Review

How antibodies fold

Matthias J. Feige[1], Linda M. Hendershot[2] and Johannes Buchner[1]

[1] Center for Integrated Protein Science, Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85747 Garching, Germany
[2] Department of Genetics and Tumor Cell Biology, Saint Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA

B cells use unconventional strategies for the production of a seemingly unlimited number of antibodies from a very limited amount of DNA. These methods dramatically increase the likelihood of producing proteins that cannot fold or assemble appropriately. B cells are therefore particularly dependent on 'quality control' mechanisms to oversee antibody production. Recent *in vitro* experiments demonstrate that Ig domains have evolved diverse folding strategies ranging from robust spontaneous folding to intrinsically disordered domains that require assembly with their partner domains to fold; *in vivo* experiments reveal that these different folding characteristics form the basis for cellular checkpoints in Ig transport. Taken together, these reports provide a detailed understanding of how B cells monitor and ensure the functional fidelity of Ig proteins.

A short overview of antibody biology

Immunoglobulin (Ig) proteins serve as cell-surface antigen receptors on B cells and upon antigen stimulation and terminal differentiation to plasma cells, they are secreted as soluble effector molecules (antibodies) that provide protection against infections and foreign antigens. In their simplest form, i.e. IgG antibodies, each molecule is composed of two identical heavy chains (HCs) and two identical light chains (LCs) that are linked by disulfide bonds. Both chains are comprised of multiple domains of ~100 amino acids each (Figure 1a). The N-terminal domains of both chains vary between antibodies. These contain particularly diverse stretches of amino acids (hypervariable regions) that provide the exquisite binding specificity of the antibody molecule and give rise to their designation as variable domains (V_H and V_L). Together these two domains form the antigen binding site (Figure 1a). The remainder of the antibody sequence is conserved within antibody classes (constant domains) and is important for effector functions such as complement activation or recruitment of macrophages and natural killer cells. Five classes of antibodies are made in most higher vertebrates, IgM, IgG, IgA, IgD, and IgE, that differ in the HC constant regions used. Only two types of LC (κ or λ) exist, which can assemble with all HC classes. In a given cell, only one HC and one LC allele are expressed, so that antibodies with a single specificity are produced [1].

The development of progenitor cells committed to the B-cell lineage is characterized by the sequential expression of HC and LC subunits. In pre-B cells, a unique HC variable region is created by combining a single variable (V_H) gene segment with one diversity (D_H) and one joining (J_H) gene segment at the DNA level on a single allele [2,3], which is initially spliced to the IgM constant region at the mRNA level [4]. During development, class switching can occur to juxtapose the rearranged V_H domain to other downstream constant regions. DNA rearrangements that give rise to HC variable regions involve imprecise joining of these three gene segments, the addition of non-templated bases at the site of joining of these gene segments, and, during later stages of differentiation, directed hypermutation of the variable region exons [5]. Once a functional HC is made, similar gene rearrangements commence to form the V_L domain of the LC [6]. These mechanisms are essential to generate antibody diversity and allow affinity maturation of the immune response, yet they clearly increase the likelihood of producing a protein that cannot fold or assemble appropriately, be transported to the cell surface or secreted, or engage the appropriate signaling molecules, thereby compromising the functioning of the immune system. B lineage cells are therefore particularly dependent on the 'quality-control system' of the endoplasmic reticulum (ER) to ensure that only correctly assembled Ig molecules are transported to the cell surface. It is accordingly not surprising that many of the major components of the mammalian ER quality-control machinery were first identified by virtue of their association with

Glossary

B cell: refers to a mature developmental stage of B cell differentiation in which heavy and light chains are synthesized and expressed at the cell surface via a transmembrane region at the C-terminus of the heavy chain that is produced by alternative splicing of the heavy-chain mRNA.
Heavy/light chain: constituent polypeptide chains of antibody molecules. Light chains are made up of two Ig domains and therefore possess a lower molecular weight than the heavy chains (which are made up of a minimum of three Ig domains).
Hypervariable regions: highly diverse portions of the variable domains of heavy and light chain that form the antigen binding site.
Ig domain: a folding unit of approximately 100 amino acids with a highly conserved twisted barrel-like β-sheet structure that is, in most cases, stabilized by a buried intrachain disulfide bond.
Ig superfamily: refers to a large group of proteins that are composed of Ig domains. It is one of the most widespread protein topologies observed in nature, and is often involved in extracellular binding and recognition processes.
Isotypes: refers to the antibody classes IgM, IgG, IgA, IgD, and IgE, which are named for the heavy-chain constant region used, i.e. μ, γ, α, δ, and ε, respectively. A single variable region of a heavy chain can be sequentially associated with different constant regions via a process known as class switching.
Plasma cell: the terminal stage of B cell differentiation that occurs after the stimulation of B cells with mitogen or antigen. These normally shortlived cells produce tremendous quantities of a single type of antibody.
Pre-B cell: an early stage of B cell development characterized by the production of heavy-chain, but not light chain, proteins.

Corresponding authors: Hendershot, L.M. (linda.hendershot@stjude.org); Buchner, J. (johannes.buchner@ch.tum.de).

0968-0004/$ – see front matter doi:10.1016/j.tibs.2009.11.005 Available online 21 December 2009

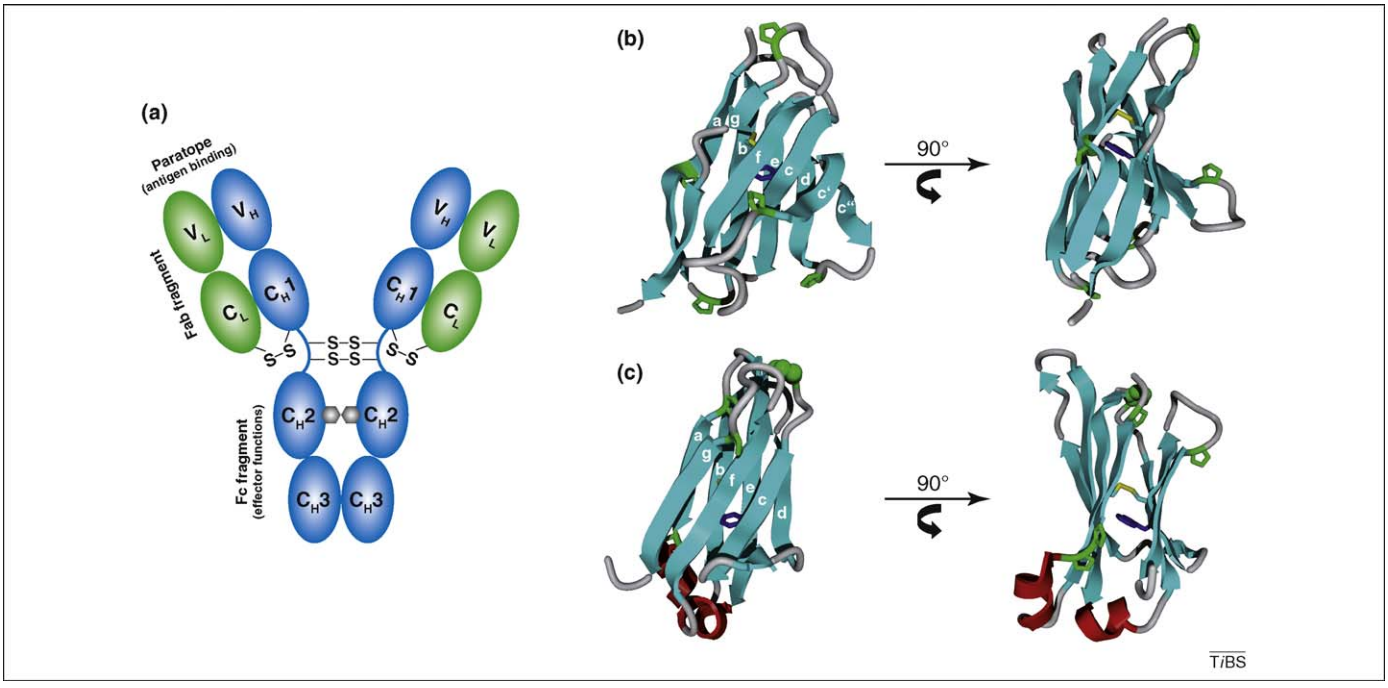

Figure 1. Overall structure and domain architecture of antibodies. **(a)** Domain arrangement of an IgG antibody molecule. The light chains are shown in green, the heavy chains in blue. The oligosaccharides between the C_H2 domains are depicted as gray hexagons. Interchain disulfide bridges and important functional elements of the antibody (antigen binding paratope, Fab fragment, Fc fragment) are indicated. Domain architecture of the light chain variable (V_L) **(b)** and constant (C_L) domains **(c)**. The strand nomenclature is indicated. The intrachain disulfide bridge (yellow) and the proximal conserved tryptophan residue (blue) are shown. The proline residues of the two domains are shown in green with the highly conserved *cis*-proline residue between strands b and c of C_L highlighted in a CPK representation. Small helices (red) connect strands a and b and strands e and f of the C_L domain.

antibody chains, and that Ig molecules are some of the earliest identified substrates of ER folding enzymes (Table 1).

HCs and LCs are co-translationally translocated into the ER, and folding begins even before the polypeptide chains are completely translated [7]. Most IgGs assemble first as HC dimers to which LCs are added covalently via a disulfide bond between the C_L and C_H1 domains [8]. IgG HC mutants with a deleted C_H3 domain do not readily form HC dimers and are often secreted as HC-LC 'hemimers' [9].

Table 1. Members of the ER folding machinery

Folding helper	Additional names	Molecular mass (kDa)	Function	Involved in Ig biosynthesis?	References[a]
BiP	Grp78, Kar2 (yeast)	78	Molecular chaperone, Hsp70 homolog	+	[58] [59]
GRP94	Gp96, Hsp90b1	94	Molecular chaperone, Hsp90 homolog	+	[87]
Calnexin/Calreticulin		65/47	Glycoprotein quality control, ER retention	+	[88]
ERdj1	Mtj1	64	Co-chaperone of BiP, J-protein, Hsp40 protein, membrane-bound	?	[89]
ERdj2	Sec63	83	Co-chaperone of BiP, J-protein, Hsp40 protein, membrane-bound	?	[90]
ERdj3	DnaJB11	43	Co-chaperone of BiP, J-protein, Hsp40 protein	+	[91]
ERdj4	DnaJB9 Mdj1	25	Co-chaperone of BiP, J-protein, Hsp40 protein, membrane-bound	+	[92]
ERdj5	DnaJC10	91	Co-chaperone of BiP, J-protein, reductase	?	[93]
ERdj6	P58-IPK, DnajC3	58	Co-chaperone of BiP, J-protein, Hsp40 protein	?	[94]
ERdj7	Gng10, DnajC25	40	Co-chaperone of BiP, J-protein, Hsp40 protein	?	[95]
GRP170	Lhs1 (yeast)	110	Nucleotide exchange factor for BiP	+	[96]
BAP	Sil1	50	Nucleotide exchange factor for BiP	?	[97]
Sig1R			Cofactor of BiP	?	[98]
PDI		55	Oxido-reductase, disulfide bond formation, isomerization	+	[99]
Perp1		20	Oxido-reductase, chaperone?	+	[100] [101]
ERp72	CaBP2	72	Oxido-reductase	+	[102]
P5	CaBP1	55	Oxido-reductase	+	[102]
ERp57		60	Oxido-reductase	+	[103]
CypB		20	Peptidyl-prolyl isomerase	+	[91]
ERp29		25	Chaperone?	?	[104]
UDP-GT		53	Glycosyl-Transferase	?	[105]

[a]References refer to the first description of the protein, or to the first demonstration of a role in antibody biosynthesis.

Thus, Fc fragment dimerization is primarily mediated by interactions between the C_H3 domains and stabilized by disulfide bonds in the hinge region. The C_H2 domains interact only via N-linked glycans (Figure 1a), which are covalently linked to this domain co-translationally [10]. They determine the orientation and spacing of the two C_H2 domains, which is crucial for the binding of downstream effectors [11–13]. The heavily glycosylated HCs of IgM require the glycans for assembly and transport, suggesting that they probably guide the folding of IgM μ heavy chains [14], whereas the monoglycosylated γ HCs of IgG mature appropriately in their absence [15]. The analysis of the basic steps of antibody biosynthesis in cells, together with *in vitro* folding studies, have provided a molecular understanding of the folding and assembly processes of Ig proteins.

Antibody structure and the evolution of the Ig fold

In the case of IgG (for which most of the *in vitro* work has been done), the 'Y'-shaped molecule is composed of two four-domain HCs and two two-domain LCs (Figure 1a). The orientation of the two arms of the Y is flexible due to an unstructured hinge region between the first (C_H1) and the second (C_H2) constant domain of the HC. The IgG molecule can be proteolytically cleaved in the hinge region, which subdivides it into three functional segments; each of which is a dimer [16,17]. The two N-terminal fragments (composed of a LC associated with the V_H-C_H1 domains of the HC) are termed 'fragment antigen binding' (Fab). The remaining 'fragment crystallizable' (Fc) comprises two identical, two domain (C_H2-C_H3) segments that are covalently linked via disulfide bonds in the hinge region. The Fc fragment is important for connecting antigen binding to antibody effector functions. Each of the Ig domains forms a highly similar 'beta sandwich' structure, known as the 'Ig fold'. The Ig fold is characterized by a greek-key β-barrel topology in which the barrel is not continuously hydrogen-bonded, but instead composed of two sheets, forming a sandwich-like structure. The variable domains (Figure 1b) comprise nine strands (abcc'c"defg) and the constant domains (Figure 1c) seven strands (abcdefg) [18]. In most antibody domains, a buried disulfide bridge, which spans ~60–70 residues, connects strands b and f [11,18]. It is oriented approximately perpendicular to the individual sheets and significantly stabilizes the folded domain [18,19]. Another characteristic feature shared among antibody domains is a conserved tryptophan residue that is located in proximity to the internal disulfide bridge. Its fluorescence is quenched only in the native state by the adjacent disulfide bond, so it can be used as a 'reporter group' for the conformational state of antibody domains [19]. Proline residues are unusually abundant in antibody domains, contributing up to 10% of the amino acids. Particularly important is a conserved *cis*-proline residue (Box 1) in the loop connecting strands b and c of the constant domains (Figure 1b).

The Ig fold, which was first discovered in antibodies, is one of the most widely used protein topologies in nature, giving rise to the Ig superfamily (IgSF). The origin of the Ig fold represents ~750 million years of evolutionary history, with the identification of IgSF members as early as in sponges [20,21]. The ability to produce antibodies is a more recent development (~500 million years), first appearing in cartilaginous fish such as sharks, skates and rays [21,22]. In vertebrates, the Ig fold is the major building block of extracellular recognition systems [23,24] whereas, in invertebrates, expression of IgSF members is mostly limited to the neural system [25]. The Ig fold has also been detected in prokaryotic and viral proteins, albeit less frequently, suggesting that it might have been acquired by horizontal gene transfer [26]. The evolutionary success of the IgSF can probably be attributed to its robust fold, which provides stability against proteases and harsh environments, and the ability to build highly diverse binding loops or edge strands on this core structure.

From the folding of antibody domains to complete IgG molecules

Dissection of the Ig protein into individual domains or fragments is necessary to detect differences in the folding of structurally similar domains. Pioneering studies on antibody folding were done on LCs that were denatured and allowed to refold *in vitro* [19]. Further studies examined isolated constant LC domains (C_L) [27,28] and IgG HC C_H3 domains [29], which revealed that these individual Ig domains can fold autonomously. A common

Box 1. Peptidyl-prolyl isomerization reactions

Within proteins, individual amino acids are covalently linked by the planar peptide bond, the product of the reaction between the carboxyl group of the amino acid number i and the amine group of the amino acid number i + 1. For 19 out of the 20 natural amino acids, this bond populates almost exclusively a *trans* state (ω=180°), i.e. the Cα atoms of amino acid i and i + 1 are on opposite sites of the CO-NH bond (Figure I). The only exception is proline, where, due to its cyclic side chain, the *cis* state (ω=0°) of the peptide bond is energetically only slightly less favorable than the *trans* state (Figure I). In mature protein structures, ~10% of all bonds between proline and its preceding amino acid (Xaa-Pro bond) are found in the *cis* state. The conversion to the *cis* state is influenced by the side chain of the amino acid preceding the proline residue [75]. The *cis* conformation is particularly pronounced in the bends and turns of proteins, suggesting a structural role [76]. The activation energy of the peptidyl-prolyl isomerization reaction is high (~80 kJ/mol), so it is an intrinsically slow reaction taking several minutes at room temperature. All Xaa-Pro bonds leave the ribosome in a *trans* state after polypeptide synthesis. Peptidyl-prolyl isomerization reactions are therefore important rate-limiting steps in protein folding, and were among the first to be identified [77,78]. Due to their unusual structural properties, prolines can be used as molecular timers and switches in conformational changes in proteins [79,80]. In the cell, peptidyl-prolyl isomerization reactions are catalyzed by the diverse and ubiquitous family of peptidyl-prolyl isomerases (PPIases) [81,82].

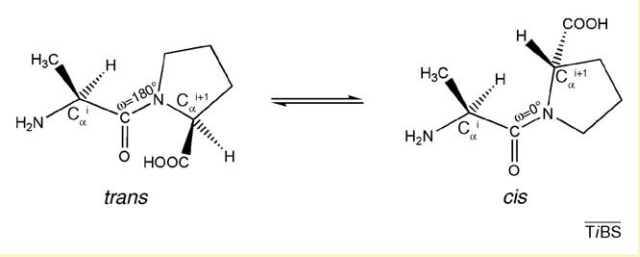

Figure I. An alanyl–prolyl isomerization reaction (schematic).

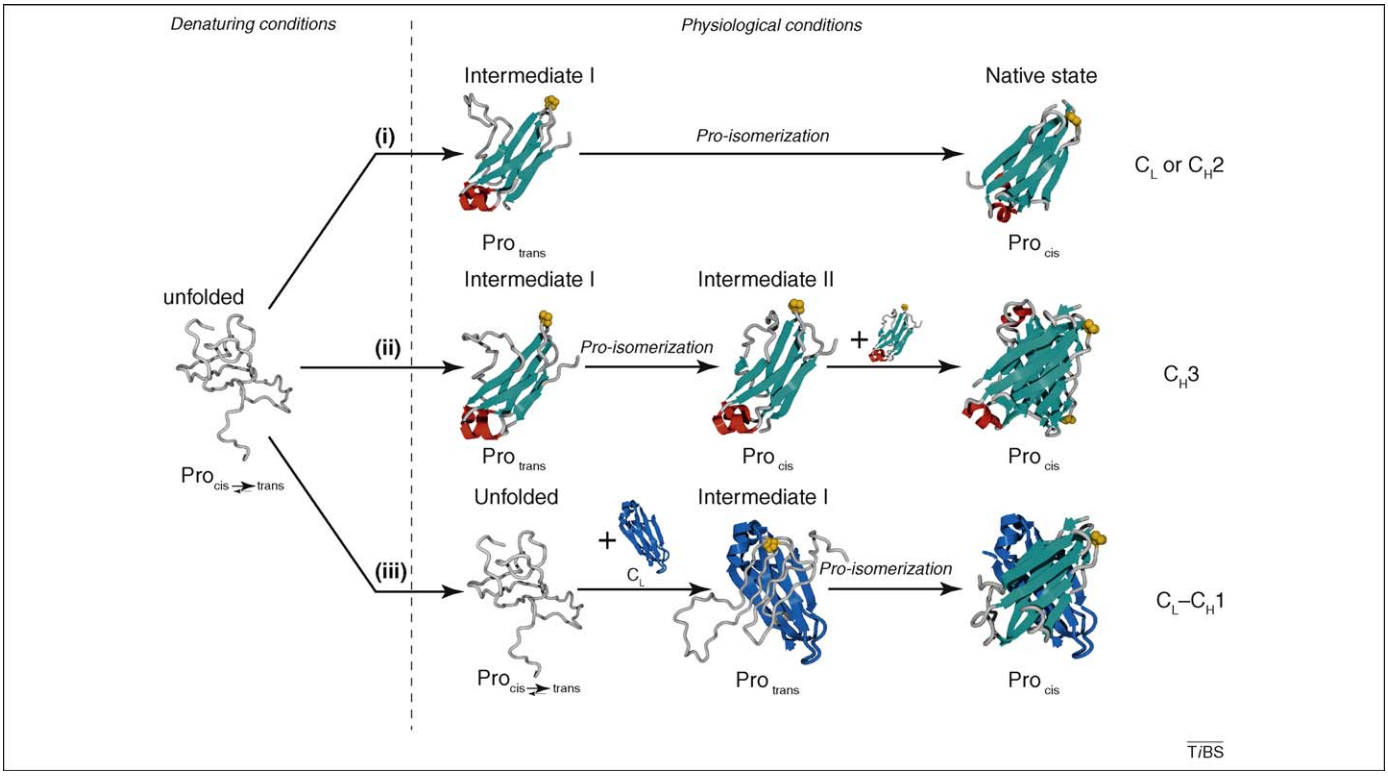

Figure 2. Three pathways of antibody domain folding. **(i)** C_L and C_H2 fold via a highly structured on-pathway intermediate that is trapped by the incorrect isomerization state (*trans* for C_L) of a proline residue in the loop connecting strands b and c (highlighted in yellow). In the intermediate, the core β-sheet structure and the two short helices connecting strands a and b and strands e and f are fully formed (shown in red). **(ii)** The obligate dimer C_H3 folds via two intermediates, both probably similar in structure to those of the C_L and C_H2 domains. In a first, rapidly formed intermediate, a critical proline residue (highlighted in yellow) must isomerize to its native *cis* state, leading to a second intermediate that can dimerize and thereby complete folding. **(iii)** C_H1 is intrinsically disordered in isolation. Upon association with C_L, it forms a loosely folded intermediate. In this complex, isomerization of the conserved proline residue between strands b and c (highlighted in yellow) limits the complete folding to the native state and formation of the interchain disulfide bridge between C_H1 and C_L.

characteristic in the folding of antibody domains are slow proline isomerization reactions (Box 1) [27,28,30–32] that often provide the rate-limiting step [33,34]. Recent *in vitro* studies addressed the folding of each of the IgG domains in more detail [30–39]. These studies showed that, although all Ig domains are very similar in terms of their final structures, they can be grouped into three folding categories (Figure 2).

In the first category, exemplified by the well-studied C_L protein, domains can fold autonomously to a monomeric state. The chemically denatured protein does not exhibit a significant residual structure, regardless of whether or not its internal disulfide bridge is present [34,36]. Once folding is initiated, the presence of an internal disulfide bridge exerts an important guiding impact on the folding pathway [27,28,30,36]. This is because the initial folding nucleus for Ig domains involves the clustering of hydrophobic residues in strands b, c, e and f, which establishes the overall topology of the protein (Figure 2) [35,40,41]. The covalent linkage of the cysteines in strands b and f facilitates the formation of this folding nucleus and of a structured, on-pathway intermediate, thus preventing unproductive interactions (Figure 2). A population of misfolded, aggregation-prone off-pathway folding intermediates for C_L was detected in the absence of the internal disulfide bridge [36].

Although folding intermediates are usually transient and therefore elusive, they can be populated for longer times if a slow reaction limits the subsequent folding step, thereby allowing their characterization. This is the case for C_L, where the major on-pathway folding intermediate is relatively long-lived due to the non-native *trans* isomerization state of a proline residue between strands b and c, which must isomerize to its *cis* state before folding can proceed (Figure 2) [27,34]. Using nuclear magnetic resonance (NMR) spectroscopy combined with molecular dynamics simulations, it was possible to follow the changes in the chemical environment of most amino acids in this domain and to obtain an atomic resolution view of the intermediate structure. In the intermediate, the core β-strands are almost completely formed, whereas the flanking strands (particularly strand d) remain flexible (Figure 2) [34], yet to a lower degree than observed for other IgSF members [42–44]. The analysis of the structural changes of individual amino acids in the course of folding unexpectedly revealed that two small helices linking strands a and b and strands e and f (Figure 1c, Figure 2) are important elements in Ig domain folding. They become natively structured very early [34] and can act as an organizing center, stabilizing the orientation and spacing of the β-strands of the Ig fold. Furthermore they correctly position bulky hydrophobic residues in the core of the protein [34]. These helices therefore render folding of the C_L domain more robust because they stabilize the conformation of a highly structured on-pathway intermediate that is poised for subsequent productive folding. Consistent with their important role in folding, these small helices are highly conserved in most constant region domains as well as in other members of the Ig superfamily

Box 2. Alternatively folded states and antibody deposition diseases

For most proteins, the accessible conformations in equilibrium include the native state, the unfolded state and non-specific aggregates; some polypeptides might additionally adopt oligomeric fibrillar structures. The situation is different for antibodies. Antibodies can adopt a specific additional conformation at low pH (pH <3) at which many other proteins would be largely unfolded. The antibody conformation at low pH had been termed 'alternatively folded state' because it exhibits characteristics of the folded state (e.g. remarkable stability against unfolding), but the available spectroscopic information suggests that it is structurally significantly different from the native state. It was first described for a complete IgG antibody [83], but single domains such as C_H3 also can adopt this state [84]. The biological significance of this process is unknown, but there are biotechnological implications for this state because antibody manufacturing processes often include low-pH steps which can induce the alternatively folded state.

Another accessible state for some antibodies is the fibrillar amyloid structure. This is associated with several protein-folding diseases. In this cross-beta structure, fibrils are formed by β-strand exchange of the individual subunits. Whereas antibodies evolved to robustly form and maintain their β-sheet structure in the human body, secreted isolated LCs and also truncated HCs have been found to form fibrils which are deposited in organs such as kidneys. Antibodies are produced and can be deposited in large quantities, so these deposits can strongly interfere with physiological functions and thus these diseases can have fatal consequences. The most prevalent of the fatal diseases is light-chain amyloidosis (AL), which results from the over-production of monoclonal LCs prone to misfolding and the formation of amyloid deposits. In the case of AL, certain V_L domains seem to be particularly susceptible to amyloid formation [85]. This is consistent with the idea that the C_L domain is protected against misfolding by helical elements which are missing in V_L [34,38,45] and the fact that all C_L domains are identical, whereas V_L domains are different. The precise fibrilization mechanism remains incompletely understood, but it seems that the mechanisms that give rise to the production of variable domains might, at times, generate less stable domains that can pass ER quality control but have a propensity to misfold outside the cell.

[18,23,24]. The structural insights gained for the major C_L folding intermediate seem to be readily transferable to some other HC constant domains, in particular C_H2 [31], where the effect of sugar moieties on the folding reaction is currently unknown. Such helices are not found in variable antibody domains (Figure 1b) or in several IgSF members that are prone to misfolding and amyloid formation [43–45]. This suggests that structural differences in folding intermediates might influence if IgSF members fold reliably (Box 2).

The second category of antibody domain folding pathways (Figure 2) is represented by the C_H3 domain of the IgG HC [29,33]. In addition to folding slower than the domains of the first category, this domain forms an obligate homodimer with the internal disulfide bridge being dispensable for folding and self-association [39]. As with the C_L domain, a partially folded species was observed which was trapped by a non-native prolyl isomerization state [33]. The monomeric intermediate could not dimerize until the native proline isomer was formed, so proline switches can regulate not only folding, but also dimerization in antibodies.

The third (and most unexpected) category of antibody domain folding is the recently discovered template-assisted folding of the C_H1 domain which interacts with the C_L domain in the intact antibody (Figure 1a). Surprisingly, the isolated C_H1 domain is intrinsically disordered as determined by various spectroscopic techniques [37]. This is in marked contrast to all antibody domains previously studied and completely unexpected from its structural similarity to other Ig domains [11]. To induce its folding, the C_H1 domain strictly requires interaction with key residues in the dimerization interface of the folded C_L domain. Unlike other antibody domains where the intramolecular disulfide bond only enhances the folding process, the covalent linkage of the two cysteines in the C_H1 domain is a prerequisite for its folding. Another unique twist is that the rate-limiting proline isomerization between strand b and c and subsequent productive folding can occur only after association with the C_L domain. These observations are in agreement with studies on the Fab fragment in which C_H1 folding was proposed to be the slowest step, occurring after association of the HC and the LC [32].

Taken together, these data reveal that the β-barrel topology of antibody domains is reached by an overall conserved mechanism, even though strikingly different pathways are employed. The biological significance of these differences becomes clear if one considers that different domains of antibodies are used for significantly different purposes in the cell such as guiding dimerization (e.g. C_H3) or as a quality-control sensor for assembly (e.g. C_H1).

Quality control of antibody folding and assembly *in vivo*

The secretion of incompletely folded or assembled antibodies would be deleterious to the immune response, so several quality-control checkpoints are required during the development and differentiation of B cells to monitor antibody integrity (Figure 3). One of the first quality-control measures employed after HC variable gene rearrangements centers on the ability of the HC protein to associate with the 'surrogate LC', which is assembled from the V_{preB} protein (contributing the 'variable domain') [46] and the λ_5 protein (supplying the 'constant domain') [47]. This LC-mimetic tests the ability of HC to fold correctly and assemble with a LC-like protein. If this step is successfully passed the pre-B cell receptor is transported to the cell surface along with signaling proteins and provides the stimulus for the further development of the pre-B cell [48]. The C_H1 domain constitutes a crucial aspect of this quality-control step because HCs that lack the C_H1 domain can be transported to the cell surface and signal without assembling with a surrogate LC [49]. The folding of the C_H1 domain in association with the LC C_L domain remains a critical focus of Ig quality-control efforts throughout the development of B-cells and differentiation of plasma cells. Once the HC is judged to be functional by successfully completing these steps, conventional LC gene rearrangements commence. Unlike LC (which can be secreted alone), HCs are retained in the ER and eventually degraded unless they assemble with LC (Figure 3) [50]. LC loss variants of plasmacytomas are very rarely observed, whereas HC loss variants occur much more frequently [51]. This was argued to be due to the 'toxicity' of free HC, which could be neutralized by LC [52]. Exceptions to this rule occur in the rare B cell lymphoproliferative disorder known as heavy chain disease in which truncated Ig

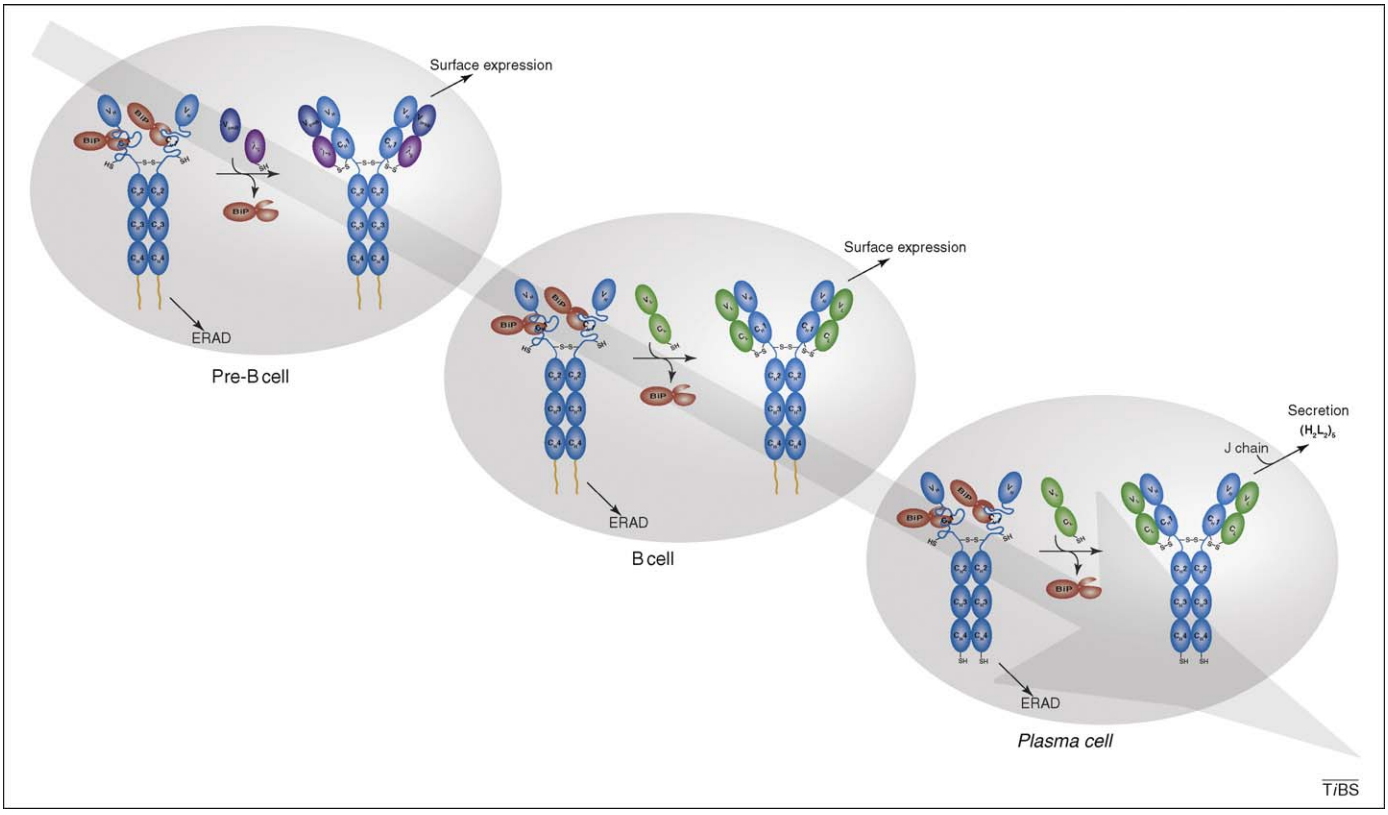

Figure 3. Immunoglobulin quality-control checkpoints at various stages in the development of B cells. After HC gene rearrangements, pre-B cells produce IgM HCs (μ HCs) (blue) bound to BiP (red). If their association with the surrogate LC (which is assembled from the V_{preB} (deep purple) and λ_5 (light purple) proteins) induces BiP release and folding of the C_H1 domain, and if the other Ig domains fold appropriately, the HC can traffic to the plasma membrane and engage signaling molecules (HC membrane anchor shown in yellow). If there is a failure in any of these steps, the μ HCs become substrates for ER-associated degradation (ERAD) and are retro-translocated to the cytosol for degradation by the 26S proteasome. Once conventional LCs (green) are produced in the B cell, they assemble with μ HCs, displace BiP from the C_H1 domain, and induce its folding. The ability of all domains of the HC to fold appropriately upon assembly was tested at the pre-B cell stage; quality control at this stage monitors the pairing and folding of the V domains. Differentiation to plasma cells leads to the synthesis of extremely high levels of antibodies. The ability of the specific HC and LC combination to assemble and fold appropriately was verified at the B cell stage of development, so quality control at this point involves monitoring the completeness of Ig assembly, focusing on the LC-induced release of BiP from the C_H1 domain and its concomitant folding. There is a shift to production of the secretory form of μ HC in plasma cells, which possess a terminal cysteine that is involved in assembly with the J chain and formation of pentamers. Thiol-mediated retention mechanisms monitor the redox state of this cysteine and prevent IgM monomers from being secreted [86].

HCs are secreted from cells without LCs. Notably, although these short HCs have been identified for several isotypes (i.e. IgA, IgG, IgM), the deletions nearly always involve portions of V_H and C_H1 domains [53]. Similarly, mouse plasmacytoma lines expressing HCs with deletions of the C_H1 domain can secrete free HC [54], whereas deletion of any of the other constant-region domains does not permit this. The serum of Camelidae contains a significant fraction of antibodies that are naturally devoid of LC. These 'HC-only' antibodies do not possess a C_H1 domain [55], further underscoring the evolutionarily conserved importance of this domain in regulating the transport and quality control of Ig proteins.

Generally, the term 'ER quality control' refers to the process of monitoring the maturation of nascent secretory proteins and allowing only appropriately folded and assembled proteins to transit further along the secretory pathway. Proteins that fail to mature correctly are retained and eventually retro-translocated to the cytosol for degradation by the 26S proteasome in a process known as 'ER-associated degradation' (ERAD) [56,57]. Immunoglobulin heavy-chain binding protein (BiP), the first component of the eukaryotic ER quality-control apparatus to be identified (Table 1), was found by virtue of its association with the unassembled, non-transported HCs produced in pre-B cell lines [58]. It interacts transiently with Ig assembly intermediates but not with completely assembled H_2L_2 molecules [59]. BiP is the ER ortholog of the Hsp70 family of chaperones [60] and is retained in the ER (along with associated proteins) by virtue of its C-terminal KDEL tetrapeptide [61]. Similar to the differences detected by *in vitro* folding experiments, folding requirements and dependence on BiP are quite different for the various Ig domains in cells. BiP binds transiently to some Ig domains (i.e. V_L, V_H, and some C_H domains), but other domains (e.g. C_L) appear to fold rapidly without ever interacting with BiP [62,63], even though they possess potential BiP binding sites [64]. Only the C_H1 domain interacts stably with BiP in the absence of LC [65] (Figure 3). The association of this domain with BiP is crucial for controlling the assembly and transport of Ig proteins because its deletion (and the resulting ablation of BiP binding) leads to the secretion of incompletely assembled Ig intermediates. *In vivo* studies that determine the folding status of proteins possessing intrachain disulfide bonds rely on the analysis of the oxidation status of cysteine residues because disulfide bonds make proteins more compact and migrate faster in non-reducing gel electrophoresis experiments [66]. Unlike other Ig domains, the C_H1 domain remains reduced in the absence of LC [63],

suggesting that it is incompletely folded. In contrast to the *in vitro* studies described above, there is no evidence that BiP can associate with oxidized C_H1 domains in cells [67]. *In vivo,* the association with LC, oxidation, and folding of C_H1 might be more tightly coupled.

In keeping with *in vitro* studies, the ATP-mediated release of BiP from isolated HCs resulted in C_H1 domain oxidation, but this was insufficient for appropriate folding [67,37]. This finding suggests that LC association is also required for the folding of the C_H1 domain *in vivo*. Only LCs in which both domains (V_L and C_L) were folded could assemble with HCs and induce oxidation and secretion of the C_H1 domain from cells. *In vivo* experiments also confirmed the requirement for proline isomerization in the C_H1 domain in these processes, because a mutation of the critical proline inhibited oxidation, assembly with LC, and secretion [37]. Thus, the evolution of a unique C_H1 domain that absolutely requires assembly with a C_L domain for its folding allows the cell to ensure that newly rearranged HCs in pre-B and B cells will be retained unless they can pass an important test, i.e. their ability to combine with a surrogate or conventional LC, respectively. It also ensures that plasma cells, which have been estimated to synthesize up to 10^3 antibody molecules per second [68], do not release partially assembled subunits that cannot appropriately bind to the selected antigen or carry out effector functions.

It appears that a comparable folding-based retention mechanism also operates on some LCs. Many LCs fold readily and can be secreted by themselves as monomers [69] or dimers [70], suggesting that the V_L domains of these LCs probably belong to folding categories 1 and 2 as described above (Fig. 2). However, LCs exist that are not secreted without HC [71] due to a failure of the V_L domain to fold appropriately by itself [72], suggesting that these V_L domains might belong to category 3. The requirement for assembly-assisted folding of some V_L, and presumably V_H domains, would limit which V_L and V_H pairings could pass ER quality control. It is likely that only self-folding V regions on one chain could complement and fold the assembly-dependent V regions on the other chain, in much the same way that a folded C_L domain is required to induce folding of the C_H1 domain. This possibility could explain why only certain possible HC and LC pairings are observed in cell lines and immune responses [73].

Concluding remarks and future perspectives

The combination of biophysical and *in vivo* studies on individual Ig domains, antibody fragments, and complete antibodies has identified common themes that together provide a detailed picture of IgG folding (Figure 4). Once the internal disulfide bridge is formed, most domains will autonomously fold in at least a three-step reaction. The first observable step is the formation of an on-pathway folding intermediate whose lifetime is increased by incorrect peptidyl-prolyl isomerization states. Subsequent peptidyl-prolyl isomerization reactions control folding to the native state, assembly and formation of interchain disulfide bridges, and might also play a part in inhibiting aggregation. In the case of most IgG subclasses, once the C_H3 domain folds it induces dimerization of the HC, which is further stabilized by the formation of disulfide bonds in the hinge region (Figure 4). At this stage, all the constant-region domains except C_H1 are folded. In general, folding of the LCs will occur independently and in parallel. Association of a folded LC with HC will induce C_H1 domain folding, and once C_H1 is completely folded, the assembly of HCs and LCs will be stabilized by an interchain disulfide bond (Figure 4). In the cell, the individual steps are

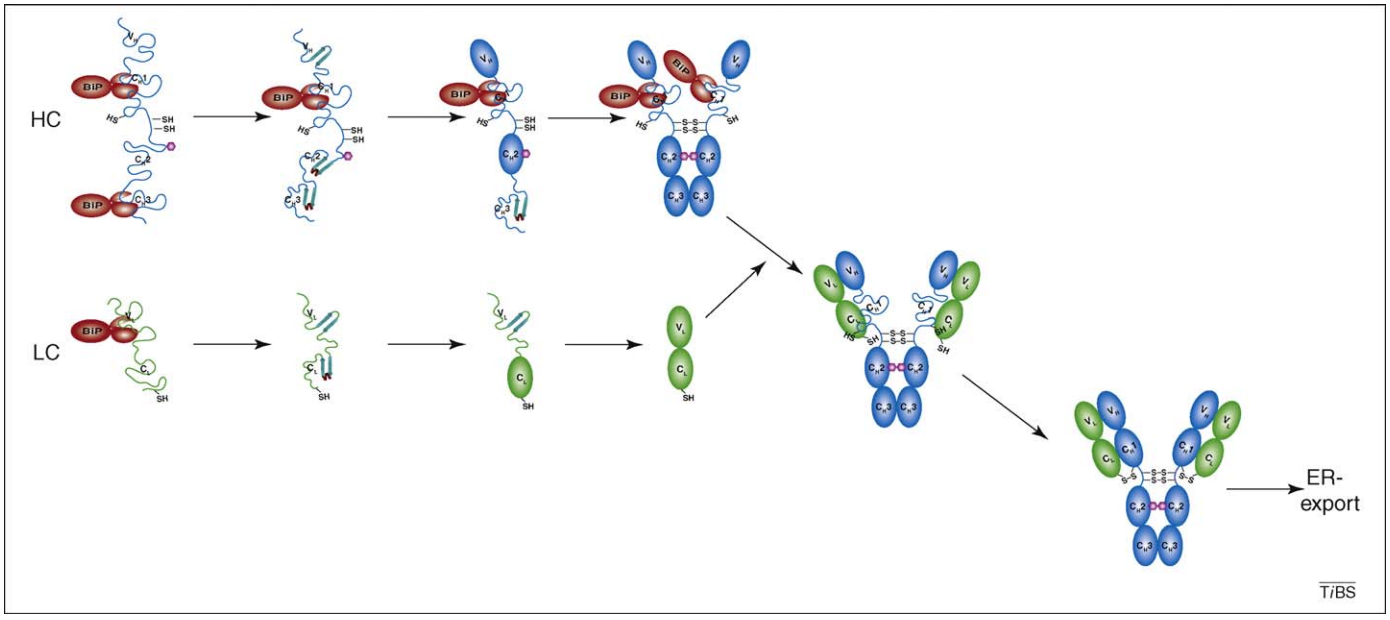

Figure 4. A comprehensive view of the folding and assembly of IgG. Folding, formation of disulfide bridges and glycosylation of the HC (blue) and LC (green) begins cotranslationally in the ER. The molecular chaperone BiP (red) interacts with most of the domains transiently before folding is completed. All constant domains except C_H1 and most variable domains fold autonomously, populating an on-pathway intermediate on the way to the native state. C_L is known to fold rapidly in the cell. Once C_H3 is folded, it induces HC dimerization, which will be solidified by disulfide bridges in the hinge region. C_H1 remains unfolded, unoxidized and stably bound to BiP until the LC displaces BiP and C_L induces folding of the C_H1 domain. Once the important C_H1 prolines are in the correct isomerization state and C_H1 is folded, a disulfide bridge between the LC and the HC forms, rendering the IgG molecules ready for secretion. Most of these steps are likely to hold for other Ig classes. Chaperones and folding catalysts, such as Grp94, protein disulfide isomerase (PDI) and the peptidyl-prolyl isomerase cyclophilin B contribute to the individual steps in Ig biogenesis.

attended by the ER chaperone machinery, which associates co-translationally with precursor HC and LC. It allows high concentrations of unfolded domains to exist without aggregating, slow steps to be accelerated and folding to take place co-translationally [74]. Future work will therefore focus on further integrating the role of the complex ER folding network in modulating, synchronizing and controlling the folding and assembly of antibodies and other IgSF members.

Acknowledgements

We thank Moritz Marcinowski and Dr. Roger Müller for helpful comments on the manuscript and Julia Behnke for help with preparing Box 1. Funding is gratefully acknowledged of: M.J.F. by the Studienstiftung des deutschen Volkes; J.B. by the DFG SFB 749 and the Fonds der chemischen Industrie; and L.M.H. by NIH Grant GM54068, the Cancer Center CORE Grant CA21765, and the American Lebanese Syrian Associated Charities of Saint Jude Children's Research Hospital.

References

1 Murphy, K.M. *et al.* (2008) *Janeway's Immunobiology, Chapters 3 & 4*, (7th edition.), Garland Science, (New York)
2 Maki, R. *et al.* (1980) Exon shuffling generates an immunoglobulin heavy chain gene. *Proc. Natl. Acad. Sci. U. S. A* 77, 2138–2142
3 Early, P. *et al.* (1980) An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: VH, D and JH. *Cell* 19, 981–992
4 Calame, K. *et al.* (1980) Mouse Cmu heavy chain immunoglobulin gene segment contains three intervening sequences separating domains. *Nature* 284, 452–455
5 Alt, F.W. *et al.* (1987) Development of the primary antibody repertoire. *Science* 238, 1079–1087
6 Bernard, O. *et al.* (1978) Sequences of mouse immunoglobulin light chain genes before and after somatic changes. *Cell* 15, 1133–1144
7 Bergman, L.W. and Kuehl, W.M. (1979) Formation of an intrachain disulfide bond on nascent immunoglobulin light chains. *J. Biol. Chem.* 254, 8869–8876
8 Baumal, R. *et al.* (1971) Synthesis, assembly, and secretion of gamma globulin by mouse myeloma cells. 3. Assembly of the three subclasses of IgG. *J. Exp. Med.* 134, 1316–1334
9 Zack, D.J. *et al.* (1981) Somatically generated mouse myeloma variants synthesizing IgA half-molecules. *J. Exp. Med.* 154, 1554–1569
10 Bergman, L.W. and Kuehl, W.M. (1978) Temporal relationship of translation and glycosylation of immunoglobulin heavy and light chains. *Biochemistry* 17, 5174–5180
11 Huber, R. *et al.* (1976) Crystallographic structure studies of an IgG molecule and an Fc fragment. *Nature* 264, 415–420
12 Krapp, S. *et al.* (2003) Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J. Mol. Biol.* 325, 979–989
13 Feige, M.J. *et al.* (2009) Structure of the murine unglycosylated IgG1 Fc fragment. *J. Mol. Biol.* 391, 599–608
14 Sitia, R. *et al.* (1984) The role of glycosylation in secretion and membrane expression of immunoglobulins M and A. *Mol. Immunol.* 21, 709–719
15 Hickman, S. and Kornfeld, S. (1978) Effect of tunicamycin on IgM, IgA, and IgG secretion by mouse plasmacytoma cells. *J. Immunol.* 121, 990–996
16 Kalmanson, G.M. and Bronfenbrenner, J. (1942) The reversal of Pneumococcus Quellung by digestion of the antibody with papain. *Science* 96, 21–22
17 Porter, R.R. (1950) The formation of a specific inhibitor by hydrolysis of rabbit antiovalbumin. *Biochem. J.* 46, 479–484
18 Bork, P. *et al.* (1994) The Immunoglobulin Fold - Structural Classification, Sequence Patterns and Common Core. *J. Mol. Biol.* 242, 309–320
19 Goto, Y. *et al.* (1979) Refolding of the immunoglobulin light chain. *J Biochem.* 85, 1427–1438
20 Du, P.L. *et al.* (2004) Immunoglobulin superfamily receptors in protochordates: before RAG time. *Immunol. Rev.* 198, 233–248
21 Hsu, E. *et al.* (2006) The plasticity of immunoglobulin gene systems in evolution. *Immunol. Rev.* 210, 8–26
22 Dooley, H. and Flajnik, M.F. (2006) Antibody repertoire development in cartilaginous fish. *Dev. Comp. Immunol.* 30, 43–56
23 Williams, A.F. and Barclay, A.N. (1988) The immunoglobulin superfamily–domains for cell surface recognition. *Annu. Rev. Immunol.* 6, 381–405
24 Barclay, A.N. (2003) Membrane proteins with immunoglobulin-like domains–a master superfamily of interaction molecules. *Semin. Immunol.* 15, 215–223
25 Rougon, G. and Hobert, O. (2003) New insights into the diversity and function of neuronal immunoglobulin superfamily molecules. *Annu. Rev. Neurosci.* 26, 207–238
26 Halaby, D.M. and Mornon, J.P. (1998) The immunoglobulin superfamily: an insight on its tissular, species, and functional diversity. *J. Mol. Evol.* 46, 389–400
27 Goto, Y. and Hamaguchi, K. (1982) Unfolding and Refolding of the Constant Fragment of the Immunoglobulin Light Chain. *J. Mol. Biol.* 156, 891–910
28 Goto, Y. and Hamaguchi, K. (1982) Unfolding and refolding of the reduced constant fragment of the immunoglobulin light chain – kinetic role of the intrachain disulfide bond. *J. Mol. Biol.* 156, 911–926
29 Isenman, D. *et al.* (1979) Folding pathways of immunoglobulin domains, The folding kinetics of the C3 domain of human IgG. *Biochemistry* 18, 3327–3336
30 Ramm, K. *et al.* (1999) Removal of the conserved disulfide bridges from the scFv fragment of an antibody: effects on folding kinetics and aggregation. *J. Mol. Biol.* 290, 535–546
31 Feige, M.J. *et al.* (2004) Folding mechanism of the C(H)2 antibody domain. *J. Mol. Biol.* 344, 107–118
32 Lilie, H. *et al.* (1995) Association of antibody chains at different stages of folding: prolyl isomerization occurs after formation of quaternary structure. *J. Mol. Biol.* 248, 190–201
33 Thies, M.J.W. *et al.* (1999) Folding and association of the antibody domain C(H)3: Prolyl isomerization preceeds dimerization. *J. Mol. Biol.* 293, 67–79
34 Feige, M.J. *et al.* (2008) The structure of a folding intermediate provides insight into differences in immunoglobulin amyloidogenicity. *Proc. Natl. Acad. Sci. U. S. A* 105, 13373–13378
35 Freund, C. *et al.* (1996) Folding nuclei of the scFv fragment of an antibody. *Biochemistry* 35, 8457–8464
36 Feige, M.J. *et al.* (2007) Influence of the internal disulfide bridge on the folding pathway of the C-L antibody domain. *J. Mol. Biol.* 365, 1232–1244
37 Feige, M.J. *et al.* (2009) An unfolded CH1 domain controls the assembly and secretion of IgG antibodies. *Mol. Cell* 34, 569–579
38 Simpson, E.R. *et al.* (2009) The folding pathway of the antibody V(L) domain. *J. Mol. Biol* 392, 1326–1338
39 Thies, M.J. *et al.* (2002) Folding and oxidation of the antibody domain C(H)3. *J. Mol. Biol.* 319, 1267–1277
40 Geierhaas, C.D. *et al.* (2004) Comparison of the transition states for folding of two Ig-like proteins from different superfamilies. *J. Mol. Biol.* 343, 1111–1123
41 Lappalainen, I. *et al.* (2008) Plasticity within the obligatory folding nucleus of an immunoglobulin-like domain. *J. Mol. Biol.* 375, 547–559
42 Mizuguchi, M. *et al.* (2003) Folding of a beta-sheet protein monitored by real-time NMR spectroscopy. *J. Mol. Biol* 328, 1161–1171
43 Kameda, A. *et al.* (2005) Nuclear magnetic resonance characterization of the refolding intermediate of beta(2)-microglobulin trapped by non-native prolyl peptide bond. *J. Mol. Biol.* 348, 383–397
44 Jahn, T.R. *et al.* (2006) Amyloid formation under physiological conditions proceeds via a native-like folding intermediate. *Nat. Struct. Mol. Biol.* 13, 195–201
45 Qin, Z.J. *et al.* (2007) Structural characterization of the partially folded intermediates of an immunoglobulin light chain leading to amyloid fibrillation and amorphous aggregation. *Biochemistry* 46, 3521–3531
46 Kudo, A. and Melchers, F. (1987) A second gene, VpreB in the lambda 5 locus of the mouse, which appears to be selectively expressed in pre-B lymphocytes. *EMBO J.* 6, 2267–2272
47 Sakaguchi, N. and Melchers, F. (1986) Lambda 5, a new light-chain-related locus selectively expressed in pre-B lymphocytes. *Nature* 324, 579–582

48 Karasuyama, H. *et al.* (1990) The proteins encoded by the VpreB and lambda 5 pre-B cell-specific genes can associate with each other and with mu heavy chain. *J. Exp. Med.* 172, 969–972
49 Shaffer, A.L. and Schlissel, M.S. (1997) A truncated heavy chain protein relieves the requirement for surrogate light chains in early B cell development. *J. Immunol.* 159, 1265–1275
50 Mains, P.E. and Sibley, C.H. (1983) The requirement of light chain for the surface deposition of the heavy chain of immunoglobulin M. *J. Biol. Chem.* 258, 5027–5033
51 Coffino, P. and Scharff, M.D. (1971) Rate of somatic mutation in immunoglobulin production by mouse myeloma cells. *Proc. Natl. Acad. Sci. U. S. A* 68, 219–223
52 Kohler, G. (1980) Immunoglobulin chain loss in hybridoma lines. *Proc. Natl. Acad. Sci. U. S. A* 77, 2197–2199
53 Seligmann, M. *et al.* (1979) Heavy chain diseases: current findings and concepts. *Immunol. Rev.* 48, 145–167
54 Morrison, S.L. (1978) Murine heavy chain disease. *Eur. J. Immunol.* 8, 194–199
55 Hamers-Casterman, C. *et al.* (1993) Naturally-occurring antibodies devoid of light-chains. *Nature* 363, 446–448
56 Lippincott-Schwartz, J. *et al.* (1988) Degradation from the endoplasmic reticulum: disposing of newly synthesized proteins. *Cell* 54, 209–220
57 Vembar, S.S. and Brodsky, J.L. (2008) One step at a time: endoplasmic reticulum-associated degradation. *Nat. Rev. Mol. Cell Biol.* 9, 944–957
58 Haas, I.G. and Wabl, M. (1983) Immunoglobulin heavy chain binding protein. *Nature* 306, 387–389
59 Bole, D.G. *et al.* (1986) Posttranslational association of immunoglobulin heavy-chain binding-protein with nascent heavy-chains in nonsecreting and secreting hybridomas. *J. Cell Biol.* 102, 1558–1566
60 Haas, I.G. and Meo, T. (1988) cDNA cloning of the immunoglobulin heavy chain binding protein. *Proc. Natl. Acad. Sci. U. S. A* 85, 2250–2254
61 Munro, S. and Pelham, H.R. (1987) A C-terminal signal prevents secretion of luminal ER proteins. *Cell* 48, 899–907
62 Hellman, R. *et al.* (1999) The in vivo association of BiP with newly synthesized proteins is dependent on the rate and stability of folding and not simply on the presence of sequences that can bind to BiP. *J. Cell Biol.* 144, 21–30
63 Lee, Y.K. *et al.* (1999) BiP and immunoglobulin light chain cooperate to control the folding of heavy chain and ensure the fidelity of immunoglobulin assembly. *Mol. Biol. Cell* 10, 2209–2219
64 Knarr, G. *et al.* (1995) BiP binding sequences in antibodies. *J. Biol. Chem.* 270, 27589–27594
65 Hendershot, L. *et al.* (1987) Assembly and secretion of heavy-chains that do not associate posttranslationally with immunoglobulin heavy-chain binding-protein. *J. Cell Biol.* 104, 761–767
66 Braakman, I. *et al.* (1992) Manipulating disulfide bond formation and protein folding in the endoplasmic reticulum. *EMBO J.* 11, 1717–1722
67 Vanhove, M. *et al.* (2001) Unassembled Ig heavy chains do not cycle from BiP in vivo but require light chains to trigger their release. *Immunity* 15, 105–114
68 Hendershot, L.M. and Sitia, R. (2004) Antibody synthesis and assembly. *Mol. Biol. B Cells* 261–273
69 Dul, J.L. *et al.* (1996) Ig light chains are secreted predominantly as monomers. *J. Immunol.* 157, 2969–2975
70 Leitzgen, K. *et al.* (1997) Assembly of immunoglobulin light chains as a prerequisite for secretion. A model for oligomerization-dependent subunit folding. *J. Biol. Chem.* 272, 3117–3123
71 Horibata, K. and Harris, A.W. (1970) Mouse myelomas and lymphomas in culture. *Exp. Cell Res.* 60, 61–77
72 Skowronek, M.H. *et al.* (1998) The variable domain of nonassembled Ig light chains determines both their half-life and binding to the chaperone BiP. *Proc. Natl. Acad. Sci U. S. A* 95, 1574–1578
73 Wiens, G.D. *et al.* (1998) Harmful somatic mutations: lessons from the dark side. *Immunol. Rev.* 162, 197–209
74 Komar, A.A. (2009) A pause for thought along the co-translational folding pathway. *Trends Biochem. Sci.* 34, 16–24
75 Reimer, U. *et al.* (1998) Side-chain effects on peptidyl-prolyl cis/trans isomerisation. *J. Mol. Biol.* 279, 449–460
76 Stewart, D.E. *et al.* (1990) Occurrence and role of cis peptide bonds in protein structures. *J. Mol. Biol.* 214, 253–260
77 Brandts, J.F. *et al.* (1975) Consideration of the Possibility that the slow step in protein denaturation reactions is due to cis-trans isomerism of proline residues. *Biochemistry* 14, 4953–4963
78 Schmid, F.X. and Baldwin, R.L. (1978) Acid catalysis of the formation of the slow-folding species of RNase A: evidence that the reaction is proline isomerization. *Proc. Natl. Acad. Sci. U. S. A* 75, 4764–4768
79 Eckert, B. *et al.* (2005) Prolyl isomerization as a molecular timer in phage infection. *Nat. Struct. Mol. Biol.* 12, 619–623
80 Lu, K.P. *et al.* (2007) Prolyl cis-trans isomerization as a molecular timer. *Nat. Chem. Biol.* 3, 619–629
81 Fischer, G. *et al.* (1989) Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337, 476–478
82 Lang, K. *et al.* (1987) Catalysis of protein folding by prolyl isomerase. *Nature* 329, 268–270
83 Buchner, J. *et al.* (1991) Alternatively folded states of an immunoglobulin. *Biochemistry* 30, 6922–6929
84 Thies, M.J. *et al.* (2001) The alternatively folded state of the antibody C(H)3 domain. *J. Mol. Biol.* 309, 1077–1085
85 Bellotti, V. *et al.* (2000) Review: Immunoglobulin light chain amyloidosis - The archetype of structural and pathogenic variability. *J. Struct. Biol.* 130, 280–289
86 Anelli, T. *et al.* (2003) Thiol-mediated protein retention in the endoplasmic reticulum: the role of ERp44. *EMBO J.* 22, 5015–5022
87 Melnick, J. *et al.* (1994) Sequential interaction of the chaperones BiP and GRP94 with immunoglobulin chains in the endoplasmic reticulum. *Nature* 370, 373–375
88 Hochstenbach, F. *et al.* (1992) Endoplasmic reticulum resident protein of 90 kilodaltons associates with the T- and B-cell antigen receptors and major histocompatibility complex antigens during their assembly. *Proc. Natl. Acad. Sci. U. S. A* 89, 4734–4738
89 Brightman, S.E. *et al.* (1995) Isolation of a mouse cDNA encoding MTJ1, a new murine member of the DnaJ family of proteins. *Gene* 153, 249–254
90 Tyedmers, J. *et al.* (2000) Homologs of the yeast Sec complex subunits Sec62p and Sec63p are abundant proteins in dog pancreas microsomes. *Proc. Natl. Acad. Sci. U. S. A* 97, 7214–7219
91 Meunier, L. *et al.* (2002) A sub-set of chaperones and folding enzymes form multi-protein complexes in the ER to bind nascent proteins. *Mol. Biol. Cell* 12, 488A
92 Shen, Y. *et al.* (2002) Identification and characterization of a novel endoplasmic reticulum (ER) DnaJ homologue, which stimulates ATPase activity of BiP in vitro and is induced by ER stress. *J. Biol. Chem.* 277, 15947–15956
93 Cunnea, P.M. *et al.* (2003) ERdj5, an endoplasmic reticulum (ER)-resident protein containing DnaJ and thioredoxin domains, is expressed in secretory cells or following ER stress. *J. Biol. Chem.* 278, 1059–1066
94 Rutkowski, D.T. *et al.* (2007) The role of p58IPK in protecting the stressed endoplasmic reticulum. *Mol. Biol. Cell* 18, 3681–3691
95 Zahedi, R.P. *et al.* (2009) Analysis of the membrane proteome of canine pancreatic rough microsomes identifies a novel Hsp40, termed ERj7. *Proteomics.* 9, 3463–3473
96 Chen, X. *et al.* (1996) The 170 kDa glucose regulated stress protein is a large HSP70-, HSP110-like protein of the endoplasmic reticulum. *FEBS Lett.* 380, 68–72
97 Chung, K.T. *et al.* (2002) BAP, a mammalian BiP-associated protein, is a nucleotide exchange factor that regulates the ATPase activity of BiP. *J. Biol. Chem.* 277, 47557–47563
98 Hayashi, T. and Su, T.P. (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. *Cell* 131, 596–610
99 Roth, R.A. and Pierce, S.B. (1987) In vivo cross-linking of protein disulfide isomerase to immunoglobulins. *Biochemistry* 26, 4179–4182
100 van Anken, E. *et al.* (2009) Efficient IgM assembly and secretion require the plasma cell induced endoplasmic reticulum protein pERp1. *Proc. Natl. Acad. Sci. U. S. A* 106, 17019–17024
101 Shimizu, Y. *et al.* (2009) pERp1 is significantly up-regulated during plasma cell differentiation and contributes to the oxidative folding of immunoglobulin. *Proc. Natl. Acad. Sci. U. S. A* 106, 17013–17018

102 Rupp, K. *et al.* (1994) Effects of CaBP2, the rat analog of ERp72, and of CaBP1 on the refolding of denatured reduced proteins. Comparison with protein disulfide isomerase. *J. Biol. Chem.* 269, 2501–2507

103 Lindquist, J.A. *et al.* (1998) ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. *EMBO J.* 17, 2186–2195

104 Mkrtchian, S. *et al.* (1998) A stress-inducible rat liver endoplasmic reticulum protein. *ERp29. Eur. J. Biochem.* 251, 304–313

105 Rao, A.K. *et al.* (1976) Biosynthesis of the carbohydrate units of immunoglobulins. 1. Purification and properties of galactosyltransferases from swine mesentary lymph nodes. *Biochemistry* 15, 5001–5009