Published in final edited form as:

Immunogenetics. 2019 March ; 71(3): 251–261. doi:10.1007/s00251-019-01105-0.

Origin and evolution of the specialized forms of proteasomes involved in antigen presentation

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Abstract

Proteasomes are a multi-subunit protease complex that produces peptides bound by major histocompatibility complex (MHC) class I molecules. Phylogenetic studies indicate tahat two specialized forms of proteasomes, immunoproteasomes and thymoproteasomes, and the proteasome activator PA28αβ emerged in a common ancestor of jawed vertebrates which acquired adaptive immunity based on the MHC, T cell-receptors and B-cell receptors ~500 million years ago. Comparative genomics studies now provide strong evidence that the genes coding for the immunoproteasome subunits emerged by genome-wide duplication. On the other hand, the gene encoding the thymoproteasome subunit β5t emerged by tandem duplication from the gene coding for the β5 subunit. Strikingly, birds lack immunoproteasomes, thymoproteasomes and the proteasome activator PA28αβ, raising an interesting question of whether they have evolved any compensatory mechanisms.

Keywords

adaptive immune system; genome-wide duplication; immunoproteasome; proteasome activator; thymoproteasome

Introduction

Eukaryotic cells have two major pathways for intracellular protein degradation. One is lysosomal autophagy (Nakatogawa et al. 2009) and the other is the ubiquitin-proteasome system (Tanaka 2009). In the latter, proteins covalently modified with a chain of ubiquitin are specifically recognized by a large protease complex, the 26S proteasome, leading to the degradation of ubiquitin-tagged proteins (Bard et al. 2018; Collins and Goldberg 2017). The ubiquitin-proteasome system is indispensable for cell survival and plays a critical role in many essential cellular processes including protein quality control, regulation of cell cycle progression, transcription, cell trafficking and responses to oxidative stress. The catalytic

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing financial interests.

core of the 26S proteasome, known as the 20S proteasome, has a cylindrical structure composed of a stack of four heptameric rings; the two outer rings are made up of catalytically inactive α subunits, whereas the two inner rings, which are made up of β subunits, contain catalytic subunits, with active sites facing the interior of the cylinder (Unno et al. 2002).

Proteasomes are of ancient origin. They are found not only in eukaryotes but also in all archaea and some eubacteria such as actinobacteria and mycobacteria (Maupin-Furlow 2011). Although the overall structure of the 20S proteasome is highly conserved between eukaryotes and prokaryotes, the complexity of α and β subunits is generally low in prokaryotes. Thus, the archaebacterium *Thermoplasma acidophilum* has only one kind of α and β subunits, respectively (Lowe et al. 1995). Eubacterial 20S proteasomes usually have one or two kinds of α and β subunits, respectively (Jastrab and Darwin 2015). In contrast, eukaryotic 20S proteasomes contain seven distinct α subunits designated as α1 to α7 and seven distinct β subunits designated as β1 to β7. Among the seven β subunits, only β1 (also known as PSMB6), β2 (also known as PSMB7) and β5 (also known as PSMB5) subunits are proteolytically active and exert caspase-like, trypsin-like and chymotrypsin-like activities, respectively, which confer the ability to cleave peptide bonds at the C-terminal side of acidic, basic and hydrophobic amino acid residues, respectively (Table 1). Archaeal and eubacterial proteasomes efficiently cleave short fluorogenic peptides only after hydrophobic residues whereas eukaryotic proteasomes exhibit three major peptidase activities (Dolenc et al. 1998). Therefore, the chymotrypsin-like activity is most likely the most ancient, fundamental one.

In the immune system, proteasomes play a critical role in the production of antigenic peptides bound by major histocompatibility complex (MHC) class I molecules (Heemels and Ploegh 1995; Kniepert and Groettrup 2014; Tanaka and Kasahara 1998). Notably, jawed vertebrates have two specialized forms of 20S proteasomes named immunoproteasomes (Akiyama et al. 1994) and thymoproteasomes (Murata et al. 2007) in addition to the standard or constitutive 20S proteasome shared by all eukaryotes (Fig. 1). These specialized forms of proteasomes differ from the standard 20S proteasome in the composition of catalytic β subunits (Murata et al. 2018). As a result, immunoproteasomes, which have β1i (also known as LMP2, PSMB9), β2i (also known as MECL1, PSMB10) and β5i (also known as LMP7, PSMB8) subunits instead of β1, β2 and β5 subunits, respectively, have distinct cleavage specificities and produce peptides with C-terminal hydrophobic residues that fit well in the groove of MHC class I molecules more efficiently than standard 20S proteasomes. Recent work has shown that the repertoire of MHC class I-binding peptides differs substantially between wildtype and immunoproteasome-deficient mice and that the presentation of class I epitopes to cytotoxic T cells is greatly impaired in the latter (Kincaid et al. 2011).

Immunoproteasomes are constitutively expressed by professional antigen presenting cells and medullary thymic epithelial cells, but are induced in response to immune and inflammatory stimuli in other cell types (Kniepert and Groettrup 2014). This induction occurs because the expression of genes coding for β1i, β2i and β5i subunits is induced by exposure to inflammatory cytokines such as interferon γ and tumor necrosis factor. Under

inflammatory conditions, immunoproteasomes rather than standard 20S proteasomes are assembled because β1i, β2i and β5i subunits are preferentially incorporated into newly assembled 20S proteasomes in place of β1, β2 and β5 subunits, respectively, partly due to preferential binding of β5i to POMP, a chaperone essential for proteasome assembly (Griffin et al. 1998; Heink et al. 2005).

Another specialized form of proteasome, the thymoproteasome, has β1i, β2i and β5t (also known as PSMB11) subunits instead of β1, β2 and β5 (β5i) subunits, respectively (Murata et al. 2007). Because β5t is expressed exclusively in thymic cortical cells (Murata et al. 2007; Tomaru et al. 2009), thymoproteasomes occur only in the thymic cortex. Unlike β5 and β5i whose substrate-binding pockets are mostly composed of hydrophobic amino acids, the substrate-binding pocket of β5t contains many hydrophilic amino acids, thereby producing a distinct set of peptides that presumably bind to MHC class I molecules with low affinity (Kondo et al. 2018; Nitta et al. 2010; Sasaki et al. 2015; Xing et al. 2013). In thymoproteasome-deficient mice, in which cortical thymic epithelial cells express immunoproteasomes instead of thymoproteasomes, $CD8^+$ T cell production is severely impaired (Murata et al. 2007). It is thought that thymoproteasomes play a critical role in thymic positive selection of $CD8^+$ T cells through producing a unique set of peptides involved in positive selection and/or minimizing the overlap of the repertoire of MHC class I-binding peptides displayed to developing T cells in the cortex and medulla where positive and negative selection takes place, respectively (Klein et al. 2014; Murata et al. 2018; Nitta et al. 2010; Takahama et al. 2012).

Here we provide a brief overview of proteasome evolution mainly focusing on immunoproteasomes and thymoproteasomes.

Evolution of adaptive immunity and the origin of specialized forms of proteasomes

Accumulated evidence indicates that lymphocyte-based adaptive immunity emerged in a common ancestor of vertebrates and once established its basic design has been maintained throughout vertebrate evolution (Flajnik and Kasahara 2001, 2010; Kaufman 2018b) (Fig. 2). Thus, the essential features of lymphocyte-based adaptive immunity, such as the clonal expression of a single type of antigen receptors with allelic exclusion, the clonal proliferation of antigen-stimulated lymphocytes and the dichotomy of lymphocytes into T and B cells are shared by all vertebrates (Boehm et al. 2012; Hirano et al. 2013). There are, however, substantial differences between the adaptive immune systems of jawed and jawless vertebrates (Boehm 2011; Boehm et al. 2018; Flajnik and Kasahara 2010; Kasahara and Sutoh 2014). In jawed vertebrates ranging from cartilaginous fishes to mammals, three cardinal elements involved in antigen recognition are T-cell receptors, B-cell receptors and MHC molecules (Flajnik 2018a). By contrast, jawless vertebrates, represented by lampreys and hagfish, have neither MHC class I nor class II molecules and use variable lymphocyte receptors (VLRs) as antigen receptors (Cooper and Alder 2006; Flajnik 2018b). Unlike T-cell and B-cell receptors, which are both members of the immunoglobulin superfamily, VLRs are members of the leucine-rich repeat (LRR) family and are composed of an N-

terminal cap, a variable number of multiple LRR modules, a connecting peptide, a C-terminal cap and an invariant stalk. Because the sequences of LRR modules are diverse, and the number of modules assembled into the *VLR* gene is variable, a single *VLR* gene can generate diversity comparable to that of antigen receptors of jawed vertebrates. Because T and B lymphocyte lineages are conserved in all vertebrates, jawed and jawless vertebrates appear to have adopted different molecules for antigen recognition within the context of specialized lymphocyte lineages (Boehm et al. 2012; Flajnik and Kasahara 2010; Kasahara and Sutoh 2014).

Initial evidence that immunoproteasomes occur in non-mammalian vertebrates was obtained in *Xenopus laevis*, from which the genes coding for β5i and β1i were identified (Namikawa et al. 1995; Nonaka et al. 1997a). Subsequently, the cartilaginous fish was shown to have genes coding for β5i and β5i-like subunits, but in the same study the gene coding for β5i was identified in neither lampreys nor hagfishes, suggesting that immunoproteasomes occur only in jawed vertebrates (Kandil et al. 1996). This suggestion is now confirmed by genome sequence information accumulated in various vertebrates and invertebrates. The gene coding for the β5t subunit, designated *PSMB11*, also occurs in the cartilaginous fish but not in jawless vertebrates or invertebrates (Sutoh et al. 2012). Considering that immunoproteasomes and thymoproteasomes apparently have functions dedicated to the production of MHC class I-binding peptides, it is reasonable that these specialized forms of proteasomes occur only in jawed vertebrates possessing MHC class I molecules.

Genome-wide duplications (GWDs) and evolution of immunoproteasomes

Susumu Ohno proposed based mainly on cytological evidence that one or two rounds of GWD took place close to the origin of vertebrates (Ohno 1970). The refined version of this proposal, which became known as the 2R (two round) hypothesis, assumes that the genome of jawed vertebrates underwent two rounds of GWD (2R-GWD) close to the origin of vertebrates (Kasahara 2007, 2013). The 2R hypothesis is now strongly supported by comparative genomics analysis, although the timing of GWD relative to the emergence of jawless vertebrates is still controversial (Mehta et al. 2013; Smith et al. 2018). One of the key observations underpinning the 2R hypothesis is genome paralogy, which refers to the occurrence of closely linked sets of paralogous genes on multiple, typically four different chromosomes. In humans and mice, the genes coding for β1i and β5i (*PSMB9* and *PSMB8* in humans, and *Psmb9* and *Psmb8* in mice) are in the class II region of the MHC on chromosomes 6 and 17, respectively. Mapping of the gene coding for the β2 subunit (*PSMB7* in humans and *Psmb7* in mice) showed that it is located on the regions of human chromosome 9 and mouse chromosome 2 containing a group of genes, the paralogous copies of which are located in the MHC, providing the first indication that the MHC region retains the traces of ancient GWDs (Kasahara et al. 1996). Subsequent studies showed that, along with the *HOX* gene clusters, the MHC is a prototypical region exhibiting genome paralogy, with an extensive number of gene families sharing paralogous copies among four major paralogous regions (the MHC on chromosome 6 and specific regions of chromosomes 1, 9 and 19) and other minor paralogous regions (Flajnik and Kasahara 2001, 2010; Kasahara 2007). Prompted by this observation, it was realized that other immune gene families such as

chemokine, JAK/STAT and B7 have paralogous copies in the regions displaying genome paralogy (Kasahara 1998, 2010).

Among the seven β subunits of the 20S proteasome, three catalytic β subunits, β1, β2 and β5, are more closely related to each other than they are to other β subunits. Also, β1i, β2i and β5i, which occur only in jawed vertebrates, are most closely related to β1, β2 and β5, respectively, indicating that each immunoproteasome subunit emerged by gene duplication from the evolutionarily more ancient, corresponding subunit of the standard 20S proteasome. Based on these observations and the finding that the gene coding for the β2 subunit maps to the region paralogous to the MHC, it was proposed that *PSMB8*, *PSMB9* and *PSMB10* arose from *PSMB6*, *PSMB5* and *PSMB7*, respectively, not by individual duplications, but by 2R-GWD (Kasahara et al. 1996)(Fig. 3). However, in mammals, the *PSMB10* gene coding for the β2i subunit is not encoded in the MHC; in addition, the genes coding for β1, β2 and β5 are unlinked and do not map to any of the four major paralogous regions initially identified, apparently contradicting the idea that the three immunoproteasome subunit genes arose by GWDs. However, genes often change their locations due to translocations and other mechanisms. It was therefore assumed that the genes coding for β1, β2i and β5 changed their locations secondarily.

Subsequent studies in non-mammalian vertebrates showed that the gene encoding β2i is encoded in the MHC region in *Xenopus*, and that the *Xenopus* β1 subunit is encoded in one of the MHC paralogous regions (Ohta et al. 2006). In zebrafish, the class I region of the MHC was found to contain the *psmb13* gene (a member of the β2 subunit family distinct from *psmb10*), although the teleost *psmb10* gene, which was initially mapped to the class I region of the MHC (Clark et al. 2000; Lukacs et al. 2007), actually maps outside of the MHC region on a separate chromosome (McConnell et al. 2016). Furthermore, the region of human chromosome 14 (14q11-q32) containing the gene for β5 is now considered to be part of the MHC paralogous regions (Flajnik and Kasahara 2010; Kasahara 2007). Therefore, it seems that the proteasome subunit genes whose chromosomal localization in mammals initially appeared contradictory to what was expected from 2R-GWD changed their locations secondarily in the course of vertebrate evolution. Considering all the evidence accumulated in various vertebrate species, it is now very likely that the genes coding for immunoproteasome subunits emerged by 2R-GWD.

Class I region in the primordial MHC

An interesting feature of the MHC in nonmammalian vertebrates is the close linkage of the immunoproteasome, transporter associated with antigen processing (*TAP*) and TAP-binding protein (*TAPBP*) genes with classical class I genes, whereas in most mammals they are closely linked to class II genes (Michalova et al. 2000; Nonaka et al. 1997b; Ohta et al. 2002; Ohta et al. 2003). These findings suggest that in the primordial MHC, the class I processing and presenting genes co-evolved so that the peptides generated and transported preferentially bound to particular class I allelic products (Flajnik et al. 1999; Kaufman 1999). Consistent with this idea, there are polymorphisms in *TAP* and immunoproteasome genes that co-segregate with class I polymorphisms in amphibians and some fish (see below). Indeed, chicken *TAP* genes are polymorphic and very closely linked to the classical

class I (*BF*) genes (Kaufman 1999). Chicken class I allelic products are either 'permissive' (generalists) and capable of binding to many peptides or 'restrictive' (specialists) binding to peptides with defined anchor residues (Kaufman 2018a); the peptides transported by a particular TAP allelic product have a propensity to bind to the class I allelic product, the coding genes of which are linked to each other (Kaufman 2015; Walker et al. 2011).

psmb8 biallelism in ectotherms

In most ectotherms studied, there are biallelic lineages of *psmb8*, most conspicuously with modifications in the catalytic site (Kandil et al. 1996; Namikawa et al. 1995; Ohta et al. 2002; Tsukamoto et al. 2012). One lineage with an alanine at position 31 is predicted to be similar to mammalian β5i, which was shown in mammals to produce peptides with hydrophobic C-termini. The catalytic site in the other allele has a conspicuous phenylalanine-31 that is predicted to modify the range of peptides generated (Kandil et al. 1996; Ohta et al. 2002). This biallelism is found in cartilaginous fish, teleosts and amphibians, strongly suggesting that this is the primordial feature of the β5i subunit. *Xenopus* also shows biallelism of *tap*, a feature that extends to all *Xenopus* species, which unfailingly co-segregates with particular *psmb8* and class I alleles. Wild frogs of both *X. laevis* and *X. tropicalis* maintain these two allelic syntenic sets, demonstrating a balancing selection within populations over 100 million years and likely a block of recombination (i.e. cold spots) between the three genes (Ohta et al. 2003). The paradigm put forward by Kaufman on selection for class I 'generalists' and 'specialists' in birds may also apply to the class I system in ectotherms (Kaufman 2018a).

This Ala/Phe-31 polymorphism of *psmb8* is found in many ectotherms, yet its loss in several fish and amphibians suggests it is under a strong pressure and it can be re-established by convergent evolution in different animal groups (Huang et al. 2013). Additionally, in sharks and some bony fish there has been an expansion of immunoproteasome genes suggesting that: 1) the peptide repertoire can be expanded in some species; and 2) ectotherm classical class I molecules are much more dependent on proteasome-generated peptides than class I molecules of most mammals (McConnell et al. 2016; Michalova et al. 2000; Ohta et al. 2003). We have known of these interesting polymorphisms for a long time, but functional experiments like those done in birds for TAP, e.g. examining peptide generation *in vitro* or impact on class I expression/function *in vivo* have not been done for ectotherm immunoproteasomes or TAP. With the ease of crispr usage in teleost models and *Xenopus*, the experiments should be relatively easy to implement.

Evolution of thymoproteasomes

Like immunoproteasomes, thymoproteasomes are an invention by jawed vertebrates. However, unlike immunoproteasomes, the *PSMB11*gene coding for the thymoproteasome-specific subunit β5t appears to have emerged by tandem duplication from the evolutionarily more ancient *PSMB5* gene coding for the β5 subunit (Sutoh et al. 2012)(Fig. 3). This is indicated by the fact that *PSMB11* is located adjacent to the *PSMB5* gene in all classes of jawed vertebrates for which information is available, expect for birds that have lost *PSMB11*. A striking feature of *PSMB11* is the complete absence of introns; most β-subunit

genes including those coding for β1, β1i, β2, β2i and β5i subunits have six to eight exons, with similar exon–intron organization (Hayashi et al. 1997; Tanaka and Kasahara 1998). The most plausible explanation for this complete absence of introns is that the *PSMB5* gene underwent tandem duplication and that the reverse-transcribed, processed mRNA derived from *PSMB5* replaced another copy of *PSMB5* next to the source gene by homologous recombination (Kaessmann et al. 2009). Interestingly, gnathostome *PSMB5* genes have only three exons and do not share any exon-intron boundaries with those of other β subunit genes, whereas lamprey and amphioxus *PSMB5* genes have exon-intron organization typical of β subunit genes (Abdulla et al. 1996; Kohda et al. 1997; Sutoh et al. 2012). Therefore, the *PSMB5* gene appears to have undergone major alterations in gene structure in a jawed vertebrate lineage. It is unclear whether this change in gene structure has something to do with the emergence of *PSMB11*. The alteration in gene structure may have taken place prior to the tandem duplication that gave rise to an ancestor of *PSMB11* (Fig. 3, panel B, top). Alternatively, *PSMB5* may have changed its structure after it had diverged from *PSMB11* (Fig. 3, panel B, middle). A third possibility is that the loss of introns in *PSMB5* took place before tandem duplication and that *PSMB5* acquired introns newly after duplication (Fig. 3, panel B, bottom).

In mammals, reptiles, amphibians and cartilaginous fishes, *PSMB11* is a single copy gene. In contrast, teleost fish have two copies of apparently functional *psmb11* genes, designated *psmb11a* and *psmb11b*, which are thought to have emerged by fish-specific GWD (Sutoh et al. 2012). The β5tb subunit encoded by *psmb11b* has a typical S1 pocket made up of hydrophilic residues, whereas the S1 pocket of the β5ta subunit encoded by *psmb11a* is less hydrophilic, suggesting that the cleavage specificities of the thymoproteasomes containing β5ta and β5tb may differ. Whether both β5t subunits are expressed in cortical thymic epithelial cells remains to be investigated.

Evolution of proteasome activators

Two additional proteasome components with a role apparently specialized for MHC class I-mediated antigen presentation are PA28α and PA28β subunits of the PA28 activator complex, also known as the 11S regulator of the 20S proteasome (Cascio 2014; Tanaka and Kasahara 1998). The PA28 activator complex, which is inducible by stimulation with interferon-γ, is a ring-shaped, cytoplasmic heteroheptamer composed of ~28-kDa α and ~28-kDa β subunits with a stoichiometry of α3β4 or a mixture of α3β4 and α4β3 (Zhang et al. 1999); binding of this complex to the outer α-rings of the 20S proteasome stimulates its peptidase activity in an ATP-independent manner and enhances the generation of class I-binding peptides by promoting the assembly of the immunoproteasome and altering its cleavage pattern (de Graaf et al. 2011). Enhanced expression of the PA28α subunit in virus-infected fibroblasts resulted in more efficient presentation of viral peptides to cytotoxic T cells (Groettrup et al. 1996). Furthermore, mice lacking the PA28α subunit (Preckel et al. 1999) or both PA28α and PA28β subunits (Murata et al. 2001) showed impaired cytotoxic T cell responses against some epitopes, demonstrating a critical role of PA28αβ in MHC class I-mediated presentation of some peptides.

The genes coding for PA28α and PA28β subunits (named *PSME1* and *PSME2*) are members of the PA28 family. A third member of this family is *PSME3* coding for the PA28γ subunit, which was originally identified as Ki antigen in sera of patients with systemic lupus erythematosus (Nikaido et al. 1990). PA28γ, which forms a homoheptamer, is a nuclear activator of the 20S proteasome that plays an important role in the regulation of cell proliferation, apoptosis, nuclear dynamics and cellular stress response (Wilk et al. 2000).

Phylogenetic analysis of the PA28 family of proteins indicates that PA28α and PA28β occur only in jawed vertebrates, whereas PA28γ occurs in wide-ranging multicellular animals including sponges and in some fungi, although it is apparently absent in plants (Fig. 4). Therefore, the PA28γ subunit is clearly of more ancient origin than PA28α and PA28β subunits; it appears that the *PSME1* and *PSME2* genes emerged from the *PSME3* gene in a common ancestor of jawed vertebrates along with other key elements of adaptive immunity including the genes coding for the subunits of immunoproteasomes and thymoproteasomes. In humans and mice, the genes coding for PA28α and PA28β subunits are closely linked to each other in a tail-to-tail orientation, indicating that they arose by tandem duplication (Kandil et al. 1997; Kohda et al. 1998; McCusker et al. 1999). They are also tightly linked in the genomes of *Xenopus tropicalis* (NCBI *Xenopus tropicalis* annotation release 103: NC_030677.1) and coelacanth (NCBI *Latimeria chalumnae* annotation release 101: NW_005821462.1), indicating that the original gene arrangement has been maintained from bony fish to mammals.

Loss of specialized immune proteasomes in birds

The MHC regions of chicken and quail (and most birds) are quite compact, with short introns and intergenic regions, and thus it was relatively easy in the early days of genome analysis to sequence the entire MHC region. Like what was described above for ectotherms, the *TAP* and *TAPBP* genes were found closely linked to class I genes (Kaufman et al. 1999). Yet, many other genes found in the mammalian MHC, including the immunoproteasome genes, were not present (Sutoh et al. 2012). Further genomic and transcriptomic studies over the next 20 years also failed to unearth these genes, and a comprehensive proteomic study of proteasome in activated chicken cells revealed only the constitutive subunits (Erath and Groettrup, 2015; Kaufman 2015). Thus, there is little doubt that birds lost their specialized proteasome genes, including *PSMB11* and proteasome activator *PSME1* and *PSME2* genes. It is tempting to speculate that bird class I's strict dependence on TAP, and the duality of their classical class I system, resulted in the loss of the immune-specialized proteasomes (Kaufman 2018a). However, birds have lost many other immune genes (Magor et al. 2013), and have broken many syntenic groupings outside of the immune system (Lovell et al. 2014), so there may be a general pressure to lose genes rather than something specific to the immune system. Nevertheless, it is doubtful that the loss of both immuno- and thymoproteasome in birds was just a coincidence, since *Psmb11* is co-expressed with *Psmb9* and *Psmb10* in mouse cortical thymic epithelial cells. The lack of the thymoproteasome in birds would call into question the 'peptide-switch' hypothesis in which $CD8^+$ T cells are positively selected on a different set of peptides on cortical thymic epithelial cells and thus would escape negative selection on an entirely different set of peptides on medullary thymic epithelial cells. Another head scratcher: while β5t and other immunoproteasome subunits

may be co-dependent and generate a different set of peptides compared to constitutive proteasomes, the PA28 subunits have an entirely different function, the rapid production of peptides at the time of viral (or other) infection, as noted above. While the loss of specialized immunoproteasomes can be rationalized by the unusual bird class I system, the loss of enhancing the efficiency of peptide production has no obvious explanation.

There is no doubt that the proteasome system was coopted and modified by gene duplication to service MHC class I antigen presentation. The loss of all of the duplicated genes in birds was either a 'use it or lose it' scenario, or the unusual bird class I system could not function well with the duplicates. Regardless of the "reason" for their loss, further studies of $CD8^+$ thymic differentiation and class I antigen presentation in birds are of interest.

Conclusions

Compared to all other 20S proteasome subunits, β5 has a special place in the immune system. As mentioned, β5 has the primordial specificity of the β catalytic proteasome subunit, and one of its paralogues encodes β5i, which is the lynchpin in the formation of 20S immunoproteasomes. As mentioned, the gene coding for β5 has a unique exon/intron organization among β proteasomal subunit genes (and only in jawed vertebrates), which might be related to the generation of the gene encoding β5t early in gnathostome evolution. Interestingly, the immunoproteasome lineages described above, by and large, are found only for β5i, not β1i or β2i. *Xenopus* speciates by polyploidization with 2n, 4n, 8n, and 12n species, but the MHC (and other genes involved in adaptive immunity) is diploidized, first shown in functional studies such as acute graft rejection (Flajnik et al. 1985; Kobel and Du Pasquier 1986). Later molecular analyses demonstrated that while classical class I, class II and *TAP* genes are diploidized within a block, other MHC region genes may be found in more copies, i.e. are not diploidized, or the functional gene might be diploidized yet not remain within the MHC. *psmb9* and *psmb10* genes may fall into this latter category, but the functional *psmb8* gene, consistent with its ancient biallelism, always remains linked to class I/*TAP*/class II genes, i.e. what we would call the 'true' MHC (Du Pasquier et al. 2009; Session et al. 2016). Lastly, data suggest that *psmb8* and classical class I genes are coexpressed in *Xenopus*, with little-to-no expression in tadpoles and high expression in many tissues in adults (Salter-Cid et al. 1998).

Clearly, there is still much to learn about the immunoproteasomes, nearly 30 years after their discovery. Peptides have yet to be purified from class I molecules on cortical thymic epithelial cells, which would aid in distinguishing between various models for $CD8^+$ T cell development. A longstanding issue is determining the types of peptides generated by ectotherm immunoproteasomes, and the specificity of those peptides for particular *tap* and class I allelic products. Lastly, the literature suggests that there are immunoproteasome responsibilities besides the production of peptides (e.g. NF-κB activation), and such functions are poorly studied (Yewdell 2005).

Acknowledgements

We thank Dr. Yoichi Sutoh, Iwate Tohoku Medical Megabank Organization, Iwate Medical University, for his help in preparing Figure 2.

Funding information Experimental work from the authors' laboratories has been supported by grants from The Ministry of Education, Culture, Sports, Science and Technology, Japan and The National Institutes of Health, USA (RO1AI140326)

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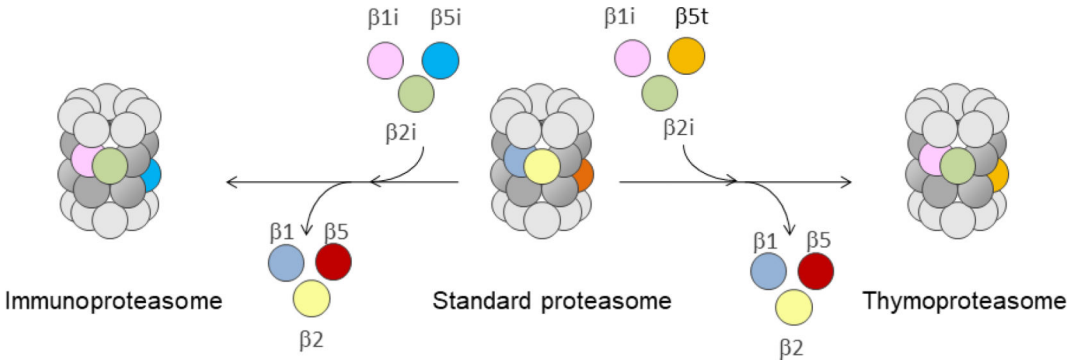

Fig. 1.

Subunit composition of immunoproteasomes and thymoproteasomes

Proteasomes have a cylindrical structure made up of four stacked rings: two outer α-rings composed up of α subunits and two inner β-rings composed of β subunits. In eukaryotes, α- and β-rings are made up of seven distinct subunits, respectively. Catalytic β subunits, β1, β2 and β5, are colored. Immunoproteasomes and thymoproteasomes, which occur only jawed vertebrates, differ from standard 20S proteasomes in the composition of catalytic β subunits.

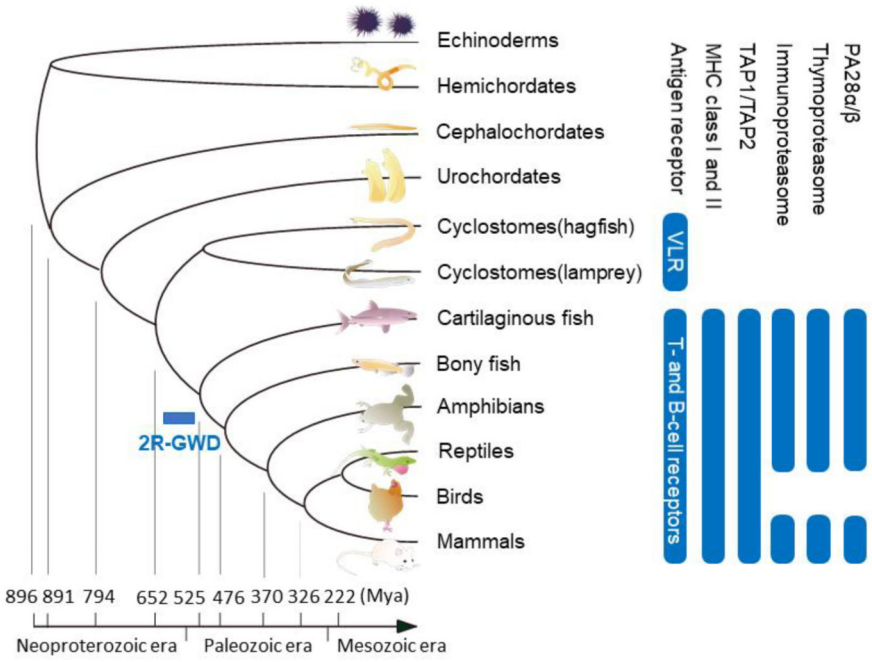

Fig. 2.

Evolution of adaptive immunity

Phylogeny of animals is shown on the left along with the divergence times based on molecular data compiled by Blair and Hedges (Blair and Hedges 2005). The phylogenetic distribution of key elements discussed in this review is shown on the right. It is conspicuous that birds lack proteasome elements specialized for antigen presentation by MHC class I molecules. This figure was modified from the previously published one (Murata et al. 2018). Mya, million years ago

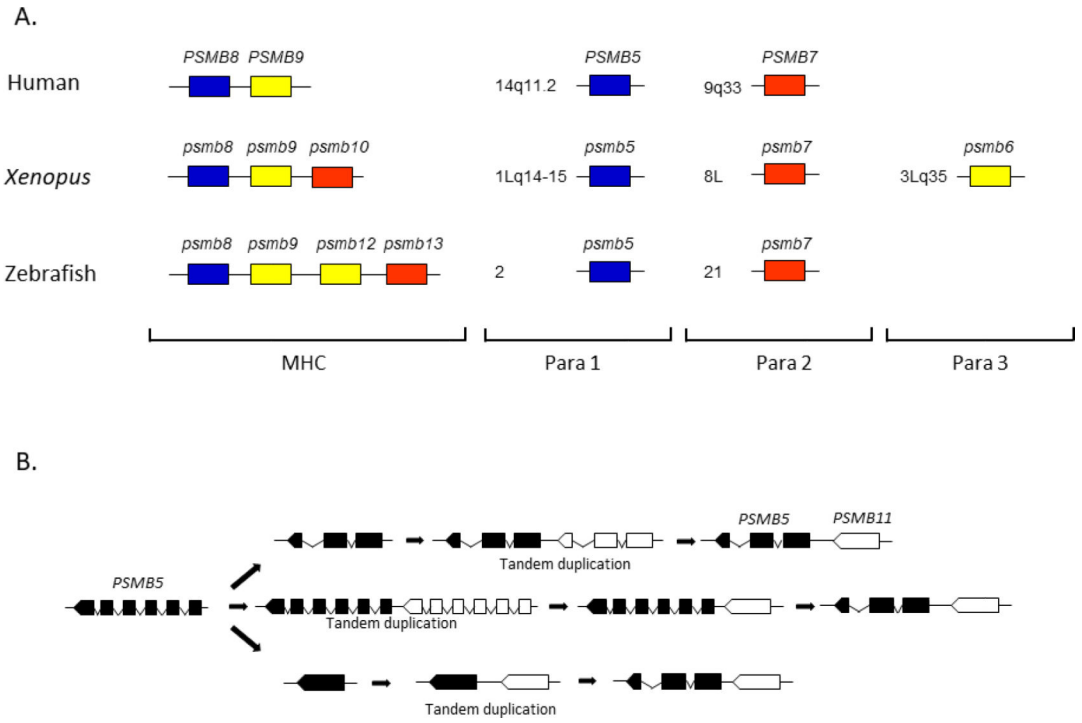

Fig. 3.

Evolution of immunoproteasomes and thymoproteasomes

Panel *A* shows that the majority of genes coding for catalytic β subunits are located in the MHC and MHC-paralogous regions. Para 1, 2 and 3 stand for MHC-paralogous regions 1, 2 and 3. In humans, *PSMB10* and *PSMB6* map outside of the paralogous regions. In *Xenopus tropicalis*, all six catalytic β subunit genes are encoded in the MHC and MHC-paralogous regions; *psmb10* maps to the class III region of the MHC and *psmb6* maps to the region syntenic to human para 3 (19p13.1–13.3). In zebrafish, *psmb12* and *psmb13*, which originated from *psmb6* and *psmb7*, respectively, are in the class I region of the MHC. Panel *B* shows three possible scenarios accounting for the emergence of the *PSMB11* gene (indicated in white) from the *PSMB5* gene (indicated in black). In bony fishes, *psmb11* and *psmb5* are located in a tail-to-head orientation as shown in the figure. In contrast, they are located in a head-to-head orientation in tetrapods (Sutoh et al. 2012). Therefore, *PSMB11* appears to have been inverted in a tetrapod lineage (not shown in the figure).

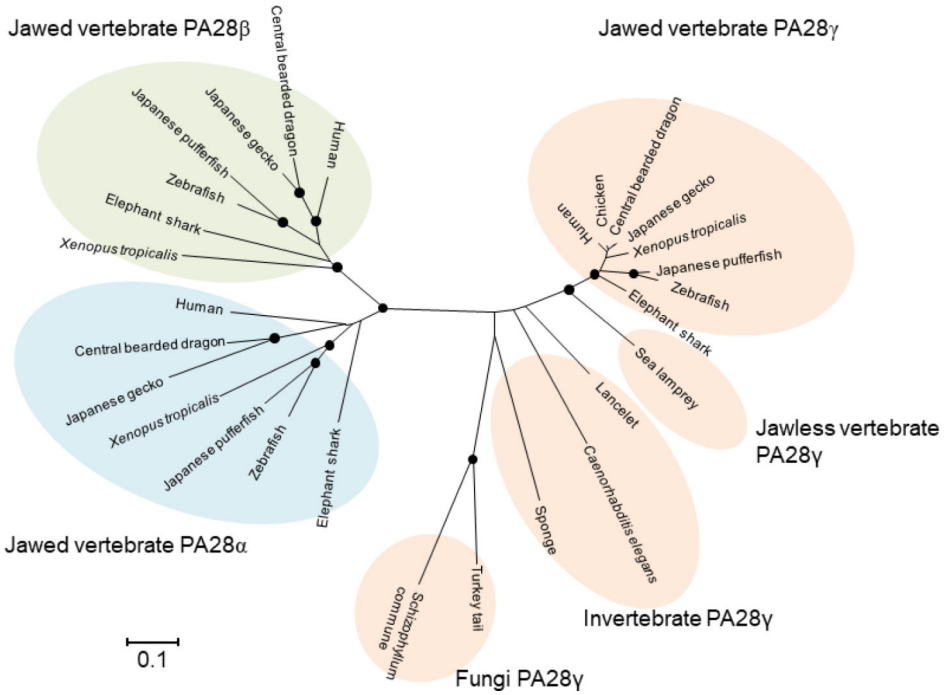

Fig. 4.

Evolution of PA28 subunits

For the construction of the phylogenetic tree, amino acid sequences of PA28α, PA28β and PA28γ subunits identified by database searches were aligned using the default Auto setting of the version 7.0 MAFFT program (Kuraku et al. 2013). The tree was constructed using the neighbor-joining algorithm implemented in the MEGA6 program package (Tamura et al. 2013). The distance matrix was obtained by calculating Poisson correction distances for all pairs of sequences. Gaps were excluded using the pairwise-deletion option. The reliability of branching patterns was assessed by bootstrap analysis (1,000 replications). Nodes supported by bootstrap values over 95% are indicated by closed circles. DDBJ/EMBL/NCBI accession numbers are as follows: human PA28α, CAG46459.1; human PA28γ, CAG46543.1; human PA28γ, CAG46545.1; chicken PA28γ, CAG31370.1; central bearded dragon PA28α, XP_020664971.1; central bearded dragon PA28β, XP_020664964.1; central bearded dragon PA28γ, XP_020651679.1; Japanese gecko PA28α, XP_015270891.1; Japanese gecko PA28β, XP_015272197.1; Japanese gecko PA28γ, XP_015282260.1; *Xenopus tropicalis* PA28α, AAH88020.1; *Xenopus tropicalis* PA28β, NP_001011494.1; *Xenopus tropicalis* PA28γ, NP_001096200.1; zebrafish PA28α, NP_571450.1; zebrafish PA28β, NP_571449.1; zebrafish PA28γ, AAF05816.1; Japanese pufferfish PA28α, XP_003967855.1; Japanese pufferfish PA28β, XP_003968209.1; Japanese pufferfish PA28γ, XP_003961088.1; elephant shark PA28α, AFM87012.1; elephant shark PA28β, JK930727; elephant shark PA28γ, XP_007907679.1; sea lamprey PA28γ, CO546357.1; lancelet PA28γ, XP_019630503.1; *Caenorhabditis elegans* PA28γ, NP_499493.1; sponge *Amphimedon queenslandica* PA28γ, XP_019856930.1; Turkey tail *Trametes versicolor* PA28γ, XP_008031792.1; and *Schizophyllum commune* PA28γ, XP_003038805.1.

Table 1

Members of the three catalytic subunit families in jawed vertebrates

Subunit	Other name	Gene symbol
β1	Y, PSMB6	*PSMB6*
β1i	LMP2, PSMB9	*PSMB9*
	PSMB12	*psmb12*
β2	Z, PSMB7	*PSMB7*
β2i	MECL1, PSMB10	*PSMB10*
	PSMB13	*psmb13*
β5	X, PSMB5	*PSMB5*
β5i	LMP7, PSMB8	*PSMB8*
β5t	PSMB11	*PSMB11*

Gene symbols given are those of humans, except for *psmb12* and *psmb13*, which are found only in fish. In humans and chickens, gene symbols are upper-case italics. Chickens have only *PSMB5*, *PSMB6* and *PSMB7*. In rodents, gene symbols are italicized, with only the first letter in upper-case. In *Xenopus* and fish, gene symbols are lower-case italics.