Regulation of Extracellular Matrix Assembly:  
In Vitro Reconstitution of a Partial Fertilization Envelope from Isolated Components

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Abstract. At fertilization, the glycocalyx (vitelline layer) of the sea urchin egg is transformed into an elevated fertilization envelope by the association of secreted peptides and the formation of intermolecular dityrosine bonds. Dityrosine cross-links are formed by a secreted ovoperoxidase that exists in a Ca$^{2+}$-stabilized complex with proteoliaisin in the fertilization envelope. By using purified proteins, we now show that proteoliaisin is necessary and sufficient to link ovoperoxidase to the egg glycocalyx. Specifically, we have found that (a) ovoperoxidase can associate with the vitelline layer only when complexed with proteoliaisin; (b) proteoliaisin binds to the vitelline layer independently of its association with ovoperoxidase; (c) proteolytic modification of the vitelline layer is not required for this interaction to occur; (d) the binding of proteoliaisin to the vitelline layer is mediated by the synergistic action of the two major seawater divalent cations, Ca$^{2+}$ and Mg$^{2+}$; (e) the number of proteoliaisin-binding sites on the vitelline layer of unfertilized eggs is equivalent to the amount of proteoliaisin secreted at fertilization; and (f) the binding of ovoperoxidase to the vitelline layer, via proteoliaisin, permits the in vitro cross-linking of these two in vivo substrates. The association of purified ovoperoxidase and proteoliaisin with the vitelline layer of unfertilized eggs reconstitutes part of the morphogenesis of the fertilization envelope.

Significant advances have been made in elucidating the nature of the diverse components that constitute extracellular matrices (ECM), yet little is known about the coordination of their assembly, or how the combination of individual constituents defines the specialized functions of the ECM. Since the ECM may determine such processes as the cell-cell interactions and cell movements that modulate developmental pathways, there must be specific controls for glycocalyx assembly and modification. These controls, isolated from the cytosolic machinery, may involve novel regulatory mechanisms. The lack of information on this issue relates in part to the paucity of model systems where specific questions can be asked about discrete glycocalyx modifications.

In this regard the sea urchin egg at fertilization provides an ideal system for investigating the assembly and modification of a specific extracellular matrix. A secretary event that follows fertilization (the cortical reaction) releases 5% of the egg protein to modify the glycocalyx layer and bring it to an elevated, hardened fertilization envelope that protects the embryo until the blastula hatches (for review see reference 13). Modification of the vitelline layer occurs in discrete steps and is complete within 10 min of fertilization, thus providing a convenient system for biochemical investigation. Analysis is facilitated by the large quantities of eggs that can be synchronously induced to undergo these modifications.

We have been studying these modification reactions by using isolated components and attempting to effect a reconstitution of the system in vitro. Two components of the fertilization envelope, ovoperoxidase and proteoliaisin, have been purified (7, 20). Proteoliaisin, which forms a Ca$^{2+}$-stabilized complex with ovoperoxidase, is thought to play a coordinating role in fertilization envelope assembly. This hypothesis was formed from the observations that the ovoperoxidase-proteoliaisin complex is found in uncross-linked fertilization envelopes and that proteoliaisin is a substrate for ovoperoxidase-catalyzed dityrosine formation in vivo (20). In this paper, we explore the possibility that proteoliaisin is responsible for integrating ovoperoxidase into complexes with vitelline layer components.

We have examined the association of ovoperoxidase with the vitelline layer by directly measuring the binding of purified, $^{125}$I-labeled ovoperoxidase and proteoliaisin to the vitelline layers of unfertilized eggs. With this in vitro system, we have also tested the possibility that proteolytic modifications...
Table I. Interaction of Ovoperoxidase with the Vitelline Layer

| Incubation conditions | 125I-Ovoperoxidase bound (%) |
|-----------------------|-----------------------------|
| A. Eggs with intact vitelline layers |                         |
| NSW                   | 0                           |
| NSW + proteoliasin    | 76                          |
| NSW + proteoliasin + nonradioactive ovoperoxidase | 2  |
| EGTA-SW + proteoliasin | 0                          |
| B. Eggs with disrupted vitelline layers |                         |
| Dithiothreitol-treated eggs in NSW + proteoliasin | 2                        |
| Trypsin-treated eggs in NSW + proteoliasin | 23                      |

The assay procedure is described in Materials and Methods. Each 250 μl sample contained 12 μg/ml 125I-ovoperoxidase (8 × 106 cpm/ml) and 10% eggs (by volume). The concentrations of nonradioactive proteoliasin and ovoperoxidase were 24 μg/ml and 120 μg/ml, respectively. Results are expressed as the percent of 125I-ovoperoxidase in the incubation mixture that was bound to the egg surface.

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Materials and Methods

Gamete Preparation and Materials

Sea urchins (Strongylocentrotus purpuratus), collected on the Straits of Juan de Fuca, were maintained, spawned, and the eggs collected as previously described (30). The egg jelly coat was removed either by brief exposure to pH 5.0 or to EGTA artificial seawater (SW) (19). Either treatment gave qualitatively similar results in our egg binding assays. In some experiments, the egg vitelline layers were disrupted by dithiothreitol (DTT) treatment (9) or by trypsin treatment (8). Eggs were washed three times in excess artificial SW before use in the assays.

All artificial SWs contained 10 mM KCl and 10 mM Tris, pH 7.8-8.0 (at 4°C). In addition, normal seawater (NSW) contained 458 mM NaCl, 10 mM CaCl2, and 50 mM MgCl2; calcium seawater (CaSw) contained 530 mM NaCl and 10 mM CaCl2; magnesium seawater (MgSw) contained 473 mM NaCl and 50 mM MgCl2; EGTA seawater (EGTA-SW) contained 500 mM NaCl and 25 mM EGTA. The divalent cation dependence of the egg binding assay was determined in seawater containing 500 mM NaCl to elute the iodinated proteoliasin. Fractions were collected and assayed for TCA-precipitable 125I. The latter fractions were pooled and the specific radioactivity of the protein was determined. In four separate labeling experiments, the average proportion of TCA-precipitable 125I was 82%. Attempts to remove residual free 125I by either repeated concentration and dialysis or by dialysis failed to significantly reduce this amount.

In the calculations, the specific radioactivity was taken to be the total cpm of labeled ovoperoxidase or proteoliasin with a washed egg suspension in the egg binding assay. 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Binding of ovoperoxidase to the vitelline layer of unfertilized eggs as a function of proteoliaisin concentration. Assays were performed in NSW as described in Materials and Methods. Each 0.5-ml assay contained 4.5 μg/ml 125I-ovoperoxidase (8 × 10⁴ cpm/ml), 10% eggs (vol/vol), and the indicated concentrations of proteoliaisin. Results are expressed as the percent of 125I-ovoperoxidase in the incubation mixture that was bound to the eggs.

Results

Binding of Ovoperoxidase to the Surface of Unfertilized Eggs

We began our investigation by examining the ability of 125I-ovoperoxidase to bind to the vitelline layers of unfertilized eggs. The results of these experiments are summarized in Table I and Fig. 1. Ovoperoxidase alone did not bind to the surface of unfertilized eggs. However, substantial binding was observed when the incubation mixture included proteoliaisin. The binding of 125I-ovoperoxidase showed a linear dependence on the concentration of proteoliaisin (Fig. 1) and was inhibited when a 10-fold excess of unlabeled ovoperoxidase was included in the incubation (Table I). Thus, ovoperoxidase appeared to bind to the egg surface only when complexed with proteoliaisin. As expected, no binding was observed in EGTA-SW, since the interaction of ovoperoxidase with proteoliaisin requires Ca²⁺ (20). These results also suggested that proteolytic processing is not obligatory for the binding of these two proteins to the vitelline scaffold, since eggs with intact vitelline layers were used. In fact, treatments which disrupt the vitelline layer (DTT and trypsin) decreased the binding of ovoperoxidase–proteoliaisin complexes to the egg surface (Table I). We conclude that proteolytic processing of the egg glycochalix was not necessary for the binding of ovoperoxidase to the vitelline layer scaffold, and that proteoliaisin was necessary and sufficient to mediate this binding.

Binding of Proteoliaisin to the Vitelline Layer of Unfertilized Eggs

To determine whether the binding of ovoperoxidase to the vitelline layer, in association with proteoliaisin, is cooperative or indirect, we examined the binding of 125I-proteoliaisin to the vitelline layer of unfertilized eggs. Proteoliaisin bound to the vitelline layer in the absence of ovoperoxidase (Table II), as long as divalent cations were present. Addition of ovoperoxidase had little effect on this binding. Changes in the divalent cation content of the seawater, however, did have significant effects on the association of proteoliaisin with the vitelline layer. Binding was optimal in NSW and decreased by 27% in CaSW. A small but significant amount of binding was observed in MgSW. Virtually no binding was observed in seawater lacking divalent cations. We conclude that proteoliaisin binds directly to the vitelline layer in a divalent cation-dependent fashion and that ovoperoxidase is not required for this interaction. Therefore, ovoperoxidase binds to the vitelline layer indirectly, by its association with proteoliaisin. In the experiments that follow, incubation in EGTA-SW was used to determine the nonspecific binding of proteoliaisin to the vitelline layer.

Divalent Cation Requirements for the Proteoliaisin–Vitelline Layer Interaction

Divalent cations are known to mediate the association of structural proteins released from the cortical granules with the vitelline layer during fertilization envelope assembly (13). Although the exact requirements are not known, reducing the total divalent cation concentration below 1 mM

| Divalent cation composition of medium | mM | 125I-Proteoliaisin bound % of Control |
|-------------------------------------|----|--------------------------------------|
| Ca²⁺                               | 10 | 98                                   |
| Ca²⁺ + ovoperoxidase               | 10 | 73                                   |
| Mg²⁺                               | 10 | 7                                     |
| Mg²⁺                                | 0  | 3                                     |
| Mg²⁺                                | 0  | 0                                     |

The total compositions of the artificial seawaters used and the assay procedure are described in Materials and Methods. Each 0.5 ml of incubation mixture contained 50 μg/ml 125I-proteoliaisin (2.4 × 10⁴ cpm/ml) and 1% eggs by volume. The concentration of nonradioactive ovoperoxidase was 50 μg/ml. Results are expressed as percent of binding observed with 125I-proteoliaisin in NSW (control), which was 1.4 × 10⁶ cpm.
allows uncross-linked fertilization membranes to partially disassemble without disrupting the interaction between ovoperoxidase and proteoliaisin (20). This suggests that the requirements for divalent cations in the interaction between ovoperoxidase—proteoliaisin complexes and other components of the fertilization envelope (i.e., the vitelline layer) must be different than the requirements for the formation of the ovoperoxidase—proteoliaisin complex, which is specific for Ca\(^{2+}\) with \(K_{d} = 50 \text{ mM}\) (20).

To explore this hypothesis, we examined the quantitative requirements for seawater divalent cations in proteoliaisin binding to the vitelline layer. As illustrated in Fig. 2 A, half-maximal binding in CaSW was observed at 200 \(\mu\text{M}\) Ca\(^{2+}\). In MgSW, half-maximal binding occurred at \(\sim 2.5 \text{ mM}\) Mg\(^{2+}\) (Fig. 2 B, closed circles). In CaSW, saturable binding was not observed at concentrations of Mg\(^{2+}\) up to 50 \(\text{mM}\) (Fig. 2 B, open circles). Note that the binding observed in seawater containing both 10 \(\text{mM}\) Ca\(^{2+}\) and 50 \(\text{mM}\) Mg\(^{2+}\) was greater than the sum of binding observed for each cation alone (Fig. 2 B), suggesting a synergistic effect of these cations on the binding interaction. This is supported by the observations presented in the next section. Thus, the divalent cation dependence of proteoliaisin binding to the vitelline layer is distinct from the Ca\(^{2+}\)-specific ovoperoxidase—proteoliaisin binding interaction.

**Quantitative Measurements of Proteoliaisin Binding to the Egg Surface**

To further characterize the proteoliaisin—vitelline layer interaction, we determined the dissociation constants and number of proteoliaisin-binding sites on each egg. The number of proteoliaisin molecules bound per egg as a function of proteoliaisin concentration is shown in Fig. 3. Saturation binding curves were fit to the observed data and the values of \(K_{d}\) and \(N\) were derived as described in Materials and Methods. For seawater containing either 10 \(\text{mM}\) Ca\(^{2+}\), 50 \(\text{mM}\) Mg\(^{2+}\), or both divalent cations, the shape of the curve was indicative of a single class of proteoliaisin-binding sites on the vitelline layer. Highest affinity binding was observed in MgSW (Fig. 3 C; \(K_{d} = 0.19 \mu\text{M}\)) with \(8.3 \times 10^{7}\) molecules of proteoliaisin bound per egg at saturation. In CaSW (Fig. 3 B), over six times as many molecules of proteoliaisin \((5.5 \times 10^{8}\) per egg).
egg) were bound at saturation but the binding was of lower affinity ($k_d = 0.47 \mu M$). The lowest apparent binding affinity was observed in seawater containing both $Ca^{2+}$ and $Mg^{2+}$ in physiological concentrations (Fig. 3A; $K_d = 1.43 \mu M$). However, at saturation, nearly twice as many molecules of proteloin (1.03 x 10^9 per egg) were bound to the egg surface as were bound in CaSW. Although these are substantial quantities of bound proteloin (see Discussion), these values represent minimum estimates since we have not corrected the data for the non-TCA-precipitable 125I associated with the radiolabeled proteloin (21% of the total 125I) or for loss of proteloin ovoperoxidase-binding activity after iodination (25% of the original ovoperoxidase-binding activity). There are significant differences in the binding observed in each of the seawaters, consistent with the observation (Fig. 2) that $Ca^{2+}$ and $Mg^{2+}$ have a synergistic effect on the binding interaction between proteloin and the vitelline layer.

**Inhibition of the Proteloin-Vitelline Layer Interaction**

Many compounds interfere in fertilization envelope assembly, but the mechanisms underlying the effects of most of these compounds are unknown (13, 19). We therefore tested the effects of several inhibitors of fertilization envelope assembly on the binding of 125I-ovoperoxidase (via proteloin) and 125I-proteloin to the egg vitelline layer. Three inhibitors were examined: glycine ethyl ester, which inhibits a characteristic morphological transition that occurs during fertilization envelope assembly, known as the I to T transition (18); glycine, which increases the amount of ovoperoxidase (and possibly other proteins) not incorporated into the fertilization envelope (11); and benzamidine, a competitive inhibitor of serine proteases that also binds to proteloin and other fertilization envelope proteins, possibly at divergent cation-binding sites (19, 20). BSA and IgG, proteins that do not interfere in fertilization envelope assembly (Weidman, P. J., unpublished data), were also assayed to determine whether other nonspecific proteins were inhibitors of the binding interaction. Of these compounds, only benzamidine significantly inhibited binding of proteloin to the vitelline layer in vitro, with $K_b$ for binding occurring at 20 mM benzamidine (data not shown). The effects of glycine and glycine ethyl ester can not be attributed to an inhibition of either the ovoperoxidase-proteloin or proteloin-vitelline layer interactions.

Evidence is accumulating that the carbohydrate elements of extracellular proteins frequently mediate specific cell surface interactions. In Xenopus eggs, assembly of the fertilization envelope proceeds in a manner similar to the sea urchin fertilization envelope and the assembly process involves a lectin-like association of secreted proteins with the egg glycoalyx (21). The vitelline layer of the sea urchin egg contains at least one cell surface receptor, the sperm receptor, in which the primary binding domain corresponds to the carbohydrate portion of a proteoglycan-like molecule (16). To determine whether carbohydrates play a role in the proteloin-vitelline layer binding interaction, we examined the effects of various simple and complex carbohydrates in the in vitro assay. No significant inhibitory effects were found with the following monosaccharides, tested at concentrations up to 100 mM: $\alpha$-methyl-D-xylose, $\beta$-methyl-D-xylose, $\alpha$-methyl-D-mannoside, D-glucose, D-galactose, $\alpha$-L-fucose, $\alpha$-D-fucose, $n$-acetyl-D-glucosamine, $n$-acetyl-D-galactosamine, and sialic acid. Fetuin, a highly glycosylated protein that frequently inhibits carbohydrate-specific interactions, also had no effect on binding. Because of the proteoglycan-like nature of some of the vitelline layer components, we examined the effects of several complex carbohydrates on the proteloin-vitelline layer interaction. Chondroitin sulfate and dextran sulfate had no effect on the binding of proteloin to the egg vitelline layer, whereas inconsistent results were obtained with different preparations of heparin and heparin sulfate. Although we can not rule out the possibility that carbohydrates play an important role in the proteloin-vitelline layer interaction, an inhibitory effect of specific carbohydrates has not been demonstrated for this interaction.

**Ovoperoxidase-catalyzed Cross-linking of Proteloin to the Egg Vitelline Layer**

In addition to binding ovoperoxidase, proteloin is also a substrate for ovoperoxidase-catalyzed cross-linking of the fertilization envelope (14; Kay, E. S., and B. M. Shapiro, unpublished data). We therefore examined the ability of pure ovoperoxidase, bound to the vitelline layer via proteloin, to cross-link proteloin to the surface of unfertilized eggs. In these experiments, we have taken advantage of the divergent cation dependence of the proteloin-vitelline layer interaction. If proteloin is noncovalently associated with the vitelline layer, then it should be possible to extract it from the egg by removing divalent cations. On the other hand, if proteloin is covalently linked to the vitelline layer, it should not be extracted by this treatment. In these experiments, several different molar ratios of ovoperoxidase and proteloin were incubated with eggs in CaSW. To prevent egg lysis during the extraction procedure, bound proteloin was extracted by diluting the eggs into 30 vol of EGTA-SW containing 2 mM MgCl$_2$ and incubating for 30 min. In control eggs that were not treated with H$_2$O$_2$, the oxidative substrate of ovoperoxidase, extraction with EGTA-SW removed $\sim$80% of the proteloin bound to the eggs (Fig. 4). After an aliquot of the same eggs were exposed to H$_2$O$_2$, the amount of proteloin bound in NSW did not change significantly (data not shown); however, as much as 70% of this bound proteloin was not extractable. These results suggest that proteloin was covalently cross-linked to the vitelline layer of the unfertilized egg by ovoperoxidase-catalyzed oxidation. That both ovoperoxidase and proteloin must be bound to the vitelline layer for cross-linking to occur is suggested by the observations that no proteloin was bound in seawater containing EGTA and H$_2$O$_2$, and that the maximum level of irreversible binding occurred only when stoichiometric amounts of ovoperoxidase were present (e.g., ovoperoxidase/proteloin molar ratios greater than or equal to one; Fig. 4). These data support the hypothesis that binding of ovoperoxidase and proteloin in our in vitro system reconstitutes one step in the fertilization envelope assembly process.

**Discussion**

In the more than a century that fertilization envelope assembly has been studied, certain mechanistic principles have emerged, which may have general validity (13). Before the
The presence of the peroxidative enzyme, ovoperoxidase, in the fertilization envelope presents an opportunity to examine a specific strategy for the regulation of enzymatic activity in the extracellular environment of a cell. Ovoperoxidase exhibits a pH-dependent hysteretic behavior that may act as a timing mechanism to delay its activity until it is assembled in the fertilization envelope (5, 6). When activated, however, ovoperoxidase is similar to most peroxidases and can oxidize a variety of substrates in addition to tyrosine (7). This suggests that part of the strategy for regulating ovoperoxidase involves rapidly sequestering the enzyme within the assembling fertilization envelope, in an orientation favorable for both interaction with specific substrates and induction of conformational changes that activate the enzyme. Our data indicate that the first step in this process is mediated by the association of ovoperoxidase with proteolaisin and the subsequent interaction of this complex with the vitelline layer scaffold. Since ovoperoxidase binds to the vitelline layer of unfertilized eggs only when proteolaisin is present, in amounts proportional to the amount of proteolaisin added (Table I), proteolaisin is probably not a component of the vitelline layer. Thus, both ovoperoxidase and proteolaisin most likely reside in the cortical granules, possibly as a preformed complex, before fertilization. Preliminary results with antibodies to both proteolaisin and ovoperoxidase verified this supposition (Somers, C., D. Battaglia, and B. M. Shapiro, unpublished data).

It is well known that some proteolytic modification of the vitelline layer occurs during fertilization envelope assembly. The best characterized of these modifications are the cleavage of attachment posts (15) between the vitelline layer and plasma membrane and the inactivation of sperm receptors (3). More extensive proteolysis has been proposed, since the morphology of the vitelline layer changes from thick fibers to thin strands during fertilization envelope assembly (4). Although this additional processing might be required for complete assembly to occur, it is clear that such processing is not obligatory for the binding of ovoperoxidase and proteolaisin to the vitelline layer scaffold. Proteolysis might, however, enhance this binding interaction, for example, by increasing the accessibility of vitelline layer binding sites.

Divalent cations have been implicated in the assembly process for many years. In this context, two features of the divalent cation dependence of the proteolaisin–vitelline layer interaction are interesting. First, this dependence is substantially different than that for the ovoperoxidase–proteolaisin interaction (20), which is highly specific for Ca\(^{2+}\) and requires relatively low concentrations of this cation (\(K_v = 50 \text{ mM}\)). In the interaction of proteolaisin with ovoperoxidase, Mg\(^{2+}\) can not substitute for Ca\(^{2+}\) nor does it enhance binding in the presence of Ca\(^{2+}\) (20; Weidman, P. J., unpublished observation). Second, the two major divalent cations found in seawater influence binding through a synergistic action. In the interaction of proteolaisin with the vitelline layer, either Ca\(^{2+}\) or Mg\(^{2+}\), can mediate binding, albeit with different efficiencies. Both cations are required for optimal binding. This suggests that proteolaisin has at least two distinct, divalent cation–dependent binding domains, one for interacting with ovoperoxidase and one for interacting with the vitelline layer.

The synergistic effect of Ca\(^{2+}\) and Mg\(^{2+}\) on the binding of proteolaisin to the vitelline layer was most striking in the saturation binding studies. There were three apparently
different classes of binding sites. Relatively low amounts (N = 8.3 × 10^7 molecules per egg) of the highest affinity sites (K = 0.19 μM) were found in seawater containing only Mg^2+. Over six times as many sites (N = 5.5 × 10^8 molecules per egg) were found in seawater containing only Ca^2+, but the affinity of these sites was approximately threefold lower (K = 0.43 μM). In seawater containing both cations, the number of binding sites increased even further (N = 1.03 × 10^9 molecules per egg). This was a nearly 10- and 2-fold increase in number of sites over MgSW and CaSW, respectively, yet the apparent affinity of these sites was considerably lower (K = 1.47 μM). This very large number of binding sites on the egg compares favorably with the number of concanavalin A binding sites on the surface of the egg (4.0 × 10^8 sites per egg; reference 17). Although we can not directly measure the number of molecules of proteolaisin per fertilization envelope, we can estimate the number of molecules of proteolaisin released at fertilization. Since the structural proteins of the fertilization envelope released from the cortical granules comprise 3.6% of the total egg protein (2), and 10% of this protein is proteolaisin (20), we estimate that there are 1.4 × 10^8 molecules of proteolaisin released per egg at fertilization. Thus, the number of proteolaisin-binding sites on the egg surface in this in vitro system is reasonably close to the levels that might be expected to act during the in vivo assembly process.

One clue to the apparently cooperative effects of Ca^2+ and Mg^2+ in the proteolaisin–vitelline layer interaction is suggested by the observation that the assembled fertilization envelope is a trilaminar structure. This results from the penetration of cortical granule proteins through the vitelline layer during assembly and the deposition of these proteins on both sides of this scaffold (4). In NSW, if we are indeed measuring the total binding capacity of the vitelline layer for proteolaisin during the assembly process, then proteolaisin must be binding to both sides of the vitelline layer, even though this structure is covalently attached to the plasma membrane in the unfertilized egg (15). The fact that almost half as many binding sites are observed in CaSW would be consistent with binding of proteolaisin to only one side of the vitelline layer. Perhaps Mg^2+ facilitates the transport of proteolaisin across the vitelline layer, thereby doubling the number of binding sites available. Since there is a distinct class of binding sites in MgSW, such sites might play a role in allowing proteolaisin to reach the other side of the vitelline layer during envelope assembly, and thus serve to prevent saturation of the plasma membrane–opposed face.

We have shown that purified ovoperoxidase can bind to the vitelline layer of unfertilized eggs when complexed with purified proteolaisin. This supports our hypothesis that the function of proteolaisin is to appropriately direct the insertion of ovoperoxidase into the fertilization envelope. The observations that ovoperoxidase can cross-link proteolaisin to the vitelline layer of unfertilized eggs and that optimal cross-linking requires stoichiometric (rather than enzymatic) quantities of ovoperoxidase indirectly demonstrate that this insertion places ovoperoxidase in an orientation that allows cross-linking. Because ovoperoxidase is bound to proteolaisin, it is likely that the binding of this complex to the vitelline layer results in highly specific and targeted cross-linking. This would serve as another mechanism for insuring specificity in the use of reactive oxygen intermediates by the egg at the beginning of its developmental program.

The data presented in this report show that it is possible to effect the in vitro assembly of a partial fertilization envelope on the surface of an unfertilized egg by using purified components. This type of in vitro morphogenesis has previously been possible only with genetically characterized systems, such as viral capsid assembly. By extending this approach, it should be possible to further elucidate the rules governing pathways and mechanisms that allow this specialized glycocalyx to be assembled in the absence of intracellular control mechanisms.

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