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A review on ovalbumin gene in poultry

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Abstract

Ovalbumin is a major protein in the egg white. It is a monomeric phosphor glycoprotein with a known complete amino acid sequence of 386 residues. Due to its structural similarities with the serine protease inhibitors (Ser PINs), it is included in the serpin super family, although it lacks inhibitory activities. The size of the entire ovalbumin gene in poultry is approximately 7.6 kb of DNA with 7 exons and 6 introns, in order to code for mRNA of 1859 nucleotides. Tubular gland cells of magnum are responsible for the production of ovalbumin protein. Ovalbumin gene in poultry have been found polymorphic in various populations in various parts of gene including coding sequence, promoter region etc., ovalbumin serve as a source of amino acid and may also have a more active/direct function on developing tissues and believed to play a crucial role in bio mineralization.

Keywords: Ovalbumin, characterization, expression, polymorphism

Introduction

Ovalbumin, a SERPIN family protein is the major protein in the egg white. Egg white is composed of ~9.7-10.6% protein by weight ^[1]. Over 24 different proteins have been identified and isolated from egg white. Some of the major proteins include ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3.5%), and lysozyme (3.4%) ^[1]. Ovalbumin has a molecular weight of 44.5kDa and is a monomeric phosphor glycoprotein with a known complete amino acid sequence of 386 residues. It is a storage protein and major source of amino acids for the developing embryo. Chicken ov-serpins are largely represented as 10 clade B serpins clustered on a 150 kb locus chromosome 2q ^[2, 3]. The size of the entire ovalbumin gene is approximately 7.6 kb of DNA in order to code for mRNA of 1859 nucleotides ^[4]. Intact ovalbumin is recovered in the extracts of many embryonic organs including the head, eye, heart, liver, intestine, spinal cord, muscle, dermis, and bone. This observation together with the absence of ovalbumin mRNA expression in these organs and with the fact that the neonate organs are no longer positive for ovalbumin shortly after hatching, suggests that egg white ovalbumin may not merely serve as a source of amino acid but may also have a more active/direct function on developing tissues ^[5]. In the vitelline membrane a total of 5 serpins have been identified with the predicted role in folliculogenesis, angiogenesis and in defence however role of ovalbumin is still unclear ^[6]. Ovalbumin in eggshell is believed to play a crucial role in calcium carbonate formation and amorphous calcium carbonate stabilization i.e. biomineralization, which is defined as the production of the hard tissue characterized by a specific minerals/organic matrix framework, by a living organism ^[7]. The objective of this study was to know the characteristics of ovalbumin gene, its expression profile and to know its polymorphism.

2. Ovalbumin

The most abundant and central protein to egg white's functional properties in foods is ovalbumin. Ovalbumin has a molecular weight of 44.5 kDa and is a monomeric phosphor glycoprotein with a known complete amino acid sequence of 386 residues ^[1]. It is a storage protein and major source of amino acids for the developing embryo. The N-terminus of ovalbumin is acetylated and contains four sulfhydryl groups and one disulfide bridge (Cys74-Cys121), which are inaccessible in the native state ^[8]. Although it is a secretion protein, ovalbumin is lacking an N-terminal leader sequence. Trans-membrane location is instead mediated by an internal sequence signal located within hydrophobic residues 21-47 ^[9]. Ovalbumin secondary structure has various motifs including α -helix (41%), β -sheet (34%),

β -turns (12%), and random coils (13%) [10]. The 3-D structure of ovalbumin is highly structured and has a α -helical reactive loop coming out of the main body of the protein on two peptide stocks and a main β -sheet A (Fig 1). The conserved reaction centre is located at Ala358-Ser359 [11]. Ovalbumin is a heterogeneous molecule with variation in its composition, which includes the degree of phosphorylation, glycosylation, and genetic variance. Two possible glycosylation sites have been identified at residues Asn 293-295 (Asn-X-Thr) and Asn 317-319 (Asn-X-Ser). The heterogeneous carbohydrate peptide chains contain a common core of mannose β (1-4) glcNAc β (1-4) glcNAc. Purified ovalbumin contains three types, A1, A2, and A3 in a ratio of 85:12:3. These types are differentiated by the degree of phosphorylation with two, one and zero phosphorylated sites respectively. The phosphorylation sites are located at serine residues 69 and 345 [9].

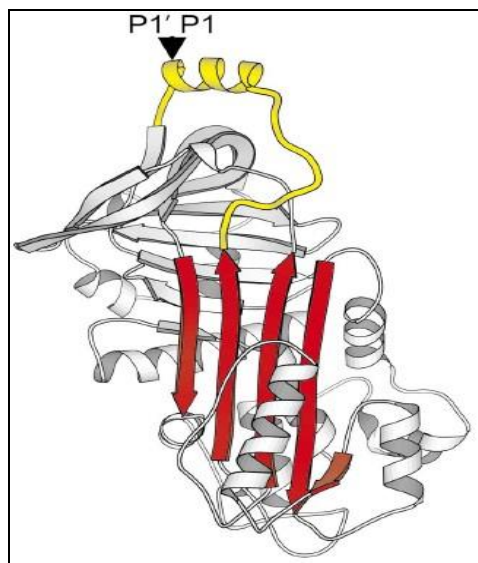


Fig 1: The 3-D crystal structure of ovalbumin with the α -helix reaction loop (yellow) and main β -sheet (red).

2.1 Serpin superfamily

Systematic analysis of National Center for Biotechnology Information and chicken Ensembl databases for identifying serpins in chicken species revealed the presence of 27 members of this family. Chicken ov-serpins are largely represented as 10 clade B serpins could be identified [2, 3]. These ov-serpins are clustered on a 150 kb locus chromosome 2q (Fig 2) and comprise Serpinb1, Serpinb2, Serpinb5, Serpinb6, two Serpinb10 homologs (Serpinb10, Serpinb10b/MENT), Serpinb12 and Serpinb14 (ovalbumin) and its related genes Serpinb14b (OVAY) and Serpinb14c (OVAX).

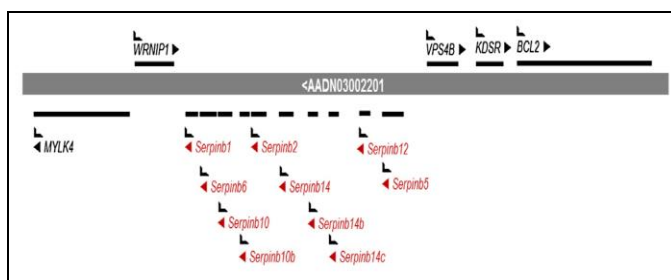


Fig 2: Localization of chicken ov-serpins including Ovalbumin (SerpinB14) in *Gallus gallus* autosome-2 (GGA-2). Serpins and flanking genes with their respective orientation (backward/forward).

Another cluster on chromosome 5 was identified containing 7 members of the Serpina family. This cluster includes 5 homologs of alpha1-antitrypsin/alpha1-proteinase inhibitors, Serpina1, Serpina3, Serpina4, Serpina5, Serpina9, which correspond to human antitrypsin, alpha1-antichymotrypsin, kallistatin, Protein C inhibitor, and serpina 9, respectively. Out of these 27 serpins, only 15 are actually recovered in the chicken egg in which the biological significance and biological activity intimately depends on the process of egg formation and on their subsequent localization (eggshell/egg white/vitelline membrane/yolk). Serpins share a highly ordered structure and a conserved reactive centerb [11].

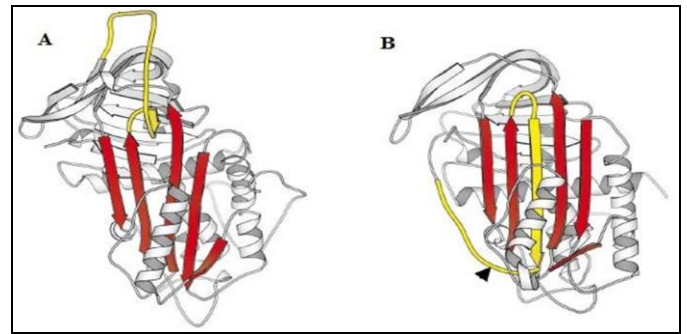


Fig 3: The crystal structures of native antithrombin (A) and activated antithrombin (B). The reactive center loop is in yellow which is inserted between β -sheet (red).

Like ovalbumin, the reactive center is protruded out of the main protein body on peptide “stalks”. When a serpin comes in contact with a protease it activates by undergoing a conformational change where the reactive center loop is cleaved and inserted in β -sheet A. This conversion is thermodynamically favourable and the resulting conformation is up to twice as stable as the native form. However Ovalbumin does not undergo this conformational change upon cleavage of its reactive loop which is the primary explanation why ovalbumin is not inhibitory. This conformational change is depicted in the Fig. 3 by taking antithrombin as example which belongs to serpin super family [9].

2.2 Phylogenetic analysis of chicken serpins

In serpin genes, some have shown a strong correlation between genomic organization, patterns of amino acids at specific sites, and insertion/deletion patterns, which contributed to identify serpin groups and to decipher vertebrate serpin evolution [12, 13]. Serpin genes have rapidly evolved; a high sequence divergence is found between all serpin clades, the sequence identity varying from 22% to 29%. The 27 serpins found in the chicken genome shows that serpin genes have been originated and duplicated before the divergence of teleosteans. Another duplication event occurred after divergence between species, for example, the clade A of *Gallus gallus* encompasses seven Serpina genes with a sequence identity around 47%. The same phenomenon is observed for mammal and fish. Using the phylogenetic trees available in Ensembl (<http://www.ensembl.org>), three serpins groups are distinguished. The first phylogenetic tree contains serpins from clade B, C, E and I, the second refers to serpins from clade A, D, F, G and H (Fig. 5), and the last tree, contains only one clade A serpin, Serpina8. The clade B serpin, present in the first tree, contains ovalbumin gene (Serpinb14) and its recently duplicated OVAY (Serpinb14b)

and OVAX (Serpib14c) [14]. The evolution of clade B serpins starts before the split of bony fishes and tetrapods, 450 million years ago, leading to at least six clade B serpin genes found in mammal and bird genomes. Out of ten clade B serpins on GGA 2, six serpins could also be found in *Homo sapiens* genome

(SERPINB1, B2, B5, B6, B10, B12). Due to recent duplication, avian Serpinb14 (ovalbumin), Serpinb14b (OVAY) and Serpinb14c (OVAX), have no human or other mammalian species orthologues and seem to be specific to oviparous species [15].

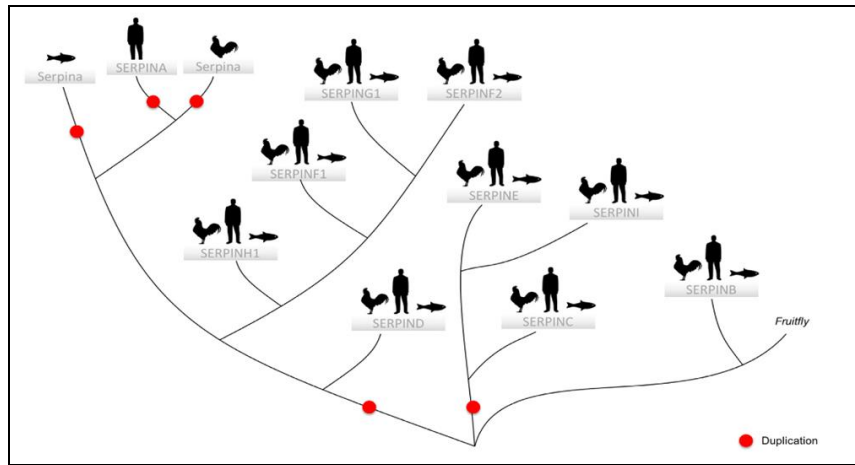


Fig 4: Evolutionary scenario of SERPIN from clade A, D, F, G and H for mammal, birds and fishes

3. Distribution of ovalbumin (Serpib14) in Egg and Developing embryo

Analyses and integration of the various proteomic data published on the cuticle/eggshell, egg white, vitelline membrane and egg yolk revealed the presence of 15 serpins in the freshly laid egg [16] (Fig. 5). With the exception of ovalbumin (Serpib14) and its related protein X (Serpib14c)

and Y (Serpib14b), most egg serpins recovered in the egg are not specifically expressed to support egg formation. Clade B serpins including ovalbumin (Serpib14), accounting for 54% of egg white proteins (about 50 mg/mL) and produced by the oviduct and more specifically by tubular gland cells of the chicken’s magnum, responsible for egg white formation [17] (Fig. 6).

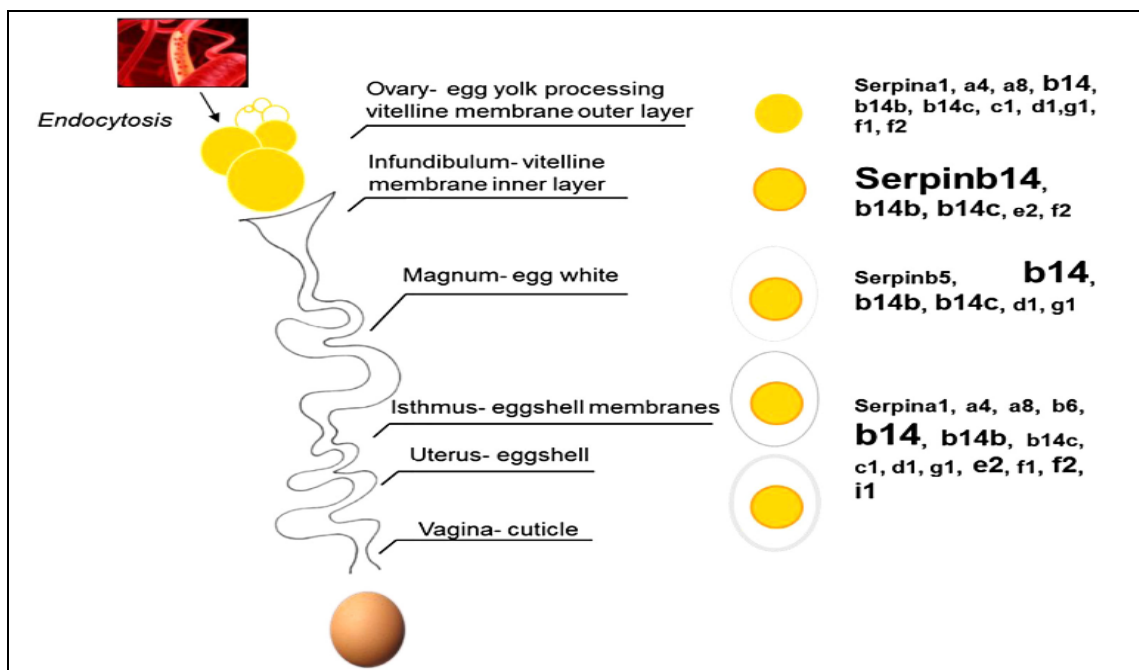


Fig 5: Distribution of SerpinB14 (Ovalbumin) in freshly laid egg.

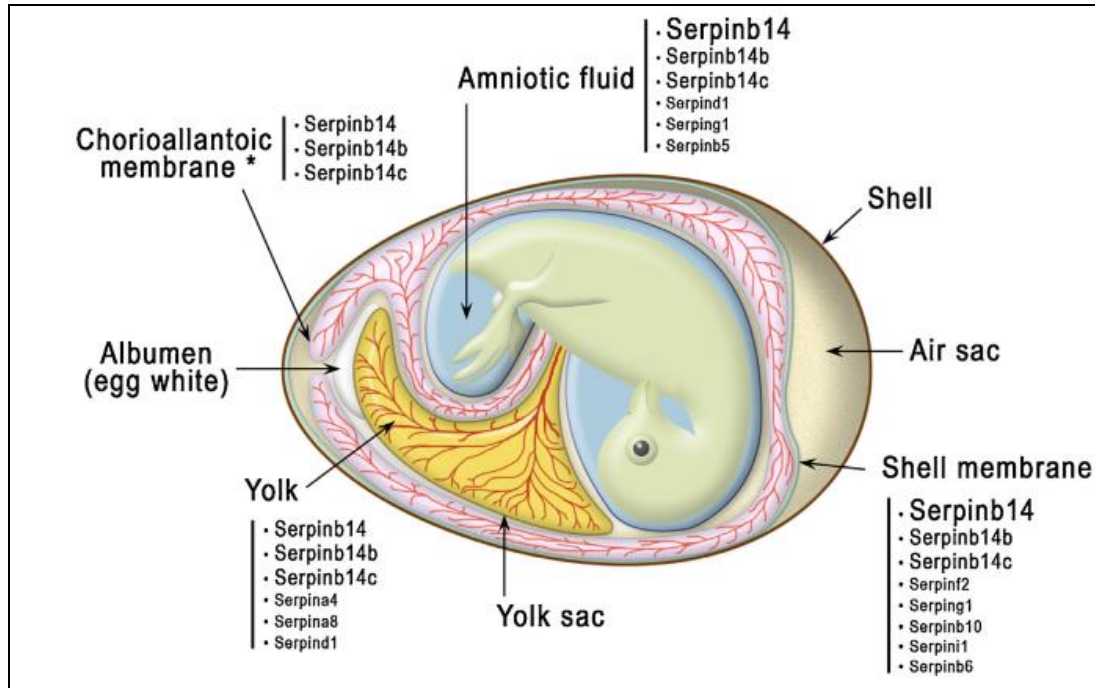


Fig 6: Schematic representation of a fertilized egg at day 16 of incubation.

4. Gene characterization and organization

The cloning of gene permitted to identify the DNA sequence that codes for the 5' end of ovalbumin mRNA. The attempt to obtain the 5' end of the structural ovalbumin gene by cloning a 3.2-kb HindIII DNA fragment and other restriction enzyme

digested DNA fragments has revealed the molecular organization of the entire ovalbumin gene. The ovalbumin gene is composed of eight structural gene segments separated by seven intervening sequences of various lengths (Figure 4) [18].

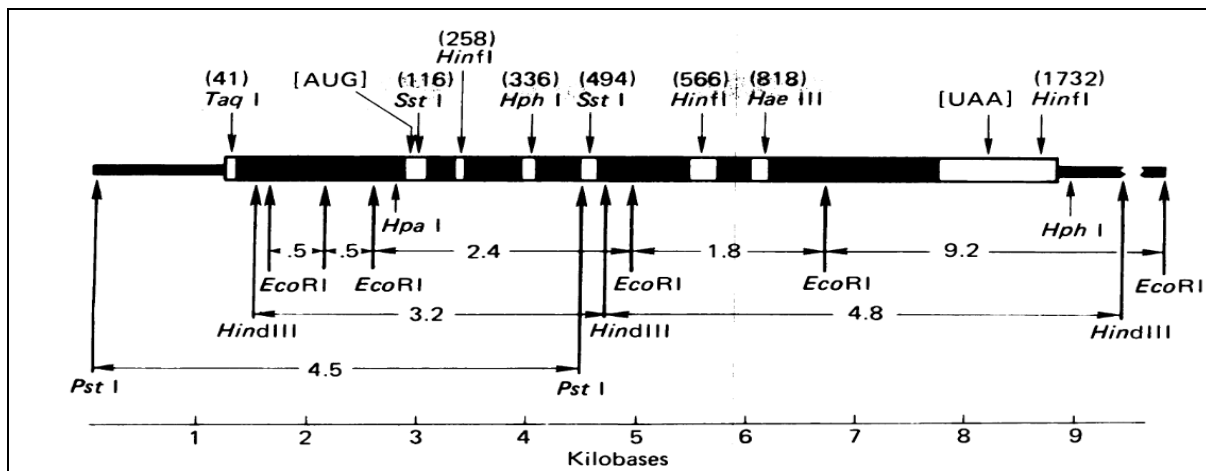


Fig 4: Physical map of the entire natural ovalbumin gene displaying some of the key restriction cleavage sites, the locations of the initiation and termination codons, and regions of interspersed structural and intervening sequences.

The size of the entire ovalbumin gene is approximately 7.6 kb of DNA in order to code for mRNA of 1859 nucleotides [4]. In another experiment cloning of the fragments of ovalbumin gene fragments after digesting with EcoRI which digests the natural ovalbumin gene into three distinct fragments of 2.4, 1.8, and 9.5 kilo base pairs in length by cleaving within the intervening regions and concluded that the structural gene sequences within the 2.4-kb and 1.8-kb fragments are subdivided into four and two regions, respectively [19].

The sequences which code for the ovalbumin messenger were interrupted in six places by intervening sequences ranging in length from 0.2-1.6 kb [20]. They showed the existence of two different alleles for the ovalbumin gene which is indicated by the absence of EcoRI fragments in some chickens and demonstrated the variation responsible for this polymorphism

lies in an intervening region, and does not seem to affect the expression of the ovalbumin gene. Finally concluded that the relative amount of intervening versus coding sequence would be the same in all chicken structural genes as in the ovalbumin gene and the ovalbumin messenger precursor should be about 3 fold longer than the sum of the coding plus intervening sequences messenger precursor should be about 3 fold longer than the sum of the coding plus intervening sequences [20].

Analysis of the EcoRI digested fragment of chicken DNA containing a sequence complementary to the 3' half of ovalbumin mRNA by molecular cloning had proven conclusively that the chicken ovalbumin gene is split [20]. This fragment contains no extensive sequence repeated elsewhere in the genome and represents the only type of organisation of

this part of the split ovalbumin gene in chicken genome and concluded approximately 1,000 base pair region of the split ovalbumin gene which codes for the 3' half of the ovalbumin mRNA should be located close to one end of the EcoRI fragment within the 2,600-base pair. Electron microscopic analysis of clone which contains the complete chicken ovalbumin gene, including its leader coding sequences inferred that the minimal size of the transcriptional unit for ovalbumin is 7.7 kilobases with 7 exons and 6 introns [21].

Cloning and sequence study of the cDNA of ovalbumin mRNA in Pigeons has found the full length pigeon OVA cDNA cloned by transcription-PCR and RACE to be 1433 bp in length (GenBank accession No. JQ345718), and consisted of a 118-bp 5'-untranslated region (UTR) and a 157-bp 3'-UTR with a poly-(A) tail of 29 nucleotides [22].

5. Gene Expression and Regulation

Various studies on expression of Ovalbumin gene suggested that the gene is expressed in Oviduct's Magnum part. Tubular gland cells of magnum are responsible for the production of ovalbumin protein. These cells are found to be highly responsive for oestrogen hormone in expression of Ovalbumin mRNA [23]. Administration of immature chicks with the hormone oestrogen resulted in concomitant proliferation of tubular gland cells in the oviduct. When the treatment ceases the number of these cells drops to 10-15% and ovalbumin synthesis becomes undetectable. A build-up of ovalbumin mRNA to approximately 50,000 molecules per cell has seen after Oestrogen administration and Ovalbumin mRNA synthesis stops when the hormone is withdrawn and within 12 days the level of mRNA has dropped to 0-10 molecules per cell [23].

Insulin is the physiological hormone that is required in addition to oestrogen to stimulate transcription of the ovalbumin gene and cyclic nucleotide derivatives, such as 8-bromo-cAMP, can mimic the effect of insulin on ovalbumin gene expression [24]. Two signal transduction pathways involving protein kinases have been implicated in the regulation of the ovalbumin gene. Primary cultures of oviduct cells after treating with phorbol 12-myristate 13-acetate (an activator of PKC) or with forskolin and 3-isobutyl-1-methylxanthine (an activator of PKA) alone, activators plus oestrogen and corticosterone, or activators plus both steroids and insulin, indicated that phorbol 12-myristate 13-acetate causes a dramatic destabilization of ovalbumin message, resulting in a reduction in ovalbumin mRNA levels. In contrast, the activators of the PKA system can substitute for insulin and, thereby, increase expression of the ovalbumin gene. Thus, in chicken oviduct cell cultures, the PKA and PKC signal transduction pathways act in opposing ways to modulate the steroid-induced expression of the ovalbumin gene i.e., PKA system causes increase in ovalbumin mRNA levels, whereas PKC system causes decrease in mRNA levels [25].

Regulation of expression of the chicken ovalbumin gene heterologous system, by cloning the entire ovalbumin gene and its flanking sequences together with the bacterial gene for xanthine-guanine phosphoribosyltransferase in plasmid pBR322 is studied. Transfection of this recombinant plasmid into an oestrogen-responsive breast carcinoma cell line (MCF-7) which is shown to possess oestrogen receptors and to be oestrogen responsive has revealed an 8 to 10 fold increase in the amount of ovalbumin mRNA to be present in cells cultured in 10^{-8} M estradiol [26]. In the Magnum,

Oestrogen and progesterone receptors act synergistically on ovalbumin gene transcription during early stages of induction but after full activation only Oestrogen receptors are implicated in Ovalbumin mRNA expression [27].

Study of expression of ovalbumin gene by Quantitative PCR analysis in Pigeon concluded pigeon OVA mRNA was highly expressed in the oviduct, and trace amounts were detected in other tissues. During the reproductive cycle, pigeon oviduct OVA mRNA expression reached its peak during the egg-laying stage, decreased with brooding, and then increased again during the squab-feeding period [22].

6. Polymorphism in ovalbumin gene

Polymorphisms of ovalbumin gene have been found in various chicken populations. About 1,210 non-synonymous SNPs (nsSNP) have been discovered in-silico in the chicken genome from EST data [28]. About 2.8 million SNPs have been identified, based on comparison of the DNA sequences of three domestic chicken breeds: a broiler, a layer and a Chinese silkie, with that of their wild relative, the red jungle fowl. It seems that at least 90% of these SNPs are valid and at least 70% are common and segregate in many domestic breeds. The mean nucleotide diversity is estimated to be about five SNPs per kilobase (1 per 200 bp) on average, for almost every possible comparison between red jungle fowl and domestic lines [28].

Analysis of polymorphism of egg white proteins in 12 local chicken populations of Indonesia using starch gel and polyacrylamide gel electrophoresis, found polymorphism at 5 loci including ovalbumin, ovoglobulins (G_2 and G_3), conalbumin and lysozyme loci [29]. Polymorphism of Ov, G_2 , G_3 and Tf_{EW} loci have been found in various chicken populations [30].

Investigation of genetic variations of seven egg white protein loci in 1,112 samples from eight Asian countries (Yunnan province of China, Mongolia, Nepal, Vietnam, Laos, Thailand, Myanmar, Indonesia) and 360 samples from two improved breeds (Isa Brown, Boris Brown) by using starch gel and polyacrylamide gel electrophoresis found five egg white protein loci (Ov, G_3 , G_2 , G_1 and Tf_{EW}) were found to be polymorphic in Asian native chicken populations [31]. They found gene frequency of the Ov^A is higher (0.848-1.000) than the Ov^B in almost all native populations in Asia. They hypothesized possible reason for disappearance of Ov^B and the Tf as genetic drift during keeping in a small colony, these alleles had been eliminated by a bottle neck effect [31].

Biodiversity study by using twenty-five single nucleotide polymorphisms (SNPs) which were analyzed in 20 distinct chicken breeds is done. These SNPs, each located in a different gene and mostly on different chromosomes. Out of these C/T SNP in ovalbumin gene is found with the expected and observed frequency of heterozygotes, 0.5 and 0.30 which was mapped to 67465920 bp in physical map of GGA2 [32].

Recent studies have shown that polymorphisms in the ovalbumin gene are significantly associated with breaking strength and shell thickness in Rhode Island Red hens [32]. A 4 SNPs in ovalbumin gene have been identified which are associated with eggshell thickness, effective layer thickness, and density of the mammillary cone and all four SNPs in ovalbumin examined in the study were in the 3' untranslated region (UTR) and 5'UTR, which may influence the regulation of ovalbumin expression, these SNPs may affect ovalbumin function during initial mineralization and alter the morphology of calcite crystals and vaterites [33].

Associations of single nucleotide polymorphism (SNP) genotypes of the duck ovalbumin gene with hatchability has found 385 C>T SNP site in the 506-base pair sequence of the ovalbumin gene. Genotyping of SNP in 187 ducks ovalbumin gene carried out by PCR restriction fragment length polymorphism and minisequencing methods, based on this SNP genotypes of the duck ovalbumin gene, there were three types: CC, TT, and CT. Birds with the CC and TT genotypes had higher hatchability (79.59 \pm 3.40, 76.35 \pm 1.77) (P <0.05) than those with a CT genotype (65.77 \pm 2.07). Hence ovalbumin gene is considered as an important candidate gene that can be used for marker-assisted selection to increase hatchability in ducks [34].

17 single-nucleotide polymorphisms of three major genes in a hen population using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry have been found. Out of 17, 12 single-nucleotide polymorphisms in Hardy-Weinberg equilibrium were used for analysis of associations with eggshell ultrastructure organization. Associations were found for (i) ovocleidin-116 with effective layer thickness (EFF), mammillary layer thickness (MAM), and average size of mammillary cones (SMAM); (ii) ovalbumin with eggshell thickness (ESH), effective layer thickness, and density of the mammillary cone (DMAM); and (iii) calmodulin1 with density of the mammillary cone. 4 SNPs were identified, 3 in 3'UTR and 1 in 5'UTR out of which the CT genotype is significantly more frequent than the CC genotype (p <0.05) in RS1, a new SNP that is significantly associated with EFF. In rs16030727, hens with the CT genotype had significantly thicker ESHs (p <0.01) and EFFs (p <0.05) than those with the TT genotype, whereas hens with the CT genotype had significantly lower DMAMs than those with the TT genotype [34].

7. Functional significance of ovalbumin

1. The functional annotation of egg yolk proteins has revealed that serpins identified in the egg yolk are essentially known actors of coagulation/fibrinolysis cascades [35].
2. In the vitelline membrane a total of 5 serpins have been identified with the predicted role in folliculogenesis, angiogenesis and in defence however role of ovalbumin is still unclear [6].
3. Ovalbumin is recovered in the extracts of many embryonic organs including the head, eye, heart, liver, intestine, spinal cord, muscle, dermis, and bone. Surprisingly enough, the presence of uncleaved ovalbumin persists in embryonic organs suggesting that at least a fraction of ovalbumin molecules could be transported intact to embryonic organs. This observation together with the absence of ovalbumin mRNA expression in these organs and with the fact that the neonate organs are no longer positive for ovalbumin shortly after hatching, suggests that egg white ovalbumin may not merely serve as a source of amino acid but may also have a more active/direct function on developing tissues [5].
4. Ovalbumin in eggshell is believed to play a crucial role in calcium carbonate formation and amorphous calcium carbonate stabilization i.e. biomineralization. Biomineralization may be defined as the production of the hard tissue characterized by a specific minerals/organic matrix framework, by a living organism [7].

8. Conclusion

Ovalbumin is the most abundant and central protein to egg white proteins. It is a secretion protein but lacking an N-terminal leader sequence. Characterization studies have shown the gene is of 7.6kb with 7 exons separated by 6 intervening sequences. Expression studies revealed that the mRNA of the gene will express in the magnum part of oviduct and sex hormones believed to regulate its expression. Polymorphism of the gene had shown association with breaking strength, eggshell thickness, effective layer thickness, and density of the mammillary cone and initial mineralization and alter the morphology of calcite crystals and vaterites in various population of chicken.

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