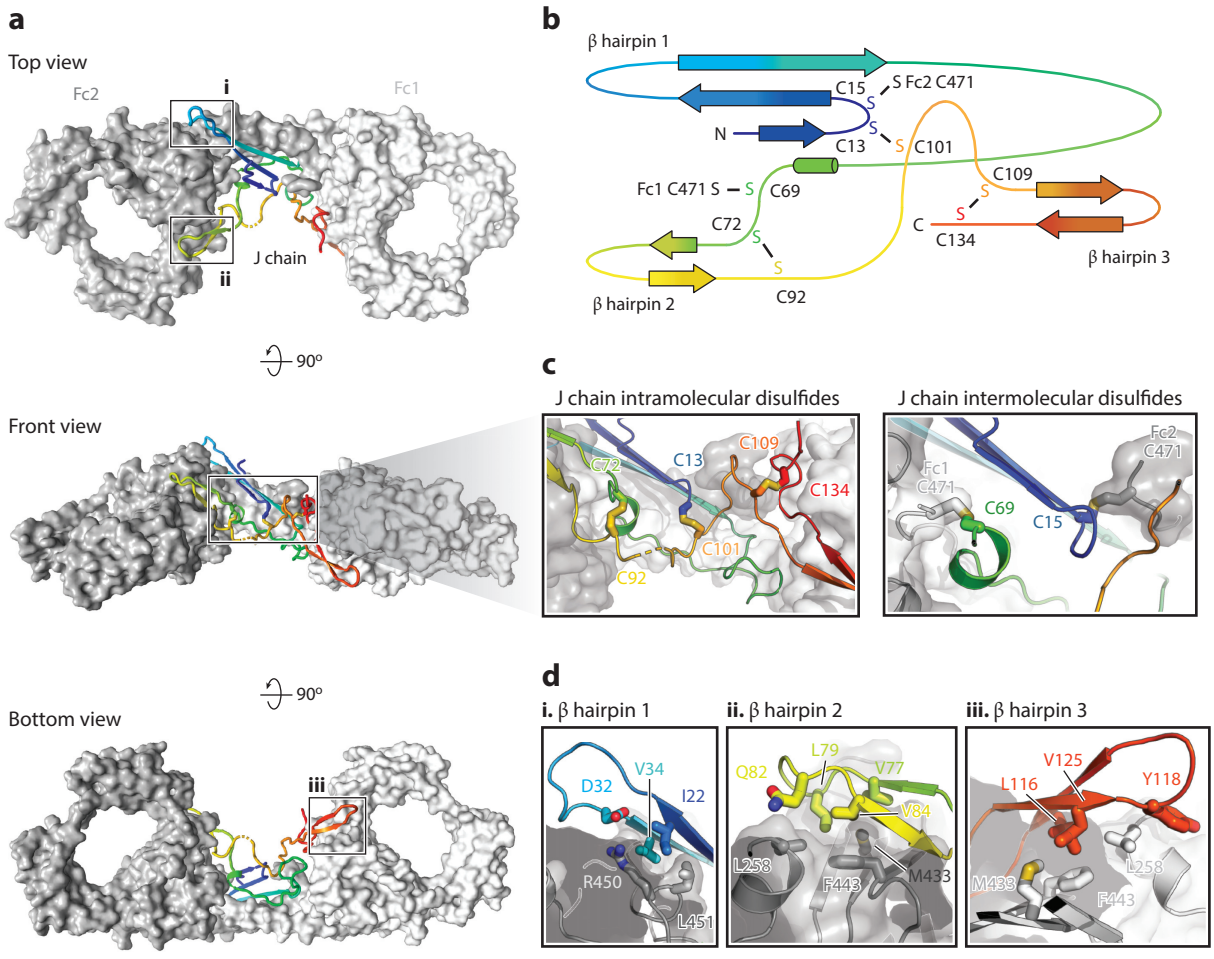


Figure 4

Structure of the J chain. (*a*) Top, front, and bottom views of the human J chain in the sIgA1 dimer (PDB 6UE7) with the SC removed for clarity. The J chain is in rainbow-sequenced colors, from the N terminus (*blue*) to the C terminus (*red*), and is rendered as a ribbon diagram. The IgA Fcs are white and gray and shown in surface representation. (*b*) Topology diagram of the human J chain in rainbow-sequenced colors, from the N terminus (*blue*) to the C terminus (*red*), with the three intramolecular and two intermolecular disulfide bonds shown. (*c*) Close-up of the box in the front view in panel *a* showing the intramolecular and intermolecular disulfide bonds of the J chain as sticks and with the corresponding cysteine residues labeled. (*d*) Close-ups of boxes *i*, *ii*, and *iii* in panel *a* showing the interaction of J-chain β hairpins 1, 2, and 3, respectively, with the IgA Fcs. Residues involved in binding are labeled and their side chains shown as sticks. Abbreviations: Fc, crystallizable fragment; PDB, Protein Data Bank; SC, secretory component; sIgA, secretory IgA. Panels *a–d* adapted with permission from Reference 84; copyright 2020 AAAS.

the Fcs form extensive main-chain hydrogen bonds with the J-chain core to extend its β sandwich (**Figure 5**). This extensive interaction of the J chain with the two IgA monomers leads to an impressive ~3,600-Å^2 of buried surface area at the interfaces (**Figure 4*a***).

5.3. Molecular Mechanisms of IgA Multimerization

The structures of the IgA dimers gave initial insight into the mechanism of multimerization (84–86). For the first time the structures of the IgA tailpieces were observed, with these 18–amino

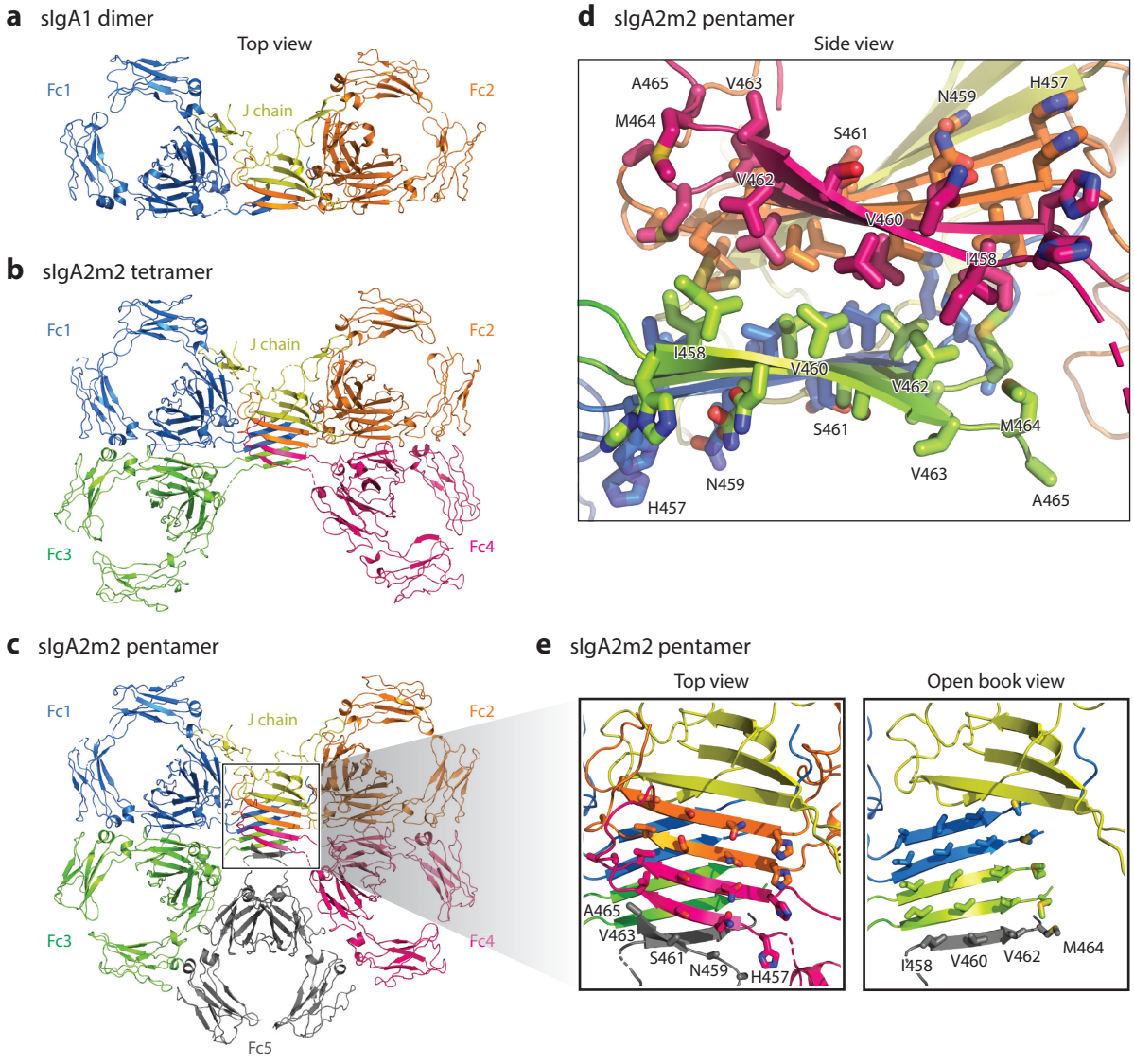


Figure 5

J chain–mediated oligomerization of polymeric IgA. (*a*) Top view of the human sIgA1 dimer (PDB 6UE7) with the SC removed for clarity. (*b*) Top view of the human sIgA2m2 tetramer (PDB 6UE8) with the SC removed for clarity. (*c*) Top view of the human sIgA2m2 pentamer (PDB 6UEA) with the SC removed for clarity. (*d*) Side view of the boxed region in panel *c*. Fc5 is removed for clarity to show a cross section of the central β sandwich with side chains of the tailpiece residues shown as sticks and labeled. (*e*) Top and open book views of the boxed region in panel *c*. Side chains of the tailpiece residues are shown as sticks and labeled. Abbreviations: Fc, crystallizable fragment; PDB, Protein Data Bank; SC, secretory component; sIgA, secretory IgA. Panels *a*, *b*, and *d* adapted with permission from Reference 84; copyright 2020 AAAS. Panels *c* and *e* adapted with permission from Reference 84, copyright 2020 AAAS; and Reference 91, copyright 2021 Cell Press.

acid extensions of the heavy chains forming two parallel β strands (**Figure 5**). In the dimer, the tailpieces extend the small β sandwich of the J chain, with one Fc contributing its two tailpieces to the extension of the top β sheet and the second Fc contributing its two tailpieces to the bottom β sheet (**Figure 5*a***). This results in formation of a slightly twisted central β-sandwich core, with

the strands of each sheet running in opposing directions. Thus, the J chain acts as a template for Fc tailpiece incorporation to facilitate formation of IgA oligomers.

These structures of different oligomeric states of IgA reveal nature's remarkably simple yet elegant solution for immunoglobulin multimerization. Comparison of the structures of the sIgA1 dimer (**Figure 5*a***) with those of the sIgA2m2 tetramer (**Figure 5*b***) and sIgA2m2 pentamer (**Figure 5*c***) reveals a structurally conserved dimer core present in the higher-order oligomers (84). Simple addition of two more Fcs to the dimer core results in the formation of the IgA tetramer. The third and fourth Fcs of the tetramer are incorporated through further extension of the central β sandwich of the dimer, again by each monomer adding two parallel β-strand tailpieces to either the top or bottom of the sandwich (**Figure 5*b***). The incorporation of the two additional Fcs results in Fc-mediated contacts at the Cα2-Cα3 junction between two adjacent monomers, with a gap large enough to fit a fifth Fc opposite the J chain. The Fc-mediated contacts are noncovalent and include both hydrophobic and polar interactions. In the IgA pentamer the fifth Fc fills that gap but breaks the pattern of the first four Fcs by adding one tailpiece to the top β sheet and one to the bottom, capping any further extension of the β sandwich (**Figure 5*c***). This capping, along with limited space around the central β sandwich, makes the pentamer the largest J chain–containing oligomer that can be formed by IgA.

The tailpieces of each Fc monomer are composed of identical sequences. Once they are incorporated into parallel β sheets to form the β sandwich, the result is a repeating array of identical residues on both halves of the sandwich that run in opposing directions (**Figure 5*d*,*e***). The tailpiece sequences contain a stretch of alternating polar and hydrophobic residues (**Figure 1*d***), a hallmark of β sandwiches. Once assembled, the center of the sandwich contains hydrophobic residues, such as isoleucine, valine, and methionine, whereas the outer sheet is lined with predominantly polar residues, such as histidine, asparagine, and serine. The hydrophobic, β-branched side chains of valine and isoleucine at the center of the sandwich interlock with each other, like the teeth of a zipper, to glue the oligomers together.

While the structures provide visualization of the final assembled product, exactly how this occurs in cells is still relatively poorly understood. Identification of the marginal zone B cell– and B1 cell–specific protein (MZB1, also known as pERp1) as a chaperone involved in IgA and IgM assembly and secretion (87–89) suggests that multimerization does not occur spontaneously but rather requires assistance from other proteins, which provide potential points of regulation. MZB1 interacts noncovalently with the penultimate cysteine in the IgA tailpieces prior to association of the Fcs with the J chain, thereby stabilizing this intermediate (89). Through an unknown mechanism, MZB1 promotes J chain binding and dimer formation to facilitate polymeric IgA assembly and secretion from plasma cells.

5.4. Structure of the Secretory Component

The structures of human and murine sIgA also revealed the mode of binding of the SC to polymeric IgA (**Figures 3** and **6**). In contrast to the closed conformation observed in the apo state (74) (**Figure 2*c***), the IgA-bound SC adopts an extended, bent conformation in all sIgA structures, binding out of the plane of the Fcs (84–86) (**Figures 3** and **6*a***). The D1-D4-D5 interface seen in the apo state is disrupted, freeing D1 to use all three of its CDR-like loops to engage in extensive interactions with polymeric IgA, consistent with previous mutagenesis studies of these loops (**Figure 6*a***) (76). In the IgA-bound state, D2 through D5 are arranged in a linear manner. A sharp bend in the linker between D1 and D2 allows a nearly 180° turn, such that D1 reverses direction and forms a back-to-back interface with D3. This extended, bent conformation of the SC allows a bipartite interaction across the dimer core, with D1 making extensive contacts with the first Fc,

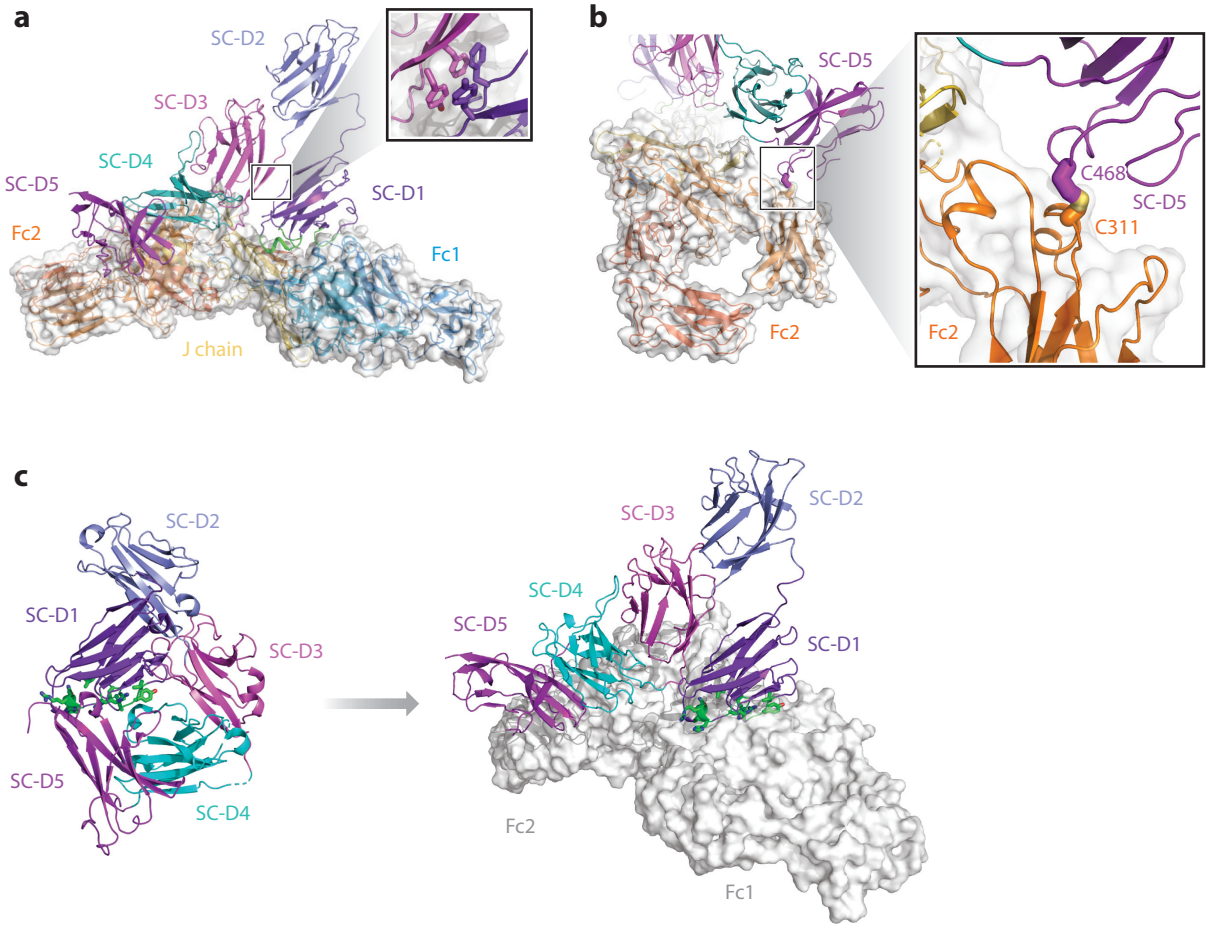


Figure 6

Polymeric immunoglobulin receptor binding and formation of sIgA. (*a*) Structure of the SC shown in the context of the human sIgA1 dimer (PDB 6UE7). The IgA dimer is rendered as a transparent surface with a ribbon diagram model underneath and the SC shown as a ribbon diagram with its five immunoglobulin-like domains labeled. The three CDR-like loops of SC-D1 are green. Magnification of the aromatic-rich interface between SC-D1 and SC-D3 is shown in the inset. (*b*) Interaction of SC-D5 with Fc2 of the human sIgA1 dimer (PDB 6UE7). The disulfide bond between C468 of SC-D5 and C311 of Fc2 is magnified in the inset. (*c*) Structure of the human SC in the apo state (PDB 5D4K) in a closed conformation stabilized by a D1-D4-D5 interface (*left*). CDR-like residues of D1 involved in polymeric IgA and IgM binding are green, and their side chains are shown as sticks. A significant conformational change is required for transition from the closed conformation of the apo SC (*left*) to the open conformation of the polymeric immunoglobulin–bound state (*right*), as exemplified by the human sIgA1 dimer (PDB 6UE7). The IgA dimer is shown in surface representation and is white. The SC is a ribbon diagram with its five immunoglobulin-like domains labeled. Abbreviations: CDR, complementarity-determining regions; D1, domain 1; Fc, crystallizable fragment; PDB, Protein Data Bank; SC, secretory component; sIgA, secretory IgA. Panels *a–c* adapted with permission from Reference 84; copyright 2020 AAAS.

the J chain, and the tailpieces of the second Fc and D5 forming a disulfide bond between the C468 and C311 of the second Fc (**Figure 6*b***). The large differences in SC structure between the apo and bound states indicate that a conformational change in pIgR must occur upon polymeric IgA binding (**Figure 6*c***), consistent with previous DEER experiments demonstrating a large separation of D1 and D5 when the SC is bound to polymeric IgA (74). Exactly how this conformational change is triggered remains unclear.

5.5. High-Resolution Structures of Secretory IgM

The first atomic-level resolution structures of human sIgM were also determined by cryo-EM, and reported by two independent groups within the same time frame as the sIgA structures (90, 91). These studies provided complementary insights into the molecular determinants of IgM multimerization. Furthermore, comparison of the structures with that of sIgA reveals the similarities and differences between these two polymeric immunoglobulins.

Li et al. (90) and Kumar et al. (91) determined the cryo-EM structures of human sIgM to 3.4- and 3.3-Å resolution, respectively (**Figure 7*a***). Both studies used recombinant constructs expressing the IgM Fc (Cμ2, Cμ3, and Cμ4 domains) plus the tailpieces, the J chain, and the SC, and the two independently determined structures are in very close agreement. The overall structure of sIgM is an asymmetric pentamer (**Figure 7*a***), highly reminiscent of the architecture of the sIgA pentamer (**Figure 3*c***). The five Fcs are arranged radially around a central β-sandwich core composed of the Fc tailpieces and the J chain, with the J chain occupying the site where a sixth Fc would be expected to form a regular hexagon. Adjacent Fcs make contact with each other through the Cμ3 and Cμ4 domains, and unlike the case of IgA this includes a covalent disulfide bond via C414. Density for the Cμ2 domains of the Fcs was poorly resolved in both structures, suggesting significant flexibility of this region relative to the Cμ3 and Cμ4 domains. This flexibility is consistent with the negative-stain images of IgM reported by Hiramoto et al. (83), where the N-terminal tip of the Fc was blurry relative to the rest of the Fc.

The IgM-bound J chain adopts the same fold observed in all of the sIgA structures (84–86), with an rmsd of only ~1.3 Å; however β hairpin 2 (residues 71–98) is disordered in both sIgM structures, for reasons that are not readily apparent (**Figures 7*b*,*c***). Interestingly, this difference in β hairpin 2 conformation is consistent with increased protease sensitivity of the IgM-incorporated J chain compared to the IgA-incorporated J chain (92). Cleavage of IgM with subtilisin produced a J chain fragment containing the amino-terminal ~67 residues, which remained associated with the IgM Fc, suggesting the cleavage site is near residue 67 of the J chain, which is at the start of β hairpin 2 (92).

The SC binds the J chain–containing IgM pentamer in the same manner as the polymeric IgA, bridging across the two Fcs directly linked by the J chain (**Figures 3** and **7*a***). It adopts the extended yet bent conformation orienting D1 to make contact through its three CDR-like loops with the first Fc, tailpieces of the second Fc, and the J chain, while D5 is in close proximity to the second Fc, with C468 of D5 adjacent to C414 of Cμ3 of the Fc. Partial density for the disulfide was observed in one of the structures, suggesting a mixture of oxidized and reduced complexes in the recombinant sample (91).

5.6. Comparison of Secretory IgA and IgM Structures

The IgA and IgM Fcs (Cα2 and Cα3 versus Cμ3 and Cμ4) and tailpieces contain only 40% sequence identity, yet both assemble with the same J chain and SC. Comparison of the structures reveals many similarities between these two immunoglobulins. While the identities of the Fc residues that interact with the β hairpins of the J chain are not absolutely conserved between IgA and IgM, the hydrophobic nature of the side chains is similar, allowing the interactions to be conserved for β hairpins 1 and 3 (91) (**Figure 7*d***). The properties of the surface residues of IgA that contact β hairpin 2 are also conserved in IgM, making it difficult to explain why β hairpin 2 is not closely associated with the IgM Fc but rather is disordered in both IgM structures (90, 91). The Fc tailpieces are also not identical between IgA and IgM (**Figure 1*d***); however, there is high sequence similarity preserving the properties required for formation of a β strand, allowing conservation of the J chain–templated β-sheet extension mechanism of multimerization for these two

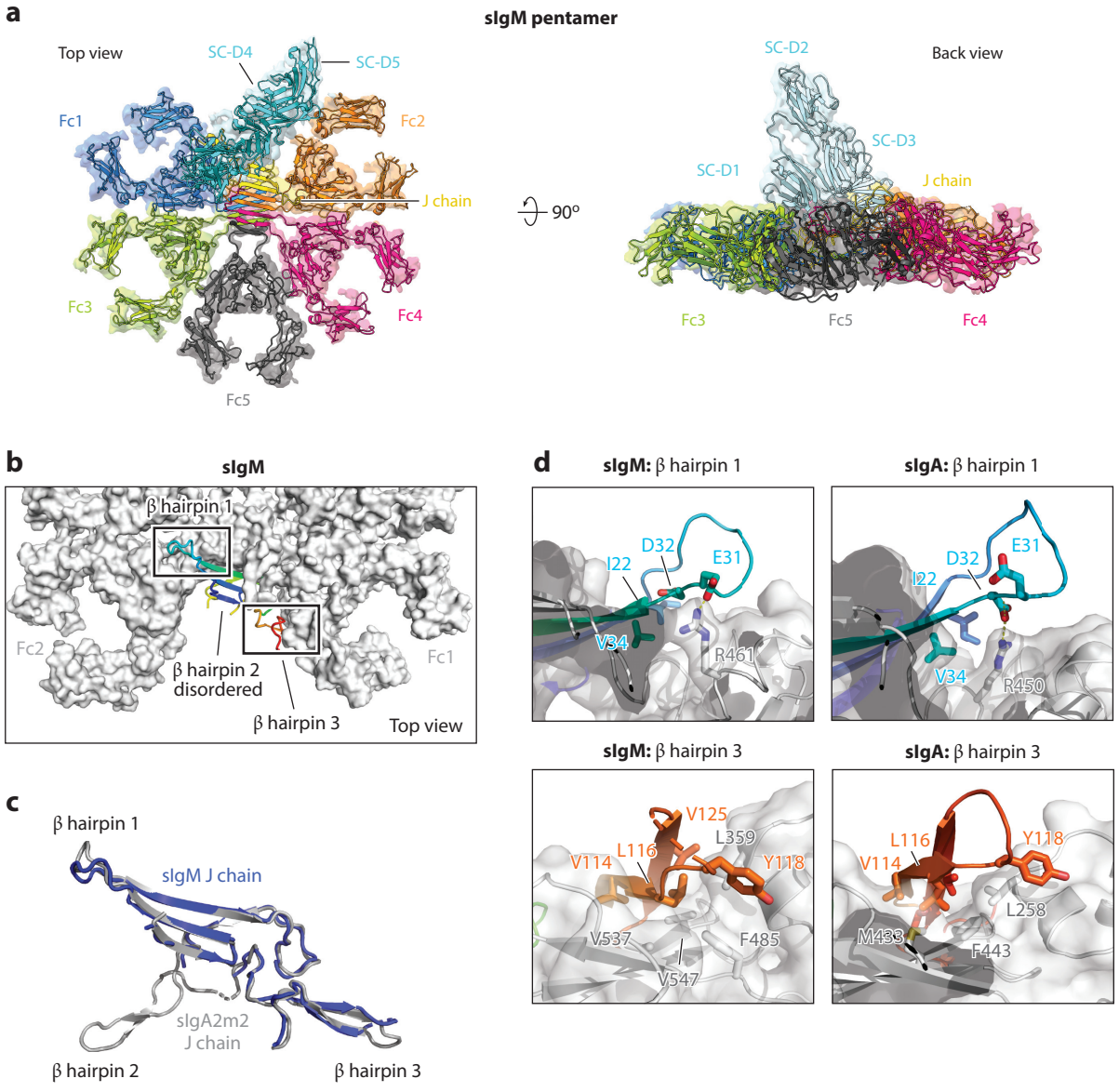


Figure 7

Cryo-EM structure of the human sIgM pentamer. (*a*) Top and back views of the human sIgM pentamer (PDB 7K0C). Transparent cryo-EM map overlaid with the corresponding model is shown. (*b*) Top view of the human J chain in the sIgM pentamer (PDB 7K0C) with the SC removed for clarity. The J chain is in rainbow-sequenced colors, from the N terminus (*blue*) to the C terminus (*red*) and is rendered as a ribbon diagram. The IgM Fcs are white and shown in surface representation. J-chain β hairpins 1 and 3 are boxed, and β hairpin 2 is disordered in the sIgM structure. (*c*) Alignment of the structure of the human J chain from the sIgA2m2 pentamer (PDB 6UEA, *gray*) and sIgM pentamer (PDB 7K0C, *blue*). (*d*) Comparison of the interactions of J-chain β hairpin 1 (*top*) and β hairpin 3 (*bottom*) with the IgM (*left*) and IgA (*right*) Fcs. Residues involved in binding are labeled and their side chains shown as sticks. Abbreviations: D1, domain 1; EM, electron microscopy; Fc, crystallizable fragment; PDB, Protein Data Bank; SC, secretory component; sIgA, secretory IgA; sIgM, secretory IgM. Panels *a–c* adapted with permission from Reference 91; copyright 2021 Cell Press. Panel *d* adapted with permission from Reference 84, copyright 2020 AAAS; and Reference 91, copyright 2021 Cell Press.

immunoglobulin isotypes (**Figures 5** and **8*a*,*b***). The mode of interaction with the SC is also highly conserved between IgA and IgM (**Figures 6*a*** and **8*c***). D1 of the SC utilizes its three CDR-like loops to bind highly conserved regions on both the IgA and IgM Fcs and tailpieces (**Figure 8*d*–*f***).

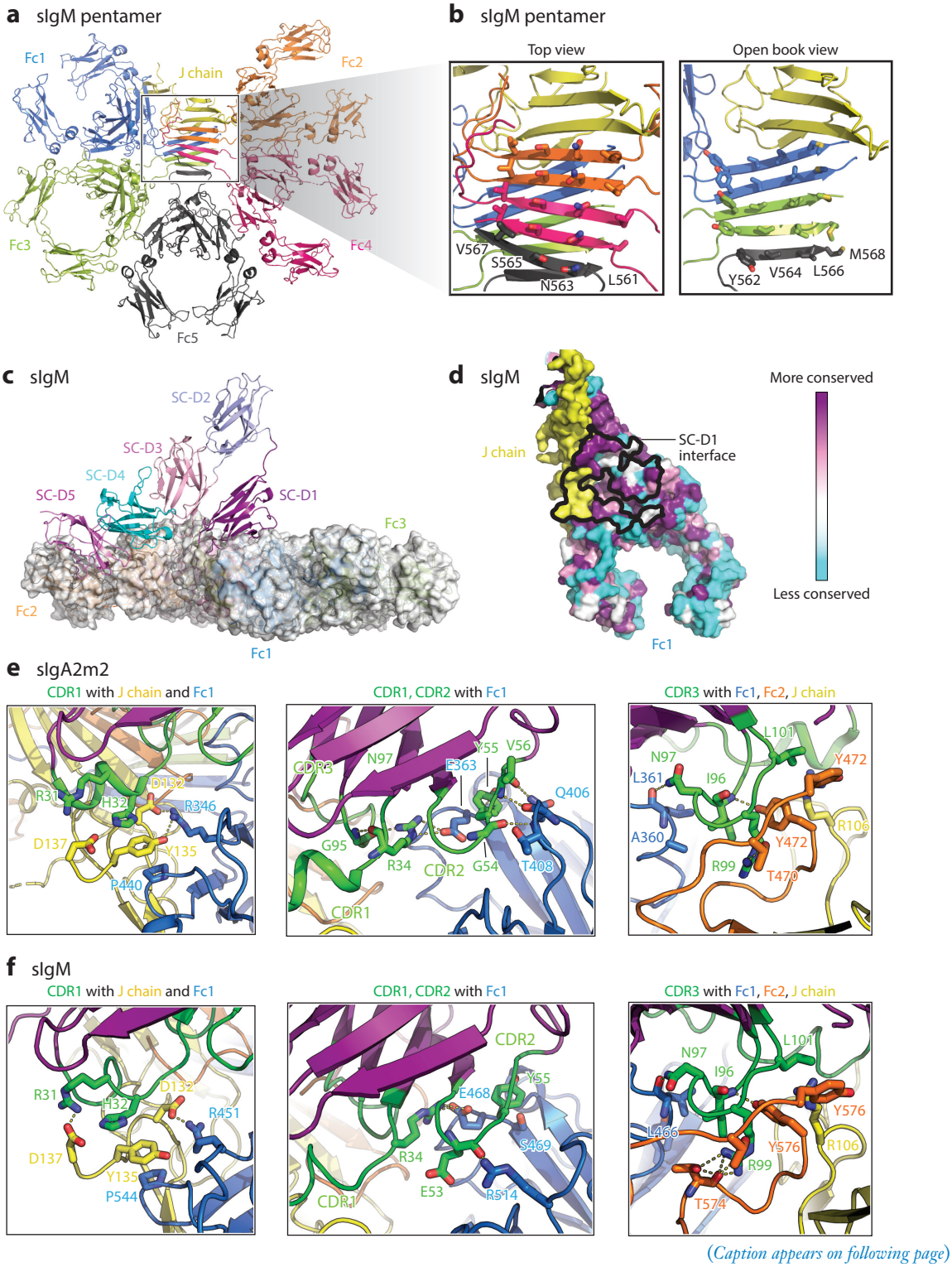
6. CONCLUSIONS

Natural abundance, ease of isolation, and the large size of sIgA and sIgM made these polymeric immunoglobulins particularly amenable to early biochemical, biophysical, and structural studies. These extensive characterizations provided significant insight into the composition, organization, and architecture of the polymeric immunoglobulins. As technological advances in molecular biology made cloning, mutagenesis, and recombinant protein production feasible, further characterization of IgA and IgM at the molecular level became possible. Whereas early structural studies provided low-resolution information on the assembled polymeric immunoglobulins, high-resolution information was limited to individual chains or domains, with atomic-level resolution structures of the assembled macromolecular complexes remaining elusive. It was not until recent advances in cryo-EM were applied to polymeric immunoglobulins that breakthroughs in structural analysis occurred, shedding light on the molecular mechanisms of multimeric immunoglobulin assembly. Through single-particle cryo-EM imaging of the sIgA-Fc and sIgM-Fc cores, the structures of the J chain and the Fc tailpieces could be visualized for the first time. A mechanism of J chain–templated oligomerization of the Fc tailpieces was elucidated, and the nature of SC incorporation was identified. Furthermore, the structures reveal how two immunoglobulins with relatively low sequence homology can utilize the same J chain and receptor for oligomerization and transcytosis.

Despite the significant progress made with recent structural characterizations, many questions remain. While we can visualize the structure of the polymeric immunoglobulin Fcs in the final assembled states, it is unclear exactly how multimerization occurs or is controlled in the cell. The structures revealed the fold of the J chain in the polymeric IgA- and IgM-bound states, revealing an intricate association with both immunoglobulins. This begs the question of whether the J chain can adopt this fold in the absence of IgA and IgM or whether its folding occurs in conjunction with immunoglobulin multimerization. If the folding occurs in conjunction with multimerization, what is the conformation of the J chain in the unliganded state? How does MZB1 facilitate J chain association and IgA multimeric assembly? Besides MZB1, are other chaperones involved in polymeric immunoglobulin folding and oligomerization?

Many questions regarding the oligomeric states of IgA and IgM also remain unanswered. Based on recombinant studies of polymeric IgA where individual components are overexpressed in protein production cell lines, relative expression levels of heavy chain and J chain can alter the relative abundance of the various oligomeric states produced. Is the oligomeric state of IgA actively controlled in plasma cells under normal expression conditions, and if so, how is this regulated? If IgA can form dimers, tetramers, and pentamers in the presence of the J chain, why does IgM form only J chain–containing pentamers? Furthermore, while IgA multimerization is dependent on the J chain, IgM is able to form hexamers without it. Although the covalent Fc-Fc contacts via the disulfides between C414 of adjacent IgM Fcs certainly lend stabilization to the hexamers, this cannot be the only explanation for why IgM can assemble in the absence of the J chain whereas IgA cannot. How does the structure of the IgM hexamer compare to that of the J chain–incorporated pentamer?

While the high-resolution cryo-EM structures of the IgA and IgM multimers elucidated the architecture of the oligomeric cores, the Fab arms were either not present in the constructs used or too flexible to be resolved in the maps. A computational conformational-search approach for murine sIgA led to modeling of possible Fab positions (86), but further work is needed to



(*Caption appears on following page*)

Figure 8 (*Figure appears on preceding page*)

J chain–mediated oligomerization and formation of sIgM. (*a*) Top view of the human sIgM pentamer (PDB 7K0C) with the SC removed for clarity. (*b*) Top and open book views of the boxed region in panel *a*. Side chains of the tailpiece residues are shown as sticks and labeled. (*c*) Structure of the SC shown in the context of the sIgM pentamer (PDB 7K0C). The IgM pentamer is rendered as a transparent surface with a ribbon diagram model underneath, and the SC is shown as a ribbon diagram, with its five immunoglobulin-like domains labeled. (*d*) Top view of the IgM Fc1 and J chain with the SC omitted for clarity. The J chain and IgM Fc1 are rendered as surfaces. The J chain is yellow. The level of sequence conservation between the Cμ3/Cμ4 domains of the IgM and the Cα2/Cα3 domains of the IgA Fc is mapped onto the IgM Fc surface. Identical residues (*purple*), residues with highly similar chemical properties (*pink*), residues with weakly similar chemical properties (*white*), and dissimilar residues (*cyan*) between the IgM and IgA Fcs are indicated. The interaction interface of SC-D1 is outlined in black on the surface. (*e*) Interaction of the three CDR-like loops of SC-D1 with the human IgA2m2 pentamer (PDB 6UEA). (*f*) Interaction of the three CDR-like loops of SC-D1 with the human IgM pentamer (PDB 7K0C). Abbreviations: CDR, complementarity-determining region; D1, domain 1; Fc, crystallizable fragment; PDB, Protein Data Bank; SC, secretory component; sIgA, secretory IgA; sIgM, secretory IgM. Panels *a–d* and *f* adapted with permission from Reference 91; copyright 2021 Cell Press. Panel *e* adapted with permission from Reference 84, copyright 2020 AAAS; and Reference 91, copyright 2021 Cell Press.

understand the full IgA and IgM structures, how the antigen-binding regions interact with the constant domains, and the roles that the various hinge sequences play in conformational flexibility and antigen recognition. Negative-stain EM and cryo-ET have provided low-resolution information on the staple-like conformation adopted by IgM in the presence of antigen (53, 56). While current structural biology techniques are not sufficient to answer such questions at high resolution, this will likely change in the coming years as cryo-ET and other in situ methods further evolve.

The structures of sIgA and sIgM revealed that the SC adopts a very different conformation in the bound state (84–86, 90, 91) than in the apo form (74), confirming that a large conformational change must occur in pIgR upon polymeric immunoglobulin binding. However, exactly how binding induces this change in the receptor is still unclear. Beyond pIgR, polymeric IgA and IgM interact with other Fc receptor (FcR) and Fc receptor–like (FcRL) proteins. FcαμR, which has homology to D1 of pIgR, binds both polymeric IgA and IgM containing the J chain, but it does not bind sIgA and sIgM, suggesting an overlapping binding site with pIgR (93, 94). While FcμR cannot bind polymeric IgA, it binds both systemic forms of IgM—the J chain–containing pentamer and J chain–independent hexamer (95, 96). Additional IgA-specific receptors also exist. FcRL3 binds sIgA but not systemic, polymeric IgA, whereas FcRL4 binds systemic, polymeric IgA but not sIgA (97–99). Further studies will be important to understand the structures of these receptors, modes of binding to the polymeric immunoglobulins, and the nature of their specificity.

SUMMARY POINTS

1. IgA and IgM are the only human immunoglobulin isotypes capable of multimeric assembly.
2. IgA and IgM monomers form higher-order oligomers through incorporation of the J chain and disulfide bond formation via their tailpiece extensions.
3. Locally produced polymeric IgA and IgM are transcytosed by the polymeric immunoglobulin receptor (pIgR) from the basolateral to the apical side of the mucosal epithelium, incorporating the proteolytically cleaved secretory component (SC) of pIgR to form secretory IgA (sIgA) and sIgM.
4. Recent advances in single-particle cryo–electron microscopy led to high-resolution structure determination of sIgA and sIgM, revealing the molecular mechanism of multimeric assembly.

5. The J chain adopts a β-sandwich fold that templates β-sheet extension by the tailpieces of IgA and IgM, forming a molecular zipper for oligomerization.
6. The SC makes extensive contact with two Fcs, their tailpieces, and the J chain, binding both polymeric IgA and polymeric IgM in a homologous manner.
7. Key residues important for both J chain and SC interaction are conserved between IgA and IgM, allowing two different isotypes to utilize the same mechanism of multimerization and transcytosis.

FUTURE ISSUES

1. What is the structure of the J chain in the unliganded state?
2. How do MZB1 and other potential molecular chaperones facilitate J chain–mediated oligomerization of IgA and IgM?
3. Is the oligomeric state of IgA (i.e., dimer, tetramer, or pentamer) actively controlled in plasma cells during expression and assembly?
4. How does the structure of hexameric IgM in the absence of the J chain compare to that of the J chain–containing IgM pentamer, and why does IgA not efficiently multimerize without the J chain?
5. Is the molecular mechanism of immunoglobulin multimerization conserved in other species beyond humans and mice?
6. What do the structures of full-length polymeric IgA and IgM look like?
7. How does polymeric immunoglobulin binding to pIgR induce the necessary conformational change required for transcytosis and SC incorporation?
8. How are polymeric IgA and IgM bound by other Fc receptor and Fc receptor–like proteins?

DISCLOSURE STATEMENT

M.L.M. was previously an employee of Genentech, Inc., a member of the Roche Group, and is currently an employee of Exelixis, Inc., and may hold stock and options in these companies. M.L.M. is an inventor on unpublished patent applications that relate to IgA antibodies and IgG-IgA fusion molecules and methods of making and using them.

ACKNOWLEDGMENTS

I thank the editors for the opportunity to contribute this review. This review is intended to be a focused discussion of the current state of the field rather than a comprehensive overview. I apologize to those whose work could not be cited due to space limitations.

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