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Meissner (1855) and Leuckart (1855) first studied the egg coverings in insects. They described the covering as vitelline membrane which was generally structure less. Riley et al., (1877) investigated the Melanoplus spretus (Orthoptera) eggshell and described two layers in the egg covering. The outer layer termed as ‘chorion’ but the inner layer left nameless. Inner layer was described as smooth, thick and translucent membrane. Chortophaga viridifasciata (Orthoptera) eggshell was studied by McNabb (1928) and two layers were described in the eggshell. Upper layer and inner layer were described as chorion and photoplasmic layer respectively. Nelsen (1931) labeled the inner layer of the chorion as vitelline membrane in Melanoplus differentialis (Orthoptera).

Jahn (1935) first investigated the chemical natures of the egg membranes and fined differences between the inner and outer layer of grasshopper chorion. Slifer (1937) also mentioned a temporary coating over chorion which was later called as exochorion by Roonwal (1954 a) in Melanoplus differentialis. Hartley (1961) gave detailed description of eggshell layers in Locusta migratoria (Orthoptera). Four layers were found in this insect, i. the vitelline membrane, ii. the endochorion, iii. the exochorion and iv. a layer which was found in Locusta, the extrachorion. In Hemipteran insect Lygus eggshell was constituted with four layers, i. vitelline membrane, ii. innermost chorionic layer, iii. air layer and iv. chorion proper (Ma et al., 2002). In Drosophila (Diptera), eggshell layers were i. vitelline membrane, ii. wax layer, iii. innermost chorionic layer, iv. endochorion and v. exochorion (Margaritis et al., 1980). Kimber (1980) divided the eggshell membrane of
Schistocera gregaria (Orthoptera) in three layers, i. vitelline membrane, ii. endochorion and iii. exochorion.

The follicle cells have been assigned a number of functions in both panoistic and meroistic insects and its differentiation involves sequential elaboration of at least three products, the yolk-associated glycoprotein, the vitelline membrane, and the chorion (Davidson, 1968; Kimber, 1980). Major function of the follicle cells was chorion secretion. In differentiation program of follicle cells, most conspicuous phase is formation of chorion. Follicle cells increase in breath and decrease in height in between the time of eggshell secretion initiation of and till the end (Huebner et al., 1975). Concomitantly with the change in the size of the cell, the nuclei of follicle cells also become enlarged (Huebner and Anderson, 1972). Prior to secretion of the vitelline membrane follicle cell cytoplasm and RER cisternae increased in number. During formation of vitelline membrane much more homogeneous electron dense layer was secreted and it became condensed down to attain its maturation (Kimber, 1980). During endochorion synthesis, follicle cells had dense RER cisternae and golgi bodies packed with electron-dense material. Proteins were secreted from the membrane-bound ribosomes present in the RER cisternae (Palade, 1975; Jamieson and Palade, 1977). Lipid droplets also increased in this stage. At the time of chorion formation, secretion of the vesicles in follicle cells starts. Endochorion tubules were clearly visible in the premature endochorion (Furneaux and Mackay, 1970, 1972). After completion of endochorion secretion also, endochorion secretion vesicles were present in follicle cells. At the time of exochorion secretion amount of RER cisternae
became lesser and golgi bodies also became smaller in size. As chorion secretion progresses autophagic vacuoles with membrane increased in number. This phenomenon has been described in silk moth and *Schistocerca* also. This feature pointed out that autolytic process was active in this stage for degeneration of these cells after completion of chorion secretion (Smith *et al.*, 1971; Kimber, 1980). Transportation of the material of chorion deposition from the follicle cells was done by oocyte membrane (Chapman, 1998).

Earlier scientists described outer structures and other chorionic features of several Acridids through light microscopy. In *Schistocerca gregaria*, the chorion had hexagonal ridges and these ridges were described as imprint of follicular cell (Roonwal, 1954 b). Like *Schistocerca*, *Romalea microptera* (Orthoptera) also had outer chorionic ridges like honeycomb pattern and the thickness of the chorion was 30µm to 100 µm (Hartley, 1961). External chorionic features of *Chorthippus parallelus* (Orthoptera) was described by Waloff (1950) and in this insect had hexagonal ridges. Chorionic thickness was 35 µm at all sides of the egg. Highest thickness was at posterior pole and it was about 100 µm. Posterior pole of this insect had special ring like external feature. Beament (1946 a) used light microscopy and histochemical techniques to study the formation of eggshell in *Rhodnius* (Hemiptera). Chorion of *Chorthippus brunneus* (Orthoptera) had no outer sculpture. Posterior pole of the egg had highest chorionic thickness (Hartley, 1961). Histochemical study of *Drosophila* chorion has given the information about chorionic components. 95% of chorion and 75% of vitelline membrane was made up of protein. Carbohydrate was also a major component
of chorion (King, 1960; King and Koch, 1963). On Orthopteran chorion light microscopic studies were done by Slifer (1937). Light microscopic study was used to investigate structural peculiarities and physiological functions of Orthoptera as well as grasshopper eggshells. Pores and spaces of the eggshell contained air and the outer layer of eggshell trap air in the pillar zones and below portion of it (Hartley, 1971). This layer was designated as the air layer. Air layer has been reported to secrete the main function of respiration through eggshell even in stress conditions (Hartley 1961, 1971). Katiyar (1960) undertook a light microscopic study of eggshell surfaces of 10 Indian acridids. Kulagin (1932) described eggshell structure of two Orthopteran insects using light microscopy and pointed out that in Locusta ectochorion and endochorion were formed after the egg left the ovary. Staining with haematoxyline of Locusta migratoria egg, the formation of eggshell layer was described. Endochorion was secreted by follicle cells in matured oocyte. Exochorion existed over endochorion as a thin layer having hexagonal structures on the surface. When the egg enters into the oviduct extrachorion was layer started to form and this extrachorion started to shrink when laid. Outer surface structures persist as granular structures after hatching of the embryo. Schistocerca gregaria eggshell also had hexagonal structures as imprints of follicular cell. No differentiation was found between exo and endochorion. Extrachorion covers the spaces among the ridges. In Romalea microptera also eggshell had honeycomb pattern ridges over the exochorion. Here also extrachorion covered the ridges by outer side. Chorthippus parallelus eggshell had prominent hexagonal sculpture on surface and endochorion was divided in two zones, the inner and the outer endochorion. Here exochorion was thinner than
extrachorion. Small pits like structures formed a ring like structure in the posterior pole in this insect (Waloff, 1950). *Chorthippus brunneus* eggshell endochorion had meshwork throughout the surface. Exo and extrachorion present as a thin layer. Ring like structure made up of small pits and was present in the posterior pole (Hartley, 1961). *Tetrix vittata* and *Tetrix subulata* (Orthoptera) eggshells were analyzed with light microscopy (Hartley, 1971). In these insects outer surface of the eggshells had hexagonal structures as imprint of follicular cell boundary. Micropylar pores were present at posterior part of the middle zone of the egg. Sectioning of the egg revealed that the posterior pole was thinner than the other parts of the eggshell. The respiratory horns present on the eggshell were completely made up of chorion. In advanced stage of development chorion cracked when hatching took place (Hartley, 1962). Polysaccharide and proteinaceous compounds of chorion with follicle cell morphogenesis were revealed in *Oxya hyla hyla* and *Gesonula punctifrons* (Orthoptera) (Shyam Roy and Ghosh, 2014 a, b). But Hinton and Service (1969) pointed out that photographs of eggshell surface taken by light microscope were not sufficient to give proper impression of surface sculpture and therefore use of Electron Microscope was essential. Light microscopy preceded the basic research which was established with Scanning Electron Microscope and Transmission Electron Microscope.

Scanning Electron Microscopic study has been carried out by earlier scientists with particular emphasis on ultrastructural features of eggshell in different insect orders. Ultrastructural modifications of Lepidopteran insect eggshell have been extensively studied by earlier
scientists (Matsuzaki, 1968). Sakaguchi et al., (1973) described arrangement of eggshell sculptures in *Bombyx mori* and denoted as imprint of edges of follicle cell. Presence of micropylar pore in the anterior pole has been described in moths (Arbogast et al., 1989; Kumar et al., 1999, 2003). Hinton (1981) confirmed presence of micropylar pore at anterior pole in Lepidoptera without any exception. *Samia ricini* egg had decorated chorion. Follicular imprints were distributed throughout the eggshell. Micropylar openings with petal shaped cells had been described in this insect. Two rows of petal shaped structures covered the micropylar pore. In the posterior pole sometimes sculptures were missing but aeropyles were always present. Aeropyles were absent in two rows of petal shape structures of the micropylar zone (Kumar et al., 2007). Chorion of silk moth had been extensively studied from ultrastructural and morphogenetic viewpoint. Chorionic sculpturing changed during development. In *Antheraea assama* drastic change in chorionic sculpturing was observed in the chorion of after laying egg and the eggshell at the time of hatching (Hamordrakas et al., 1983; Hamordrakas, 1984; Dey et al., 2003). Phylogenetic and developmental analysis had been carried out in *Antheraea* which helped classification of these insects by introducing morphological characters (Regier et al., 2005). Among 72 species of *Antheraea*, eggshell of 24 species has been studied. These 24 species represents all three subgenera, groups and subgroups of *Antheraea*. Aeropyle crown was present in 17 species. In 8 species aeropyle crown was present throughout the eggshell surface except in the micropyle area. In other 9 species aeropyle crown was present at anterior-posterior axis. Size of the aeropyle crown also varied from species to species. Evolution of aeropyle
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crown was as follows: uniformly distributed fully developed aeropyle crown, aeropyle crown of rudimentary condition and aeropyle crown present in restricted areas of eggshell (Regier et al., 2005). Eggshell synthesis from the follicular cells and radial complexity had been studied in Mayflies. Eggshell morphology of 29 species of mayflies representing 8 families and 18 genera were investigated using Scanning Electron Microscope and light microscopy. Micropyle of mayfly comprised micropylar pore, sperm guide and micropylar canal. Sperm guide specially described in may flies, which was a depression of chorion and its size varied from round to oval. Reticular chorionic sculpturing was found in the surface of these eggshells and these reticular structures were varied extensively in shape and size (Ubero-Pascal and Puig, 2007). Life cycle of *Euproctis chrysorrhoea* with reference to eggshell sculpturing and micropylar opening were studied by Candan et al., (2008). In *Anastrepha obliqua* (Diptera) eggshell, respiratory horn and aeropyle were present in the apex. Surface of the eggshell was smooth without any sculpturing. Terminal parts of the eggs were reticulated with granular structure. *Anastrepha fraterculus* (Diptera) egg, polygonal structures were present in the anterior pole. Aeropyle crown was also present with the polygonal structures. Micropyle was conspicuous than the micropyle of *Anastrepha obliqua* and present at the apex of the anterior pole (Murillo and Jiron, 1994). Stick insect (Phasmatodea: Heteronemiidae) eggshell surface ultrastructure revealed that micropylar pore was not present but micropylar plate was surrounded by ridges in the anterior pole. On the surface of the egg median line was present from the micropylar plate towards the posterior pole. Operculum was covered by several ridges and formed the capitulum (Stark and Lentz, 1986). Eggshell
of *Electrogena zebrata* (Ephemeroptera) contained geometrically arranged thin strands inter connecting with each other. These structures were not present in the micropylar region (Gaino and Mazzini, 1987). In Hemiptera eggshell ultrastructure of *Triatoma infestans* (Hemiptera) has been documented in details (Chauvin *et al*., 1973; Chauvin and Barbier, 1979). *Lygus* eggshell had operculum in anterior pole. Respiratory horns were also present in the operculum (Hinton, 1981). Aeropyle were situated at the tip of respiratory horns. Aeropyle canals were facilitating the air penetration in the respiratory horn (Ma *et al*., 2002). In Diptera, Linley (1990) studied ultrastructure of eggshells of different species of *Aedes*. Musso (1981) studied immature eggshells of robber flies from morphogenesis and developmental point of view. Eggshell of *Echthistus cognatus* had ridges all over the surface and single micropylar opening in micropylar region. Eggshell sculpturing was missing in the micropylar zone. Eggshell contained aeropyles throughout the surface in between ridges. Mushroom like sculptures were also present in the egg surface (Candan *et al*., 2004). In arrangement of micropylar pore in the eggshell of several Diptera wide range of variation has been reported. Number and position of micropylar pore also varied. No micropyle was found in egg of *Dissmeryngodes anticus* (Castillo *et al*., 1994). Scanning Electron Microscopic observation of eggshell ultrastructure allowed taxonomic differentiation of blow flies. *Lucilia cuprina* eggshell was characterized with a wide plastron present through out the eggshell. In the micropyle region plastron was bifurcated. Chorion had polygonal and hexagonal architecture (Kitching, 1976; Colwell *et al*., 1999; Sukontason *et al*., 2004). *Drosophila* (Diptera) eggshell also had specialized regions which were involved in performing
specialized functions. Anterior pole of the eggshell was characterized by operculum and micropylar pore was embedded in it. Micropylar pore facilitated sperm entry into the oocyte and the area of the micropylar pore was designed to restrict single sperm entry at a time. Posterior pole contained two respiratory appendages to allow gaseous exchange in the egg when in water (Margaritis, 1984). Uvarov (1928) first pointed out the systematic importance of the eggshell sculpturing in Orthoptera eggshell. Orthoptera eggshell and its formation were studied by earlier scientists (Favard-Séréno, 1966, 1971; Goltzene, 1977). Physiological properties of eggshell were studied in Acheta by electron microscopy (Furneaux, 1969). Panoistic ovary has been described in cricket and dragonfly by Matsuzaki (1971). Tuck and Smith (1939) made detailed study of eggshell sculpturing of 48 grasshopper strains to identify the acridid eggs. Ganguly et al., (2008) for the first time studied the surface sculpturing of two Indian acridids of two different subfamilies using Scanning Electron Microscope. These two species showed completely different roof network of chorion.

Regarding formation of eggshell Transmission Electron Microscopy provided detail information about eggshell secretion, its formation and different eggshell layers in several insects of different insect orders (Kimber, 1980; Margaritis et al., 1980; Ma et al., 2002). In Lygus developmental changes of ovary and ultrastructural features were demonstrated by Ma and Ramaswamy (1987). In this insect four layers of chorion i. e. vitelline membrane, innermost chorionic layer, air layer and chorionic proper had been documented. Innermost chorionic layer was
electron lucent layer. These electron opaque granules disappear after a period of time and forms tiny pores in the innermost chorionic layer. These pores allowed penetration of gases in the developing embryo. Formation of air layer started with the secretion of electron dense granules. When these electron lucent granules disappears a perforated strcture remains which act as an air layer. Wax layer was not present in this insect. Paracrystalline structures were present in the surface of the operculum and these structures were assumed to play role in sperm attachment. Thread like structures of protein and polysaccharide droplets were found in the chorion of this insect. It was hypothesized that electrophoretic polarized movement of the proteins secreted by anterior columnar follicle cell happened towards the posterior pole (Ma et al., 2002). Formation of eggshell in milkweed bug, Oncopeltus had been studied by Transmission Electron Microscopy (Dorn, 1976). Eggshell of Drosophila has been subdivided into five layers: vitelline membrane, wax layer, innermost chorionic layer, endochorion and exochorion (Margaritis et al., 1980). EM studies confirmed the secretion of vitelline membrane from the follicle cells in Drosophila and Aedes (Cummings et al., 1971; Anderson and Spielman, 1973). In last stage of follicle cell development, follicle cells migrate between the nurse cells towards the extreme anterior pole and formed the micropylar apparatus in Drosophila (King and Koch, 1963; Cummings and King, 1969). Ultrastructural analysis of chorionic layers of Drosophila described endochorionic structure, in which vertical pillars separated the roof and fenestrated floor that facilitated gaseous exchange for respiration. Outer roof had the ridges and gave chorion a characteristic look. Globular structures were also identified in endochorion inter connected by fine fibrils. Exochorion
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consisted of loose fibers (Margaritis and Mazzini, 1998). In Drosophila eggshell presence of polysaccharide droplets and thread like structures of protein deposition were confirmed but details of their formation await further investigation (Cummings et al., 1971; Margaritis, 1985). These thread like structures were hypothesized to play role in the attachment of sperm (Margaritis, 1985). TEM observation of Lucilia cuprina (Diptera) gave detail information about the chorionic layers. Exochorion, outer endochorion, vertical pillars in aeropyle region, inner endochorion, innermost chorionic layer and wax layer constituted the chorion (Sukontason et al., 2007). Among Orthoptera Schistocerca eggshell consisted of a thin electron-dense vitelline membrane, endochorion and exochorion (Kimber, 1980). From the investigation of Gryllus chorion it was confirmed that formation of eggshell was a secretory event of 5 different secretion vesicles of follicle cells (Favard-Sérêno, 1971). In house cricket Acheta domesticus, chorion was composed of endocuticle, epicuticle, vitelline membrane, serosal epicuticle and serosal endocuticle. The water uptake through the eggshell was been controlled by two successive layers of eggshell which directly enhanced the embryogenesis. Epicuticle was recognized to have tyrosinase activity and this epicuticular tyrosinase activity regulated the water permeability of the eggshell (Furneaux et al., 1969).

Chorion has been proved to be a proteinaceous structure and at the last stage of egg development chorion goes through hardening process (Margaritis, 1985). Hardening of chorion or its insolubilization had been attributed to protein-protein interaction and formation of di-and tri-tyrosine
bonds among proteins. Di-tyrosine bond formation was considered as a major event for hardening of chorion which leads to insolubilization of chorion (Konstandi et al., 2006). Different chemicals including denaturing and reducing agents had been used to dissolve the chorion of insects of different insect orders. For dissolving Aedes aegypti chorion, 1% CHAPS, 2M Urea, 0.15M KCl, 2mM PMSF and 2mM EDTA-Na$_2$ were used with mechanical homogenization and sonication (Li et al., 2004). In another study of Aedes aegypti, chorion was solubilized in 0.125mM Tris, pH 6.8, 2% SDS, 10% glycerol and 0.1M DTT at 70°C for 30 minutes (Li and Li., 2006). Peroxidase characterization of Aedes aegypti chorion was done with solubilization in 10mM phosphate, 1mM PMSF, and 5mM EDTA, pH 6.5 (Han et al., 2000). For spectroscopic analysis, chorions of two Lepidoptera were dissolved with 6M Urea, 0.4M Tris-HCl, 4% 2-Mercaptoethanol, pH 8.4. This solution was able to dissolved chorion completely (Orfanidou et al., 1995). Silkmoth chorion was insoluble due to crosslinking of the proteins by disulphide and non-covalent bonds. Di-tyrosine and tri-tyrosine bonds were not found in the silkmoth chorion. High pH and high temperature enhanced the solubilization of chorion. Rhodnius prolixus eggshell was solubilized with 8M Urea, 0.36M Tris-HCl (pH 8.4), 0.03M dithiothreitol and 0.1M PMSF. Chorions were completely dissolved in this solution and the solution was used for electrophoretic studies (Bouts et al., 2007). A combination of denaturing agents and reducing agents (8M Urea, 0.36M Tris-HCl and 0.3M dithiothreitol, pH 8.4) were able to solubilize chorions of silkmoth (Regier et al., 1978). In Drosophila chorions of stage 14 were dissolved invariably with 7M guanidinium hydrochloride, 1% SDS and 6M Urea. But laid eggs were 20
to 50% soluble in this solution. Solubility was not increased with thiol reagents. This observation suggested about the presence of covalent bonding of the proteins at late stage and absence of disulfide was confirmed (Petri et al., 1976). Chorion of Gryllus mitratus was dissolved in 8M Urea, 10mM dithiothreitol, 30mM EDTA, 0.2M Tris-HCl buffer, pH 8.6. Minor fraction of insoluble residue remained when chorion dissolved in this solution. Protein solubility was influenced by ionic strength of the solution and pH. Buffers and salts were used for increasing the solubility of the proteins. KCl, Phosphate buffer and Tris base were used to increase the pH of the solution (Berkelman et al., 2004). Chaotropic agents like Urea disrupted the hydrophobic interaction and hydrogen bonds within and between proteins. Secondary protein structures were also disrupted by these agents. Urea can modify amino groups and give artifactual charge to them. Heat can promote the hydrolysis of urea (Rabilloud et al., 1997). To cleave the disulfide bonds between and within proteins reducing agents were used. Reducing agents were DTT and β-mercaptoethanol. These compounds prevented proteins to re-aggregating and precipitating (Berkelman et al., 2004). Detergents were also used for disrupting the hydrophobic interactions within and between proteins. CHAPS, a zwitterionic detergent increased the solubility of the proteins. Anionic detergent SDS formed a tight association with proteins and efficiency of SDS to solubilize the proteins was unparalleled to any other compound (Perdew et al., 1983; Chevallet et al., 1998; Berkelman et al., 2004).

Eggshell composed of protein provided protection to the embryo and these proteins were synthesized one layer over another (Giorgi,
Choriogenesis is the process of synthesizing the eggshell proteins. Before choriogenesis, vitelline membrane was formed just after vitellogenesis. After vitellogenesis, vitelline genes were switched off and chorion genes were turned on, and chorionic protein deposition started at stage 9 of oogenesis in *Drosophila* and in stage 11-14 in *Rhodnius* (Margaritis, et al., 1980; Bjornsson and Huebner, 2004; Bouts et al., 2007). In choriogenesis, expression of 100 structural genes had been reported to be present (Goldsmith and Kafatos, 1984). Oocyte membrane became the vitelline membrane, and chorion proteins were deposited on it (Beament, 1946). Eggshell proteins varied from species to species in insects. From 2-D gel electrophoresis, in *Drosophila* 20 and in *Antheraea polyphemus* 186 eggshell proteins were resolved (Regier et al., 1980). In a termite *Pseudacanthotermes spiniger*, one protein has been identified with antimicrobial activity (Lamberty et al., 2001). Eggshell layers were sequentially secreted, and this sequential secretion was controlled by gene expression and chorion proteins were synthesized within five hours by major chorion gene expression (Petri et al., 1976). Follicle cells became specialized to secrete the proteins of specialized regions of eggshell like the micropyle and respiratory appendages. Chorion proteins were secreted in sequential order and after deposition, minor chorion proteins were undergo proteolytic process at the N and C terminal regions. But the major chorion proteins did not undergo proteolytic process (Pascucci et al., 1996). The enzymes responsible for the proteolytic processing remain unclear. 11 minor chorion proteins have been detected in *Drosophila* eggshell (Fakhouri et al., 2006). Chorionic mRNA synthesis coincides with the chorion protein secretion in *Drosophila*. In oogenesis stage 8 to 14, all eggshell genes were transcribed within follicle
cells. Chorion genes were transcribed from the stage 11 (King, 1970; Waring, 2000). s36 and s38 chorion genes have been reported to be first transcribed in stage 11 of oogenesis. Transcription rate was highest in stage 12 and 13 and gradually decreased in stage 14 (Parks et al., 1986). For massive amount of protein production in limited time period the X-chromosome linked gene clusters amplified ~16-fold and third chromosome linked clusters amplified ~60-fold. Control element amplification of ACE1 from X-chromosome and ACE3 from the third chromosome was essential for the protein amplifications (Spradling et al., 1987; Orr-Weaver, 1991; Carminati et al., 1992). These two chorion gene clusters were programmed to be transcribed and amplified within a limited period of time and located in completely separate areas of nucleus. It has been suggested that if compartmentalization of function was required these two gene clusters may came to close vicinity from the separate areas (Tiwari and Lakhotia, 1990). In silkmoth more than 100 chorion proteins were found. These proteins surround the oocyte extracelluarly and interconnected together by cross linking to form the eggshell. It has revealed that in these proteins polymorphism also do exists. Large number of distinct structural chorion genes have been found to be associated with the synthesis of these chorion proteins. NH2-terminal sequencing of the proteins were also done for the chorion proteins in silk moth. These proteins had average molecular weight of 11,500 and comprising 69% of non-polar residues (Regier et al., 1978). In *A. polyphemus*, each and every cell-specific protein were synthesized under a specific developmental programme. Specific protein synthesis was controlled by specific mRNA synthesis or specific mRNA incorporation. At 25°C, 2.1 to 2.6 chorionic polypeptide chains were synthesized per mRNA per
minute (Paul et al., 1972; Kafatos, 1972). In *Gryllus mitratus* (Orthoptera) two type proteins have been identified. First type was phosphoprotein which was the main protein fraction and second type of proteins contained less amount of phosphorous. These two types of proteins differed in solubility also. The phosphoprotein was rich in serine and phosphorous and the second type of protein was with less phosphorous was termed as phosvitin. The phosvitin probably served storage function for the chorion. Phosphoproteins were mainly structural proteins (Kawasaki et al., 1971). *A. polyphemus* and *A. cecropia* chorion proteins were compared with structural morphogenesis and their gene expression has also been analyzed. It has been found that the eggshells were differing from morphological and protein profile also varied in various developmental stages (Hatzopoulos and Regier, 1987). Genes for two proteins have been identified in *Rhodnius* namely Rp30 and Rp45. These two proteins have similar sequences in central domain having repeats in VPV sequence. Genomic sequence of Rp30 protein was VXPNAGXFPFGFAAPFYGXYGVXP and that of Rp45 was XGPXGLVGDAGYLTGAPYYDXFH. ‘R&R consensus’ sequence was identified in Rp30 gene which indicated about the chitin binding ability of this protein (Iconomidou et al., 2005). This chitin binding feature of this protein indicated that this protein may remain intact throughout the embryogenesis process. Rp45 protein have been identified as having similarity with glycine rich structural protein elastin. These two proteins because of their glycine rich feature were designated as protein with anti-microbial activity (Bouts et al., 2007). In *Blatella germanica*, Suppression Subtractive Hybridization library has been constructed and with help of this 34 genes had been identified. These
genes were activated at late vitellogenesis and remain activated during choriogenesis. Among all these, two genes correspond to two proteins i.e. yellow-g and follicle-c proteins were involved in eggshell formation. Yellow-g protein was specialized for catalyzing the outer and inner chorionic layer. These functions of yellow-g protein had also been established in ants and Drosophila (Claycomb et al., 2004; Drapeau, 2001). Four ribosomal proteins were also identified which were responsible for secretion of large number of chorionic proteins in short period of time (Irles et al., 2009a).

Inorganic components may play role in chorion hardening as these varied with the change of developmental stages. Changes of inorganic components may be for mechanical movement of chorion surface ultrastructure (Dey et al., 2003; Williams, 1990). In silk moth Magnesium, Iron, Copper, Cobalt, Zinc, Calcium and Nickel have been found in all the developing stages of chorion. Concentration of all these components also increased with increasing time of maturity of chorion (Dey et al., 2003). Magnesium considered responsible for the colouration of chorion. Iron, Zinc and Copper has been predicted to be associated with protein and participated in photobiological, thermal and optical properties of chorion (Dey et al., 1998; Strobel, 1973). In Gryllus, chemical analysis of the chorion revealed the presence of calcium, magnesium, sodium, phosphorous and potassium. It was concluded for Gryllus chorion that all the metal cations with phosphoserine were designed to perform protective function of the chorion (Kawasaki et al., 1971).
Amino acid composition of chorion protein has been evaluated in silk moth chorion. Amount of different amino acids were varied in different proteins of chorion. Overall concentration of glycine, serine, cysteine and tyrosine was high and content of alanine, arginine, tryptophan was low. Some other protein fraction contained high amount of galactosamine, serine, proline, threonine, methionine, histidine while low in valine, isoleucine and leucine. Tryptophan was completely absent. Amino acids showed high variation in distribution. But NH$_2$– terminal part of the proteins was relatively rich in cystein, lysine and arginine. Proteins had repeats for some amino acids without known significance (Regier et al., 1978). *Drosophila* chorion has been reported to be composed of tryptophan, lysine, tyrosine, aspartic acid, histidine, arginine, cysteine, threonine, serine, proline, glycine, glutamic acid, alanine, methionine, valine, isoleucine, leucine and phenylalanine (Petri et al., 1976). Among these amino acids chorion had 17% serine, 18% proline and 29% alanine. Vitelline membrane had also different in composition and had 11% proline, 15% alanine and 16% glycine. Eggshell contained unusual amino acid also and from this it was convenient to distinguish different eggshells (Petri et al., 1976). Chorion of *Gryllus mitratus* contained glucosamine, aspartic acid, galactosamine, lysine, histidine, arginine, threonine, serine, proline, glycine, glutamic acid, alanine, cystine, valine, isoleucine, methionine, leucine, phenylalanine and tryptophan. Cystine and serine was present in high amount in this chorion proteins. Presence of serine with phosphorous defined the chorion proteins as a structural proteins (Kawasaki et al., 1971).
Maas spectral analysis of whole purified eggshell proteins was done for *Drosophila* eggshell. 2-D electrophoresis was done for chorion and a wide range of proteins from 30-150kDa. From 2-D gel 22 distinct proteins were identified. These proteins were categorized into different groups based on their functions. These secreted proteins were categorized as, eggshell structural proteins, basement membrane protein, enzyme-related proteins and oocyte surface proteins. Three putative genes CG13997, CG9050 and CG13992 were localized adjacent to the vitelline membrane genes sV17, sV23 and 26A. CG13114 and CG11381 which were minor chorion proteins and that were proline and glutamine rich protein respectively (Scherer *et al*., 1988; Waring, 2000). CG15570 was present adjacent to Femcoat chorion gene. Other proteins had no known functions related to the eggshell (Fakhouri *et al*., 2006). MALDI/TOF/MS analysis of three major chorion proteins of *Aedes aegypti* was done. SDS-PAGE analysis revealed three major chorion proteins and these were subjected to MS/MS analysis. c-13, c-15 and c-16 sequences correspond to the vitelline envelop protein 15a2, 15a1 and 15a3 respectively. 19, 16 and 13 residues from the N-terminal portion of the proteins were processed during choriogenesis. Proline, alanine, valine or leucine was present in high amount in these proteins and these feature indicated the hydrophobic nature of the protein. Conserved tyrosine and cysteine residues indicated about the formation of disulfide and di-tyrosine crosslinking within these three proteins or other chorion proteins which leads to hardening and insolubilization of the chorion. These 15a2, 15a1 and 15a3 proteins considered as most abundant chorion proteins. Sequencing for these c-13, c-15 and c-16
proteins have also been done and compared with 15a2, 15a1 and 15a3 protein sequences (Li and Li, 2006) in Aedes aegypti.