Ganoine Formation in the Scales of Primitive Actinopterygian Fishes, Lepisosteids and Polypterids

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The scales of primitive living actinopterygian fishes, lepisosteids and polypterids, have retained ganoine, a hypermineralized layer which covered the scales of the osteichthyan ancestors. To know finally its tissue origin in the actinopterygian lineage, ganoine formation was described in Lepisosteus oculatus, with scales devoid of dentin, and was compared to ganoine formation in two polypterids, Calamoichthys calabaricus and Polypterus senegalus, with scales possessing a dentin layer. The events taking place before, during and after ganoine deposition were studied in experimentally regenerated scales using light and transmission electron microscopy. In spite of differences in tissue composition and in organization of the epidermal cells on the scale surface, ganoine formation is similar in both types of scales. Preganoine is deposited by epidermal cells and constitutes a thick layer which mineralizes progressively to become ganoine, a true enamel. The cellular processes involved in ganoine formation were compared to those described for enamel in mammalian teeth.

Key Words: actinopterygians, scales, ganoine, enamel, development

INTRODUCTION

Ganoine is a superficial hypermineralized layer covering the scales in two lineages of primitive living osteichthyan fishes, lepisosteids and polypterids.1,2 These ganoid scales are of a primitive type. After millions of years they have conserved most of the characters of the rhombic scales of the osteichthyan ancestors: a thick basal plate covered by tooth-derived material.3,4

The structure and organization of the ganoid scales have been known for a long time.1,2,5-8 The lepisosteid scales differ from the polypterid scales in the lack of dentin between bone and ganoine, in the scarcity or absence of vascular canals and in the presence of canaliculi of Williamson which exist only in the bone of holostean fishes (see, e.g., Sire and Meunier).5 Ganoine early caught the attention of morphologists, both with regard to its developmental origin and nature, and to the evolutionary relationships existing between the ganoid scales and the scales of Selachians, Dipnoi and Teleostees (see discussion in Sire et al.,10). In spite of numerous studies, the embryological origin of the ganoine (dermal or ectodermal) was not solved until Sire et al.10 clearly demonstrated that ganoine in the polypterid Calamoichthys calabaricus is an epidermal product and, consequently, true enamel.

The aim of the present work is (1) to answer the question whether the ganoine in lepisosteid scales is also an epidermal product and therefore whether true enamel can be deposited in absence of dentin, and (2) to compare the cellular processes involved in ganoine formation in these scales to those occurring in polypterid scales and in enamel deposition in teeth. For these purposes, and because it is difficult to obtain growth series, regeneration has been used to induce ganoine formation in the scales of the lepisosteid Lepisosteus oculatus, and of the polypterids Calamoichthys calabaricus and Polypterus senegalus.

MATERIAL AND METHODS

Scale regeneration was provoked in two Lepisosteus oculatus (255 and 280 mm total length), two Calamoichthys calabaricus (295 and 320 mm TL) and two Polypterus senegalus (260 and 280 mm TL). Scales were dissected out on the posterior region of the flank. Regenerating scales were removed one, two and three months after the first surgery to examine ganoine formation10 and were fixed for 2 hrs at room temperature in a mixture of 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M cacodylate buffer. For decalcification, samples were immersed for 7 days at 4°C in the fixative solution to which 0.1 M EDTA was added. Decalcified and undecalcified samples were rinsed in the buffer to which 10% sucrose was added. Postfixation was performed for 2 hrs at room temperature in 1% osmium tetroxide in cacodylate buffer. The samples were next dehydrated in a graded series of
ethanol, then embedded in Epon. One-µm thick sections were stained with toluidine blue and thin sections were contrasted with uranyl acetate and lead citrate and examined at 80 kV with a Philips 201 Electron Microscope.

RESULTS

Brief Overview of the Scale Structure

The ganoid scales of *Lepisosteus oculatus* (Fig. 1) belong to the lepisosteoid type: they are thick, cellular bony plates covered by ganoine. They do not possess any kind of dentin between bone and ganoine matrix. The bony plate is characterized by a poorness in vascular canals and by the presence of canaliculi of Williamson. The latter house the cytoplasmic processes of the typical cells of Williamson. These are located at the scale surface and may have a nutritive function, probably compensating for the lack of vascular canals.9 The ganoid scales of *Calamoichthys calabaricus* and *Polypterus senegalus* (Fig. 2) belong to the palaeoniscoid type: the thick, cellular, bony plate is covered by a layer of modified dentin (probably osteodentin11 but referred hereafter as dentin) which is itself overlaid by ganoine. In polypterus scales only, a small, central region of isopedine, is sandwiched between the dentin layer and the bony plate.11 The dentin layer contains a vascular network which tends to be obliterated in old fish, and the bony plate shows numerous vascular canals mainly located in its upper region.

In both types of scales, ganoine is composed of several layers, which are deposited periodically. The edge of each ganoine layer is partially overlapped by bone and this results in a series of short serrations (Figs. 1, 2). Epidermis either covers the ganoine or is separated from it by dermal elements, but the ganoine surface is always covered by an unmineralized, 500 nm-thick layer, the ganoine membrane (see below).

Regeneration of the Bony Plate

In both types of scales, wound healing requires at least two weeks before the first elements of the regenerated scales appear. After one month of regeneration, the location of the regenerating scale anlage slightly differs between the two scale types. In *Lepisosteus* the bony plate is developing in the middle part of the dermis whereas in polypterus the first elements of the scale (dentin) are depositing in the upper part of the dermis, close to the

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FIGURES 1 and 2 Drawings of non-regenerated scales from 1-µm thick sections of EDTA decalcified scales. Anterior part of the fish on the left. Fig. 1: *Lepisosteus oculatus*; Fig. 2: *Polypterus senegalus*. Polypterus scales possess a layer of dentin, isopedine and vascular canals which are not present in lepisosteid scales but where the basal plate contains canaliculi of Williamson. BM = bony matrix; BP = bony plate; CW = canaliculi of Williamson; D = dermis; De = osteodentin; EBC = epidermal basal cell; Ep = epidermis; Ga = ganoine; GM = ganoine membrane; IGE = inner ganoine epithelium; Is = isopedine; MF = mineralization front; Ob = osteoblast; OGE = outer ganoine epithelium; Os = osteoid; PGa = preganoine; RER = rough endoplasmic reticulum; SF = Sharpey fiber; VC = vascular canal.
epidermis. Both regenerating scales thicken until they regain approximately their initial size, two months after scale removal. Then the deposition of bone and dentin matrix slows down at the scale surface.

The following description concerns the events occurring at the scale surface leading to ganoine deposition. They have been divided into four stages: end of collagen matrix deposit at the scale surface; epidermal contact on the scale surface; preganoine deposition; and ganoine maturation.

End of Collagen Matrix Deposition at the Scale Surface

Bone and dentin matrix deposition stops at the scale surface approximately after two months of regeneration. At this stage the upper surface of the scale is close to the epidermis and the layer of osteoblast-like cells is separated from the epidermis by dermal elements (Figs. 3, 4). The basal layer of the epidermis is composed of interdigitated, closely juxtaposed cells. In both scales, the last deposited collagen fibrils (bone or dentin matrix) are perpendicular to the scale surface and to the lining cell layer, and are still not mineralized (Figs. 5, 6). This is a particular type of osteoid. The osteoblasts covering this region are roughly rectangular cells with some cytoplasmic extensions located within the osteoid and parallel to the vertical fibrils.

Epidermal Contact on the Scale Surface

In both types of scales, approximately three months after scale removal, a part of the surface is covered by an epidermal layer originating from the basal layer of the epidermis (Figs. 7, 8). The epidermal covering first occurs on the surface of the posterior region of the scale and extends anteriorly. The interface between the epithelial cells entering in contact with the scale surface and the scale lining cells is characterized by the presence of cellular debris. Moreover the lining cells adjacent to the epithelial cells either have a rounded shape and are equipped with numerous bundles of microfilaments, or some of these cells have a necrotic aspect. The epidermal contact differs depending on the scale type. In polypterids, the epidermis itself enters in contact with the scale surface by means of its basal layer (Fig. 8). In *Lepisosteus* an epithelial sheet issued from the basal layer of the epidermis spreads on the scale surface; it is only connected to the epidermis by a thin epithelial bridge (Fig. 7). Elsewhere the epithelial sheet is separated from the basal layer of the epidermis by dermal components.

In *Lepisosteus*, the epithelial cells organize into a bilayered epithelial sheet as soon as they cover the bone (Fig. 9). This epithelial sheet is divided into an inner ganoine epithelium (IGE) which is in contact with the scale surface and is responsible for ganoine formation, and an outer ganoine epithelium (OGE) facing the dermis. The IGE is composed of cuboidal, polarized cells perpendicular to the scale surface, whereas in the OGE the cells are elongated and parallel to the scale surface. The OGE cells are linked to one another and to the IGE cells by short desmosomes. In polypterids the epidermal layer which covers the dentin surface is differentiated into one cell layer, the inner epidermal layer (IEL). There is no differentiated outer layer similar to the OGE. The IEL cells are similar to the IGE cells in *Lepisosteus* in organization and function. Both layers are homologous and will be referred as IGE in the following descriptions.

The IGE cells are closely juxtaposed and connected to one another by desmosomes. Their cytoplasm is rich in RER cisternae, Golgi sacules and mitochondria (Fig. 9). The Golgi apparatus is located close to the rounded nucleus that generally occupies a large part of the central region of the cell. The apical region, which is in contact with the osteoid, shows small vesicles, free ribosomes and microfilaments only (Figs. 9, 10). The contact is established in the region where the last deposited collagen fibrils of the scale matrix are vertically oriented. Here the basal lamina has disappeared and approximately one μm-long cytoplasmic extensions of the IGE cells penetrate the osteoid where they parallel the last deposited vertical collagen fibrils (inset Fig. 9 and Fig. 10).

Ganoine Matrix Deposition

In both types of scales, the first elements of the ganoine matrix (preganoine) are deposited approximately three to four months after scale removal. Newly deposited preganoine is well defined at the light microscopical level as dark (metachromatic) spots located below the columnar IGE and contrasting with the clear osteoid matrix of the basal plate surface (Figs. 11, 12).

The first elements of the preganoine matrix are electron-dense fibrils and granules first localized close to cytoplasmic extensions of the IGE cells (Fig. 13). Then preganoine matrix organizes into rounded patches (300 to 500 nm in diameter) in which the electron-dense material radiates from the center (Fig. 14). Some patches can enlarge to reach 1.5 μm in diameter. Others can be directly in contact with the plasmalemma of the IGE cells which shows thickenings and caveolae (Fig. 14). The first mineral crystals are seen early, as soon as the preganoine patches form (Fig. 14, inset). Mineral crystals are oriented along the fibrils of preganoine so that the patches look like small sea urchins.

In older stages preganoine patches have fused and a preganoine layer is formed (Figs. 15, 16). The IGE cells producing preganoine are 10 μm high, regularly arranged cells (Fig. 15). The apical region is devoid of cytoplasmic organelles but it possesses numerous, large bundles of
FIGURES 3 and 4  End of bone matrix deposition. One μm-thick sections of the posterior part of two month-regenerated scales. The surface of the basal plate (BP) is lined by a layer of osteoblast-like cells at a small distance from the epidermis (Ep). Fig. 3: Leptosteus; Fig. 4: Polysternum. Bars = 25 μm; ×400.

FIGURES 5 and 6  Detail of the upper surface of the central region of two month-regenerated scales. The last deposited collagen fibrils in the unmineralized osteoid (Os) are mostly perpendicular to the osteoblast surface. Fig. 5: Leptosteus; Fig. 6: Polysternum. Bars = 500 nm; ×30,000.
microfilaments (Figs. 17 inset, 18). These bundles are issued from desmosomes connecting the plasmalemma of neighboring cells (Fig. 17). The interface between the preganoine and the collagen matrix of the basal plate is constituted by an approximately 2 μm-thick layer of mixed tissue in which both preganoine and collagen fibrils are intermingled (Fig. 19). The preganoine is mainly composed of thin, roughly parallel fibrils perpendicular to the IGE surface (Figs. 17, 18). The fibrils are 15 nm in diameter in average and are separated by electron-lucent spaces sometimes obliterated by an amorphous background substance (Fig. 20). In the last deposited preganoine, mineral crystals are scarce. They are approximately 10 nm thick and are oriented along the preganoine fibrils, perpendicularly to the scale surface (Fig. 21).

**Ganoine Maturation**

The preganoine layer thickens by addition of new matrix at its surface, directly below the IGE cells to reach approximately 15 μm (Fig. 15). Then preganoine deposit stops and the maturation phase begins. Mineralization takes place throughout the preganoine layer and the amount of mineral progressively increases to reach a high level of mineralization. This maturation process leads to ganoine. In decalcified samples, the process of maturation of the preganoine appears to result in the progressive disappearance of its organic matrix, which is removed along with the mineral (Figs. 22–28). When decalcified in the first stages of maturation, the ganoine matrix looks less well organized in the basal region of the layer than in its upper region (Fig. 22). It looks also looser when compared to the stages preceding the maturation phase (compare Figs. 22, 23 to Figs. 9, 17). The organic matrix of the ganoine completely disappears after decalcification when the maturation process is finished (Figs. 24, 25). Undecalcified samples reveal that the mineral crystals of the ganoine are disposed perpendicularly to the scale surface (Fig. 26).

Some time after the maturation phase has started, a layer of homogeneous, unmineralized substance appears between the IGE cells and the ganoine surface (Fig. 23); this constitutes the anlage of the ganoine membrane. This 200 nm-thick anlage is composed of a granular, electron-dense layer located close to the IGE cell plasmalemma, which itself presents numerous hemidesmosomes (Fig. 24). During the maturation phase, the ganoine membrane thickens and reaches a final thickness of approximately 600 nm (Fig. 25). Then it progressively acquires its typical organization along with ganoine is completely formed (Figs. 27 inset, 28). In *Lepisosteus*, the well-formed ganoine membrane is composed of three zones (Fig. 27 inset): a 50 nm-thick, clear zone containing thin dark granules and filaments, and facing the plasmalemma of the epidermal basal cells; a 50 nm-thick, striated zone with alignments of dark granules separated by electron-lucent spaces parallel to the scale surface; and a 500 nm-thick zone constituted of a homogeneous thin granular substance. In polypterids, the ganoine membrane is different only in the clear vertical striation of the thick zone (Fig. 28).

During the maturation process of the ganoine, the well-differentiated IGE cells change progressively to finally become typical, plump, basal epidermal cells when ganoine is finally formed: large intercellular spaces, interdigitations, short desmosomes linking adjacent cells, dark cytoplasm rich in bundles of microfilaments, free ribosomes and small vesicles (Figs. 27, 28).

**DISCUSSION**

The present study clearly confirms that experimental regeneration is a useful manner to induce ganoine production in the ganoid scales of primitive living actinopterygian fishes, lepisosteids and polypterids. The processes involved in regeneration of such scales, and especially their upper part, repeat those occurring during ontogeny. Experimental regeneration has the advantage that it needs a shorter time than normal development and that it uses adult or subadult instead of young fish, especially in a case where artificial reproduction is difficult as it is for lepisosteids or polypterids.

The origin of the cells depositing ganoine in ganoid (lepisosteoid and palaeniscoid) scales is now finally elucidated. In both types, ganoine is exclusively epidermal in...
FIGURES 11 and 12  Preganoine deposit. One μm-thick sections of the posterior part of three month-regenerated scales. The darkly stained (metachromatic) preganoine has been deposited at the scale surface (arrowhead and arrow, respectively) below the cuboidal cells of the IGE. Bars = 25 μm. Fig. 11: Lepisosteus, ×400; Fig. 12: Polypterus, ×550.
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origin: (1) it is synthesized by the inner ganoine epithelium (IGE) which thus appears to be equivalent to the inner dental epithelium in mammalian tooth enamel formation; and (2) pregnogane does not contain collagen fibrils and progressively matures to become a highly mineralized tissue, ganoine. As enamel, ganoine is secreted at the interface between dentine (or bone in the case of lepisosteid scale) and epithelium, and grows centrifugally from the dentine-ganoine junction. In ganoine, the ameloblast products are not secreted through a basal lamina and they are not mixed with products from the odontoblasts as in enameloid. Moreover the mineral crystals in ganoine form a prismatic arrangement as in enamel. Consequently ganoine is true enamel. Moreover in a recent survey of various types of ganoine, Richter and Smith concluded that there are no criteria to distinguish between ganoine and enamel and that the term of ganoine is adopted as more descriptive than systematical. These results close a long controversy, starting with Agassiz, on the origin of the ganoine in the ganoid scales of living primitive actinopterygian fishes. One can also reasonably assume that other types of ganoine which have been reported in living and fossil osteichthyian fishes are formed in the same way and consequently are enamel too.

Ganoine differs from tooth enamel in being stratified, in being sometimes covered by dermal elements and in never being exposed to the external environment. Ganoine deposition is a periodical phenomenon. At given times, probably in relation to epidermal-dermal interactions, the epidermis withdraws from the ganoine surface and dermal elements invade the space on the entire surface of the scale in Lepisosteus and on a large part of its surface in polypterus. Later the epidermis again contacts the ganoine surface, whereupon the dermal elements withdraw. The superimposition of numerous layers of ganoine in adult specimens suggests that this to-and-fro movement of the epidermis persists throughout the life span of the fish. Bone is deposited on the ganoine surface in the marginal regions only, in both types of scales, and this gives rise to the typical ganoine serrations (already described in some lepisosteids by Thomson and McCune. In contrast to bone formation, ganoine deposition occurs during a relatively short period; it is probably why during normal scale development it is difficult to observe stages in which ganoine is being deposited.

In contrast to tooth enamel deposition, ganoine in lepisosteid scales is deposited in the absence of dentin. Lepisosteid scales have no dentin but this does not prevent ganoine to be deposited directly upon the bony matrix of the basal plate. Moreover in polypterus scales, the "dentin" layer located below the ganoine might be considered osteodentin rather than typical dentin. In mammalian tooth development dentin forms before enamel and this sequence is regulated by epithelial-mesenchymal interactions. This is not true for ganoine deposition in ganoid scales. In place of odontoblasts, osteoblasts can interact with epidermal cells to induce ganoine deposition. Some events occurring before ganoine deposition can be interpreted as the result of epidermal cell-osteoblast interaction. For example the perpendicular collagen fibrils are deposited by the osteoblasts shortly before the epidermal cells enter into contact with the scale surface. When pregnogane is deposited, this particular organization allows the penetration of the ganoine matrix in between the collagen fibrils and this results in a better anchorage of the ganoine. Also when the epithelial cells reach the scale surface, morphological observations indicate that dermal elements have withdrawn or have been eliminated from the scale surface. This process allows the epithelial cells to cover the scale rapidly over a great surface. Then the withdrawing of the epidermis from the ganoine surface, especially in Lepisosteus, facilitates dermal elements to penetrate the space between the epidermis and the ganoine. In contrast to polypterus, Lepisosteus scales do not possess a vascular network to provide the scale surface with nutrients when it is covered by the epidermis. Consequently, the withdrawing of the epidermis is probably necessary to allow some capillary blood vessels to bring nutritive elements to the basal surface of the epidermis.

There are no reports in the literature dealing with the protein content of the organic matrix of the ganoine. Are there enamelines or amelogenins, or both? There are controversies about the evolutionary origin of enamel versus

FIGURES 13 and 14  Detail of the apical region of the IGE cells producing pregnogane. Fig. 13: Polypterus. First patches of pregnogane matrix (arrow) are deposited within the scale matrix (Os) close to the cytoplasmic extensions (arrowhead) of the IGE cells. Bar = 500 nm; ×20,000. Fig. 14: Lepisosteus. Rounded patches of pregnogane (arrow) are formed in the osteoid (Os) at a distance from the IGE cell surface. Arrowhead indicates a pregnogane patch which is formed close to the cell surface. Bar = 1 μm; ×15,000. Inset: Detail of a pregnogane patch from an undecalcified sample. The first mineral crystals soon appear and are oriented (arrowheads) along the fibrils of the pregnogane. Bar = 500 nm; ×30,000.

FIGURES 15 and 16  Pregnogane layer. One μm-thick sections of the posterior part of three month-regenerated scales. Fig. 15: Lepisosteus; Fig. 16: Polypterus. Note that ganoine maturation has started on the left. Bars = 25 μm; ×400.

FIGURES 17 and 18  Detail of the pregnogane layer formed after fusion of the patches and thickening by addition of new material on its surface. Note that the fibrillar matrix of the pregnogane (PGa) is oriented perpendicularly to the scale surface. Fig. 17: Lepisosteus. Arrowhead points to a desmosome. Bar = 1 μm; ×7,000. Inset: The apical region of the IGE cells facing the pregnogane layer is poor in organelles and rich in bundles of microfilaments. Bar = 500 nm; ×22,000. Fig. 18: Polypterus. Bar = 1 μm; ×10,000.
FIGURE 19 *Polypterus*. Boundary between bone matrix (BM) and preganoine (PGa) showing intermingled matrices. Arrowheads point to centers of former preganoine patches. Bar = 1 μm; ×14,000.

FIGURE 20 *Leptosomus*. Preganoine matrix. Bar = 250 nm; ×45,000.
enameloid. Some immunohistochemical studies have shown that enamelin is probably present in enameloid of sharks and teleost teeth and that amelogenins are not present.\textsuperscript{17, 18} Based on the finding that in mammalian tooth enamel, enamelines represent 10% and amelogenins 90%, of the protein content,\textsuperscript{19} authors have suggested that evolution involved the development of a gene for amelogenins in Vertebrates from sarcopterygians; consequently enameloid was developed earlier than enamel in vertebrates. The present study demonstrates that true enamel (ganoine) is present in the primitive actinopterygians, the other lineage of osteichthians, and, in our laboratory, a preliminary work using immunocytochemistry has shown the possible existence of amelogenins in the ganoine. Recently, Smith\textsuperscript{20} has proposed a new theory based on comparative studies on the dental skeleton in primitive vertebrates; she claims that enamel was present earlier than enameloid, and she considers the "reduction" of the enamelines as an evolved character linked with the earlier time of secretion of the ameloblasts throughout heterochrony.

The processes involved in ganoine deposition in \textit{Lepisosteus} and polypterid scales only differ in the manner in which the epidermis comes into contact with the scale surface. In \textit{Lepisosteus}, an epithelial sheet spreads on the scale surface and it is linked to the epidermis only by a short epithelial bridge; in polypterids the epidermis itself comes in direct contact with the bone surface by means of its basal layer. Also, in \textit{Lepisosteus}, the epithelial sheet that covers the scale is composed of two layers, an outer and an inner ganoine epithelium (OGE and IGE) whereas in polypterids the basal epidermal layer cells differentiate to constitute one layer, called the inner epidermal layer (IEL). Both IGE and IEL synthesize ganoine (i.e., enamel). Consequently both are homologous and are homologous to the inner dental epithelium (IDE) in tooth development; their differentiated cells are ameloblasts. Moreover, in \textit{Lepisosteus}, the bilayered organization of the epithelial sheet recalls the classical enamel organ described in vertebrate tooth development, both are homologous structures. However on the lepisosteoid scale the "enamel organ" invests a large surface and it is covered by dermal elements. The typical bell shape does not form as in tooth development because ganoine is flat. In \textit{Lepisosteus} scales the OGE is homologous to the outer dental epithelium (ODE) in tooth development. In both types of scales, the columnar organization and the cytoplasmic content of the IGE cells (ameloblasts) during ganoine formation are comparable to what is known for ameloblasts during tooth formation.\textsuperscript{21}

The early deposits of the ganoine matrix in \textit{Lepisosteus} (and also in polypterid scales) are morphologically similar to the globular patches of early enamel matrix in the teeth of the urodile, \textit{Triturus pyrrhogaster}.\textsuperscript{22} Moreover this newt enamel is reported to be composed of two layers: an outer layer of true enamel and an inner layer interpreted as a mixed form of dentin-enamel matrices.\textsuperscript{22} A similar organization has been described herein for the interface between bone and ganoine which shows a 2 \textmu m-thick region composed of interpenetrated bone and ganoine matrices. I consider this region as an anchorage of the preganoine within the osteoid, and there is no reason to consider it as a special layer. Ganoine is, on the contrary, totally different from the so-called enamloid, a hard tissue containing ectodermally\textsuperscript{23, 24} or mesenchymally\textsuperscript{25} derived collagen fibrils, which covers the teeth of

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{\textit{Calamoichthys}. Organization of the mineral crystals (arrowhead) in the upper part of the preganoine. Bar = 250 nm; \times45,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{\textit{Polypterus}. Beginning of the maturation stage of the preganoine (PGa). In the deep region of the preganoine layer, the matrix has been partially removed during decalcification. The apical region of the IGE cells shows vesicles with electron-dense or electron-lucent content (arrowheads) and bundles of filaments. Bar = 500 nm; \times30,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{\textit{Calamoichthys}. Upper region of the scale at the beginning of ganoine maturation. The first elements of the unmineralized ganoine membrane (GM) appear between the IGE and the ganoine matrix (Ga). Bar = 500 nm; \times30,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure4.png}
\caption{\textit{Polypterus}. Advanced stage of maturation of the ganoine (Ga). Ganoine matrix has been removed during the decalcification and the ganoine membrane (GM) begins to form. Bar = 500 nm; \times30,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5.png}
\caption{\textit{Lepisosteus}. Final stage of ganoine maturation. Ganoine matrix (Ga) has been totally removed and the ganoine membrane (GM) is thick. Bar = 500 nm; \times30,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6.png}
\caption{\textit{Lepisosteus}. Same stage, undecalcified sample. The ganoine is well mineralized and the mineral crystals are attached to the deep surface of the ganoine membrane which remains unmineralized. Bar = 500 nm; \times30,000.}
\end{figure}

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\includegraphics[width=\textwidth]{figure7.png}
\caption{\textit{Lepisosteus}. Finally formed ganoine. The epidermal basal cells (EBC) are lining the ganoine membrane (GM) which covers ganoine (Ga). Bar = 1 \textmu m; \times10,000. Inset: Ganoine membrane. Note the thickening of the plasmalemma (arrowhead) of the epidermal basal cell facing the ganoine membrane, the upper layer of which is delaminated. Bar = 500 nm; \times30,000.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure8.png}
\caption{\textit{Calamoichthys}. The ganoine membrane shows vertical striations. Arrowhead points to a caveola fusing with the epidermal basal cell plasmalemma. Bar = 500 nm; \times30,000.}
\end{figure}
most osteichthyans and chondrichthyans (see also Kawasaki and Fearnhead, for a review).

This is the first TEM description of the ganoine membrane in *Lepisosteus* scales. Such a membrane has already been observed on the ganoine surface in polypterid scales (the "intermediate layer.") This thick organic layer is always seen to cover the ganoine in both types of scales. The fine structure of the ganoine membrane is roughly the same in the lepisosteid and polypterid scales except for a distinct vertical striation in polypterids. The ganoine membrane resembles a basement membrane but it is attached to the ganoine surface and persists on this surface even when secondarily overlapped by dermal elements. Zylberg et al. hesitated between considering it as a basement membrane with a thick lamina densa or as a special structure developed in relationship to the ganoine. The ganoine membrane is synthesized by the IGE cells when preganoine is mineralizing but it does not mineralize itself. These data support the hypothesis that the ganoine membrane is a special structure formed in relationship with the presence of a thick, well-mineralized tissue, ganoine. The ganoine membrane is rich in mucous substances (proteoglycans). Proteoglycans could prevent mineralization of the ganoine membrane. Moreover, the ganoine membrane probably contains adhesive substances facilitating fixation of the basal layer cells of the epidermis. The ganoine membrane could allow the mineral crystals of the ganoine to fix to its deep side and the soft tissues to stick on to its superficial side. Such a membrane has probably developed in relation to the presence of a hard (enamel)-soft (epidermis) interface to prevent the epidermis to slip during swimming. A similar function as an "antislip pad" has been suggested for the ganoine membrane in polypterid scales. Such a well-developed structure does not exist as a lining of tooth enamel.

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