Abalone lysin: the dissolving and evolving sperm protein

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Summary

Abalone sperm lysin is a non-enzymatic protein that creates a hole for sperm passage in the envelope surrounding the egg. Lysin exhibits species-specificity in making the hole and it evolves rapidly by positive selection. Our studies have focused on combining structural, biochemical, and evolutionary data to understand the mechanism of action and evolution of this remarkable protein. Currently, more is known about lysin than about any other protein involved in animal fertilization. We present an hypothesis to explain lysin's rapid evolution and the evolution of species-specific fertilization in this order of mollusks. We also propose a two-step model for lysin's action in which a dimer of lysin binds species-specifically to its glycoprotein receptor, and then monomerizes and binds the receptor in a non-species-specific manner. This experimental system yields data relevant to the general problem of molecular recognition between cell surfaces, and is also important to our thinking about how new species arise in the sea. ***BioEssays*** **23:95–103, 2001.**

Introduction

Fertilization is a poorly understood fundamental biological phenomenon. All organisms must reproduce. The survival of the species depends on the success of fertilization. Yet, despite the importance of this process, comparatively little is known about fertilization at the molecular level. Few of the cell surface proteins mediating fertilization have been identified,[1–3] and only two have had their three-dimensional structures determined.[4–8] We have combined X-ray crystallography with biochemistry, molecular, and evolutionary biology to attempt to understand the molecular mechanism of fertilization in the abalone and how it has evolved.

Why study abalone?

Fertilization has been studied in a variety of species from humans to nematode worms, with each organism having its own merits and disadvantages. While investigating fertilization in mammals is appealing due to the potential applications to human biology, the scarcity and high cost of mammalian gametes makes biochemistry and crystallography difficult. Marine invertebrates are ideal for the study of fertilization, because they produce a great abundance of gametes that are easy to harvest. Abalone are large, single-shelled marine mollusks of the genus *Haliotis*. They have separate sexes, both of which spawn gametes into seawater. Abalone sperm and eggs can be recovered in the laboratory by artificial induction of spawning in hydrogen peroxide,[9] or by dissection of the gonad. Approximately 60 species of abalone exist worldwide.[10,11] Many of these species have overlapping breeding seasons and habitats, yet hybrids are rarely found.[10–12] For example, there are seven abalone species in California, each named for the color, shape, or patterning of its shell (red, white, flat, pinto, pink, black, and green). When sperm from one species is added in vitro to eggs from a different species, approximately ten times the normal amount of sperm is needed to obtain the maximum percentage of fertilized eggs.[13] Since fertilization in abalone occurs externally, the only explanation for this cross-species fertilization incompatibility is that the molecules on the cell surface of sperm and egg do not recognize each other. Could the evolution of species-specific fertilization be part of the process by which new species arise?

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Abalone reproduction

Upon encountering an abalone egg, the abalone sperm swims easily through the egg's first protective barrier, which is a thick jelly coat (Fig. 1A). Underneath the jelly lies a more formidable barrier, the elevated vitelline envelope (VE), 0.6 μm thick and made of tightly intertwined glycoprotein fibers. Contact between the sperm and the VE induces the exocytotic acrosome reaction of the sperm (Fig. 1B). The reaction causes the acrosomal vesicle to release protein onto the surface of the VE. Simultaneous with acrosomal exocytosis is the polymerization of actin to generate the 7 μm long, membrane-covered, acrosomal process coated with acrosomal protein (Fig. 1).[14,15] The acrosomal proteins cause the VE fibers to unravel and splay apart, forming a hole in the VE (Figs. 1C, 2). The sperm swims through the hole and the tip of its acrosomal process fuses with the egg plasma membrane (Fig. 1D). Fusion of male and female gametes activates the egg's contractile cytoskeleton, which draws the sperm into the egg cytoplasm and towards the egg's pronucleus. The two haploid

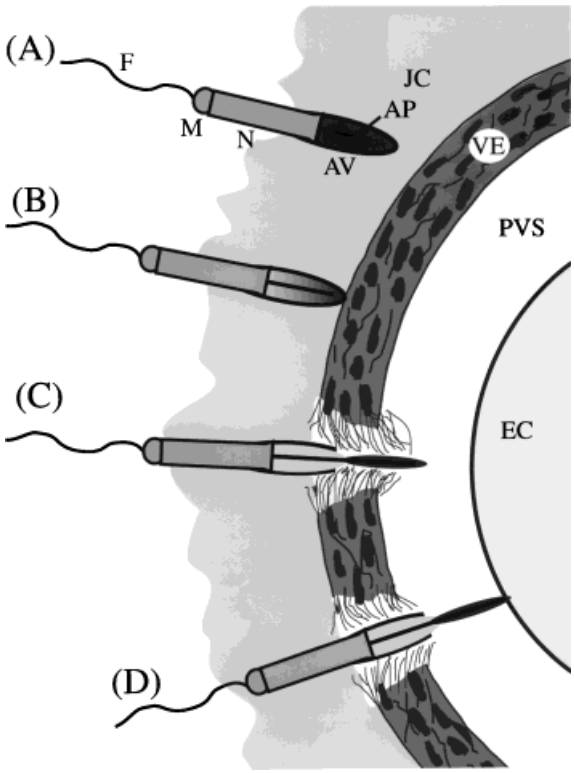

Figure 1. An overview of fertilization in abalone. **A:** The sperm (F, flagellum; M, mitochondrion; N, nucleus; AP, acrosomal process; AV, acrosomal vesicle) swims through the egg jelly coat (JC) and contacts the vitelline envelope (VE). **B:** Contact initiates the acrosome reaction resulting in release of acrosomal proteins and the elongation of the acrosomal process. **C:** The acrosomal proteins unravel the tightly intertwined fibrous molecules creating a hole in the VE. The sperm passes through the hole into the perivitelline space (PVS). **D:** The membrane covering the tip of the acrosomal process fuses with the egg plasma membrane and the sperm is drawn into the egg cytoplasm (EC).

nuclei fuse to restore the diploid genome and the egg is activated to begin dividing to form the abalone embryo.

The contents of the acrosomal vesicle

A key event in animal fertilization is the acrosome reaction. Sperm that are unable to release their acrosomal proteins cannot fertilize eggs. In abalone sperm, the acrosomal vesicle is extremely large, implying that large amounts of protein are needed to dissolve the VE and mediate sperm–egg membrane fusion.[14–16] What are the molecules released by exocytosis of the acrosomal vesicle, and how do they allow the sperm to cross the barriers surrounding the egg?

Lewis et al.[16] analyzed the contents of the acrosomal vesicle and found two major proteins of molecular masses 18,000 daltons (18 kDa) and 16,000 daltons (16 kDa) (originally reported to be 15,000 and 13,000 daltons). When abalone eggs were exposed to each purified protein, the 18 kDa protein had no effect on the VE, while the 16 kDa protein rapidly dissolved the VE. The 16 kDa protein was named "lysin" because of its ability to "lyse" (destroy) the VE.[17] Analysis of purified lysin revealed that it contains no carbohydrate and that 20% of its amino acids are positively charged (basic), accounting for its isoelectric point of greater than 9. Lysin's high affinity for paraffin-coated glass hinted that some of its surface amino acids are hydrophobic.[16] VE dissolution assays using isolated VEs and purified lysins from different abalone species showed that lysin's ability to dissolve VEs is species-specific, indicating that specific molecular recognition resides in the purified sperm protein and isolated VE. For example, significantly more red abalone lysin was needed to dissolve pink abalone VEs than was needed to dissolve red VEs.[18,19] The 18 kDa acrosomal protein preferentially coats the acrosomal process after the acrosome reaction, and is a potent fusagen of artificial lipid vesicles. These two properties imply a possible role for the 18 kDa protein in membrane fusion between the gametes.[20,21]

Lysin is not an enzyme

How does lysin cause the VE fibers to unravel and splay apart to form a hole for sperm passage (Fig. 2)? The VE is made of 6 to 10 glycoproteins forming a meshwork of tightly interwoven fibers.[16,22] Examination of the lysin-created hole with an electron microscope shows it to be full of loosely associated VE fibers, 13 nm in diameter (Fig. 2). VE dissolution was measured as a function of lysin concentration. Dissolution occurred for a short time and then stopped, implying that lysin had been inactivated. Quantification revealed that VE dissolution required more than catalytic amounts of lysin. Also, there was no reduction in mass of VE proteins after exposure to large quantities of lysin.[16] Together, these observations suggested that a stoichiometric, non-enzymatic interaction between lysin and the VE creates the hole in the VE. Hypothetically, a non-enzymatic mechanism would be advantageous because release of enzymes from the acrosome could result in complete degradation of the VE, a structure that functions as a protective microchamber for embryonic development. By using a large amount of a non-enzymatic protein, the 1 μm diameter sperm is able to create a 3 μm diameter hole in the VE, thus keeping intact this protective envelope surrounding the developing embryo.

Experimental evidence suggests that hydrogen bonds, and not hydrophobic interactions, among VE molecules give the VE its structural integrity. For example, isolated VEs melt if heated to 60°C, they dissolve between pH 5.0 and 4.5, and they also dissolve in relatively low concentrations of urea (D50% = 2.7 M). Hydrogen bonds among VE molecules[22] would involve glutamic and aspartic amino acids, both of which have a pKa of 4.75, accounting for the dissolution of VEs when

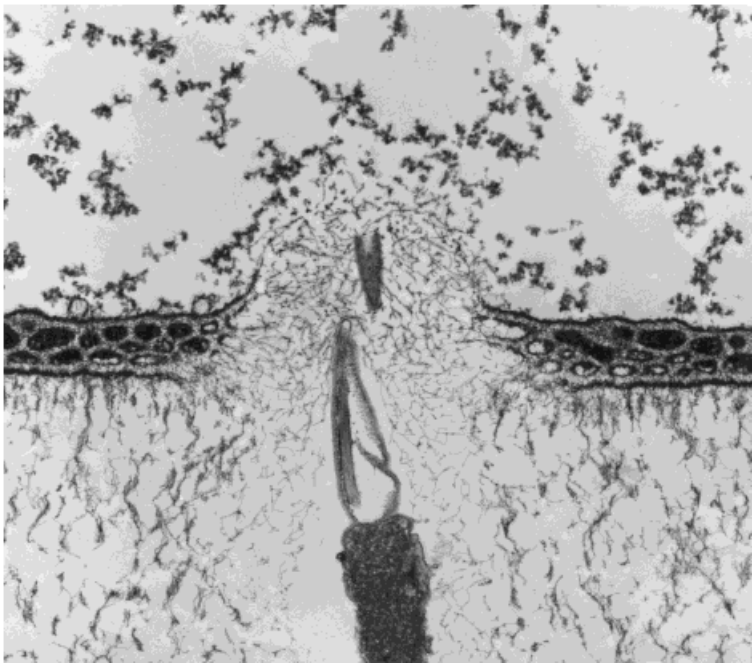

Figure 2. Thin section transmission electron micrograph of a sperm making a hole in the egg VE.[16] The sperm is coming in from the bottom and most of it is out of the plane of section. The unraveling of the 13 nm diameter VE fibers is seen at the edges of the hole. The intact VE is 0.6 μm in thickness. The magnification is approximately × 26,000.

the pH is lowered from 5.0 to 4.5. Evidence against hydrophobic interactions holding VE molecules together is that heat strengthens, not weakens, hydrophobic interactions. Also, inclusion of the nonionic detergent Triton X-100, which disrupts hydrophobic interactions, does not enhance VE dissolution by heat, low pH or urea (V. D. V., unpublished data). Furthermore, VEs do not dissolve when treated with 5% Triton X-100 or other non-ionic detergents regardless of their micelle size.

Amino acid sequences of lysin

The basis for lysin's species-specific molecular recognition remained unknown. Could answers be found in lysin amino acid sequences? To attempt to solve this puzzle, the sequences of lysins from 25 abalone species from the United States, Japan, Taiwan, Australia, New Zealand, South Africa, and Europe were determined.[23–25] All lysins are approximately the same length (126–138 amino acids), all have a highly conserved signal sequence, are very basic (positively charged), have no cysteines, and no sites for asparagine-linked glycosylation (Fig. 3). Despite their similarities, the lysin sequences are surprisingly divergent, with percentage sequence identities ranging from 49% to 92%. Two regions with high sequence variability are the amino- and carboxyl-terminal amino acids 2–15 and 99–134 (Fig. 3). Could these hypervariable termini be the basis for lysin's species-specificity? Evidence supporting this possibility came from experiments in which the termini of red and pink lysins were switched. These chimeric lysins were less adept at dissolving their own VEs, but better at dissolving the heterospecific VEs.[19] Internal sequence elements, such as positions 103–108, are also important for species-specific recognition.[19]

Positive Darwinian selection promotes lysin's rapid divergence

In pairwise comparisons between California abalone species, the number of conservative amino acid replacements in lysins is below 50%.[23] This means that the majority of amino acid replacements between lysins change the class of amino acid at a particular position. This is contrary to what is found in most proteins, where amino acid replacements are expected to conserve the class of amino acid. Calculation of the number of silent (Ds) and amino acid altering (Dn) nucleotide substitutions in cDNA coding for lysins showed that in pairwise comparisons of the seven California lysins, in all cases except one (flat versus green), Dn is greater than Ds. In some pairwise comparisons, there were more nucleotide substitutions in the exons than in introns and mitochondrial cytochrome c oxidase subunit I.[26] A $Dn:Ds > 1$ is indicative of positive Darwinian selection promoting the divergence of a protein. In other words, a $Dn:Ds > 1$ shows that there is great adaptive value in rapidly altering the amino acid sequences of lysins.[24–26] Adaptive evolution was also found in the diversification of the abalone sperm 18 kDa protein.[20] Lysin and the 18 kDa protein appear to evolve 4–50 times faster than the fastest evolving proteins between mice and humans.[26]

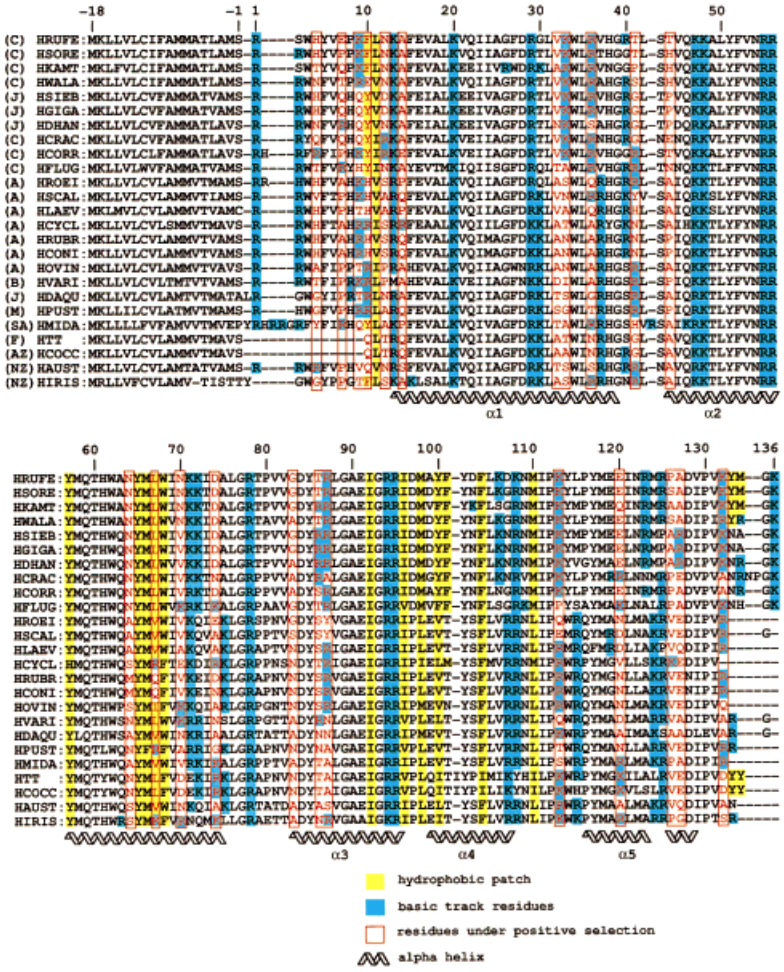

Figure 3. Amino acid sequence alignment of lysins from 25 abalone species.[25,40] Positively charged basic track amino acids are highlighted in blue and hydrophobic patch amino acids in yellow. The 23 amino acids under strong positive selection are outlined and in red letters.[40] Numbering refers to the HRUFE sequence. Dashes are added for maximum alignment. The positions of the α-helices in the structure of red abalone lysin are indicated below the sequences as spirals. The signal peptide spans positions −18 to −1, and is not present in lysin released from the acrosome. Collection sites of adult specimens are indicated in capital letters in parentheses; C, California; J, Japan; A, Australia; B, Borneo; M, Madagascar; SA, South Africa; F, France; AZ, The Azores; NZ, New Zealand. Abbreviated species names are HRUFE, *H. rufescens*; HSORE, *H. sorenseni*; HKAMT, *H. kamtschatkana*; HWALA, *H. walallensis*; HSIEB, *H. sieboldii*; HGIGA, *H. gigantea*; HDHAN, *H. discus hannai*; HCRAC, *H. cracherodii*; HCORR, *H. corrugata*; HFLUG, *H. fulgens*; HROEI, *H. roei*; HSCAL, *H. scalaris*; HLAEV, *H. laevigata*; HCYCL, *H. cyclobates*; HRUBR, *H. rubra*; HCONI, *H. conicopora*; HOVIN, *H. ovina*; HVARI, *H. varia*; HDAQU, *H. diversicolor aquatilis*; HPUST, *H. pustulata*; HMIDA, *H. midae*; HTT, *H. tuberculata tuberculata*; HCOCC, *H. coccinea*; HAUST, *H. australis*; HIRIS, *H. iris*.

The rapid evolution of proteins involved in reproduction, which in some cases show positive Darwinian selection (adaptive evolution), has also been observed in sea urchins,[27–29] the snail *Tegula*,[30,31] *Drosophila*,[32–34] nematodes,[33] and the algae *Chlamydomonas*.[35] Why are reproductive proteins evolving so rapidly? One hypothesis is sexual antagonism.[36] In order to reduce the chances of fusion with more than one sperm (which will abort development), the egg surface ligands change in an attempt to slow sperm fusions; sperm must constantly adapt to an ever-changing egg receptor. A second hypothesis is sexual selection, which can act at the level of gametic properties as well as secondary sexual characteristics. In this instance, egg receptors might have a higher affinity for certain mutant sperm proteins, thereby selecting for particular sperm and rejecting others. A third hypothesis is immunological defense. Microbes attacking the surface of the egg cause it to change its surface proteins to ward off bacterial colonization; one of these proteins is the lysin receptor. This would indirectly put selective pressure on sperm proteins to adapt to the change in egg surface proteins.[37]

VERL: The VE receptor for lysin

Lysin must bind to a molecule in the VE. To find the lysin "receptor", VEs were dissolved by pH 4.5 treatment, the pH was readjusted to 8, and the VE molecules were passed over an affinity matrix of immobilized lysin. Elution of the bound molecules yielded a giant glycoprotein of about one million daltons.[18] Sucrose density gradient ultracentrifugation of dissolved VEs mixed with radioactive lysin showed that all the lysin migrated with this giant glycoprotein. This glycoprotein, named VERL, inhibits lysin-mediated VE dissolution, is 50% carbohydrate and makes up at least 30% of the VE. VERL is rich in acidic and hydrophobic amino acids, complimentary to lysin's basic and hydrophobic amino acid composition. Electron micrographs show that VERL is a long, thin unbranched rod that is relatively straight and 13 nm in diameter

(the same diameter as the fibers seen in Fig. 2). The isoelectric point of VERL is 4.7, the same pH at which VEs dissolve, suggesting again that hydrogen bonds are important to VE stability, and demonstrating that VERL is a major structural molecule of the VE.

Isolated VERL molecules exhibit species-specific binding of lysin. About 60 lysin molecules bind to each VERL, suggesting that VERL contains a repetitive lysin-binding motif. The affinity of lysin for VERL is approximately 10^{-9} M, and binding is cooperative, meaning that it is more difficult for the first lysins to bind VERL, but less difficult for subsequent lysins once the binding reaction has been initiated. Amino acid microsequencing of VERL fragments allowed degenerate oligonucleotide primers to be made and PCR was used to obtain VERL DNA sequences that were translated into amino acids. VERL proved to contain approximately 26 tandem repeats of a 153 amino acid sequence, suggesting that two lysins bind one repeat.[38] Within a species, the repeats are very similar to each other in sequence, and further analysis showed that they had been subjected to the process of concerted evolution.[39] This is the same process by which the repetitive ribosomal genes evolve.[39] In concerted evolution, unequal crossing over and gene conversion homogenize tandem repeats so that all repeats of one species are more similar to each other than they are to repeats of another species.[39] Analysis of the pattern of nucleotide substitutions in VERL repeats indicate that it is subject to weak purifying selection and not positive selection.

The evolution of species-specific fertilization in abalone

The discovery that concerted evolution homogenizes VERL repeats allowed the formulation of an hypothesis to explain the evolution of species-specific fertilization in abalones. Since all mollusks in the Order Archeogastropoda so far examined use sperm lysins to make holes in egg VEs, this scheme is general to all species within this taxonomic order; this is a significant number of molluscan species. First, a mutation occurs in one VERL repeat. The mutant repeat has greatly lowered affinity for lysin. Because there are many other wild-type repeats in one VERL molecule, the one mutant repeat is tolerated and fertilization proceeds, thus the mutant repeat is inherited. Concerted evolution[39] propagates the mutation to other VERL repeats. As the mutant repeat spreads in the population, there is an increasing selective pressure for lysin to adapt to the changing array of VERL repeats. Thus, mutant lysins would be continuously selected for their ability to recognize new VERL repeats. At a mutation rate of 10^{-9}, approximately one out of every one million sperm could carry mutant lysin protein. One male abalone can produce billions of sperm per year, and may live for decades. With such high sperm numbers, the chances of an egg encountering a sperm with a mutant lysin gene might be significant. The lack of polymorphism (sequence variation) in lysin genes in individuals of the red abalone species, which are polymorphic in other genes,[26] suggests that when a favorable mutant lysin appears, it sweeps the population. The differences between species seen today in lysin amino acid sequences are the results of many selective sweeps in the past.

The main features of this hypothesis are that, VERL, the female fertilization protein, changes first. The change in one VERL repeat is tolerated, inherited, and spread to other VERL repeats in the population by concerted evolution. As the mutant VERL repeat becomes established there is an ever-increasing selective pressure for the cognate male protein, lysin, to adapt to the changing VERL. If populations split apart and no longer exchange genes, their fertilization systems would diverge and become exclusive. In this model, species-specific sperm-egg interaction arises as a by-product of VERL's evolution by the process of concerted evolution and lysin's adaptation by positive selection to the changing VERL repeat. Such a process could be continuous, occur at different rates in different lineages,[40] and require no external forces. In other words, the model could be a mechanism for speciation. This is the first hypothesis, based on molecular data, to explain the evolution of a species-specific gamete recognition system.[38]

The crystal structure of red abalone lysin

Could lysin's three-dimensional structure tell us how it can species-specifically and non-enzymatically destroy the structural integrity of the VE? After screening purified lysins from four abalone species in hundreds of crystallization conditions, X-ray diffraction quality crystals of red abalone lysin were grown and the three-dimensional structure solved.[4–7,41,42]

The crystal structure of red abalone lysin monomer revealed a five-helix bundle with a right-handed twist (Fig. 4).[4] Three remarkable features are seen in the structure. (1) There is a large patch of 16 hydrophobic and aromatic amino acids confined to one face of the molecule (Fig. 4A). It is unusual to have so many hydrophobic amino acids on a protein's surface. Ten of these amino acids are completely conserved, or have undergone conservative amino acid replacement, in all 25 lysins (Fig. 3). This feature explains lysin's affinity for hydrophobic surfaces. (2) A pair of parallel tracks of positively charged amino acids (basic tracks) run the length of the opposite face of the molecule (Fig. 4B). Red abalone lysin contains 12 lysines and 12 arginines. Seven of these amino acids are completely conserved in all 25 lysins, while 14 are conserved in the seven California species (Fig. 3). Thus, one side of lysin is positively charged and the opposite side is hydrophobic, making lysin a highly "amphipathic" protein. (3) The amino-terminus, composed of hypervariable amino acids (positions 2–12), extends away from the main helical bundle, and is adjacent to the moderately variable carboxyl-terminus (Fig. 4). The amino terminal sequence is

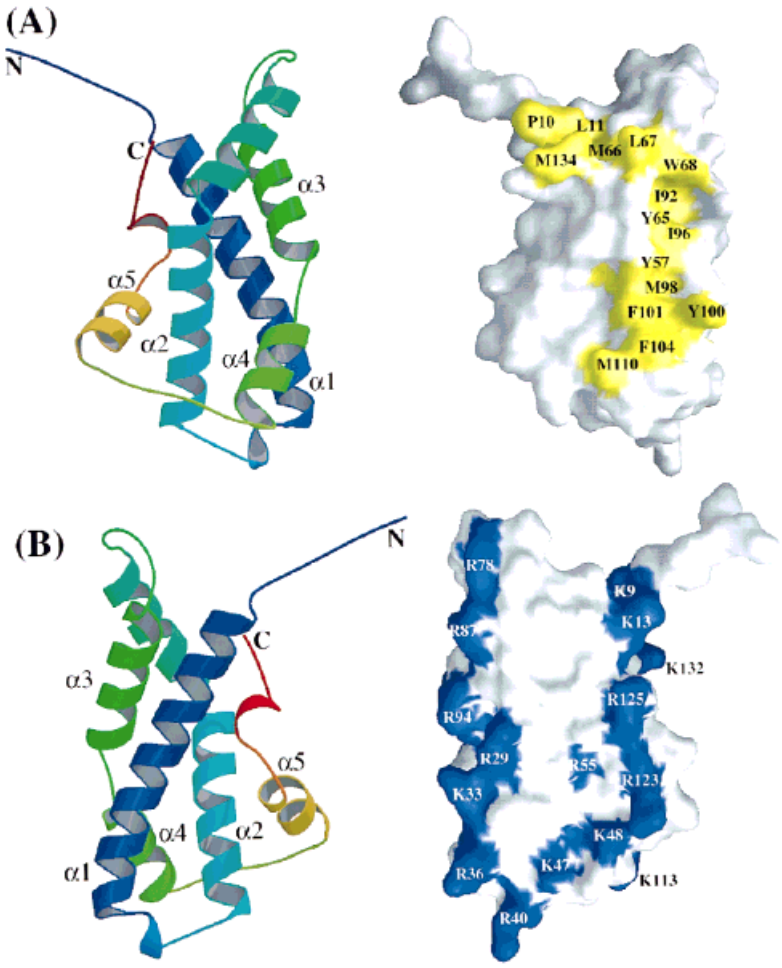

Figure 4. Corresponding ribbon-(left) and surface-area (right) diagrams showing the overall fold of the red abalone lysin monomer and the locations of the positively charged basic tracks and hydrophobic patch. Amino acids highlighted in Fig. 3 are plotted onto the surfaces. **A:** Surface containing the hydrophobic patch with patch amino acids numbered and colored yellow. **B:** 180° rotation from (A) showing the surface containing basic tracks in blue.

always species-unique. Consistent with the non-enzymatic mechanism of VE dissolution, monomeric lysin has no visible binding pockets or clefts characteristic of enzymes and lectins.

Lysin is a dimer

Dynamic light scattering[41] and chemical cross-linking followed by gel electrophoresis[5] showed that lysin is a dimer. Fluorescence resonance energy transfer (FRET) experiments showed that the monomers in the dimer interact weakly, with an affinity constant of 1 μM, and an 8 minute half-time for monomer exchange between dimers.[5] FRET analysis demonstrated that lysin dimers rapidly monomerize when isolated VEs are added to lysin solutions.[5] This suggested that the dimer is involved in the initial recognition of the VE, but it is the monomer that unravels the VE molecules.

The crystal structure of the lysin dimer from red abalone revealed that the dimer is formed by the association of the hydrophobic patches of two monomers (Fig. 5A).[5,7] The central feature of the dimer interface is the intercalation of aromatic amino acids such as phenlyalanines 101, 104 and tyrosine 100 (Figs 3, 4A). Dimerization buries only 30% of the hydrophobic patch surface area, leaving 70% exposed to the aqueous environment.[6,7] In the dimer, the positively charged basic tracks of each monomer are on the outer surfaces. Their repulsive electrostatic forces account for the low-affinity constant of the dimer and its rapid dissociation and exchange of monomers. A further consequence of dimer formation is that two clefts, 2 nm wide and 1.3 nm deep,[7] are formed on opposite sides of the dimer, adjacent to the amino- and carboxyl-termini (Fig. 5A).

The dimer of a second species

Of the seven species of California abalone, green abalone lysin is the most divergent from red lysin, having different amino acids in 48 out of 136 positions.[23] Crystals of green lysin were grown and the structure of the dimer was solved.[6] The green lysin monomer has a similar three-dimensional

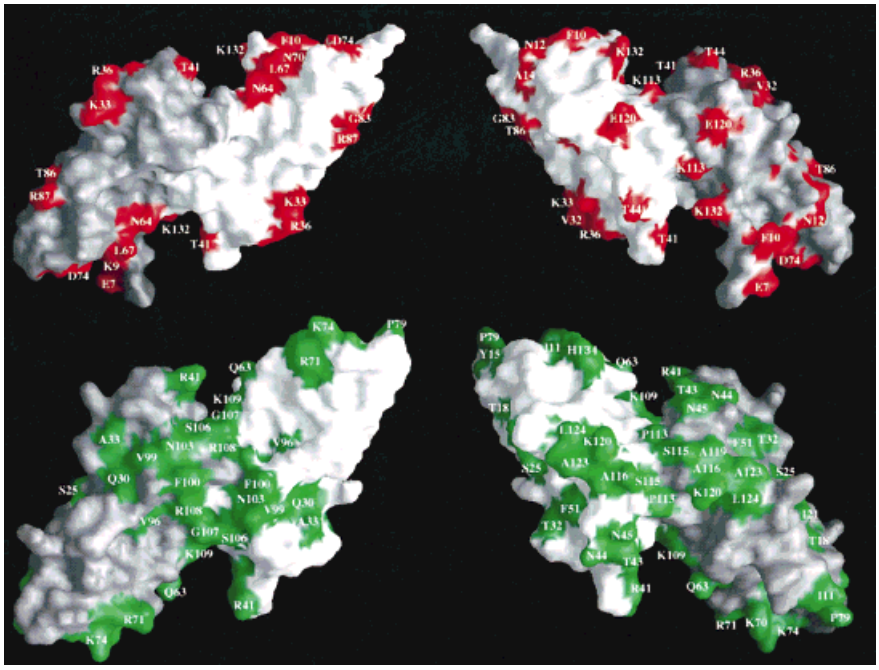

Figure 5. The red and green abalone lysin dimers with subunit 1 in white and subunit 2 shaded gray. **A:** Two views (180° rotation about a vertical axis) showing the surface of the red abalone lysin dimer and the 23 amino acids under strong positive selection in red.[40] **B:** The same two views of the green abalone lysin dimer showing the 48 residues which differ between red and green abalone lysin in green. Differences in the shapes of the two deep clefts can be clearly seen.

structure to that of the red lysin, with a hydrophobic patch and two positively charged tracks similar in size and shape.[6] However, a distinct difference between the two lysins is seen in the shape of the two deep clefts formed by dimerization (Fig. 5A versus 5B). When the 48 amino acids that differ between the red and green species are plotted onto the surface of either dimer, they cluster around the two clefts, and extend into the central region of the dimer (Fig. 5B).[6] In both red and green species, there is also a concentration of species-variable amino acids around the two clefts. Taken together, these features suggest that it is most probably the two deep dimer clefts that mediate species-specific recognition between lysin and a VERL repeat. The electrostatic surfaces of dimers from the two species are also quite different.[6] The red lysin dimer is less positively charged than green lysin dimer and has more electrically neutral patches on its surface than does the green.[38]

Twenty-three amino acids in lysin are subjected to strong positive selection as the protein evolves (Fig. 3).[40] How do these residues map onto the structure of the dimer? If they are the residues involved in species-specific recognition of VERL, they should all be on the surface. When mapped onto the surface of the lysin dimer, all 23 amino acids are indeed found on the surface of the molecule (Fig. 5A). Several of them cluster on the amino and carboxyl termini and are found in the dimer cleft (Fig. 5A). Their location supports the idea that they are involved in species-specific recognition.

How does lysin unravel the tightly intertwined, fibrous VERL molecules?

The 23 amino acids of lysin subjected to positive selection, and the four termini of the lysin dimer, vary in sequence between species. The two deep clefts formed by lysin dimerization in red and green abalone differ in the shape and charge (Fig. 5). The overall electrostatic surfaces of the green and red abalone lysin dimers are also quite different. All these species-variable features must be involved in species-specific recognition between lysin and VERL repeats.[6] Conversely, lysins also have features that are highly conserved between species, such as the hydrophobic patch amino acids that hold the lysin dimer together, and the two positively charged basic tracks on the opposite surface. These conserved features are general to all abalone lysins; we propose that they mediate the general mechanism abalone lysins use to unravel VERL molecules and destroy the integrity of the egg VE.

Remember that the VERL molecules are the major structural element of the egg VE, and that VERLs are largely made of tandem repeats of a 153 amino acid sequence

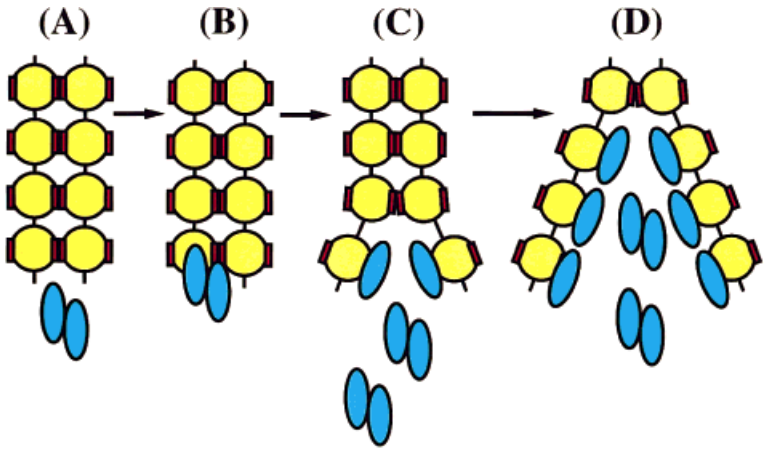

Figure 6. Model for the interaction of lysin with VERL. **A:** Lysin dimers are depicted as blue ellipses. VERL repeats within VE molecules are shown as yellow circles connected by primary structure (black sticks) and hydrogen bonds are red rectangles. **B:** The deep cleft of a lysin dimer binds species-specifically to a VERL repeat. **C:** Binding brings the dimer close enough to VERL that lysin's positively-charged amino acid tracks competitively displace hydrogen bonds among VERL repeats on intertwined VERL molecules. This causes the lysin dimer to monomerize. **D:** The binding between lysin monomers and VERL repeats exposes other repeats to lysin, facilitating the unraveling of the VERL molecules.

of which 11% (17 residues) are negatively charged amino acids.[38] As we mentioned earlier, we propose that VERL molecules are held together primarily by intermolecular hydrogen bonds. It is not known if these are VERL to VERL bonds, bonds between smaller VE proteins and VERLs, or a combination of both.

This is the only animal model in which the cognate sperm and egg gamete recognition proteins are known. Many models can be proposed for lysin's action on the VE. We propose one such model (Fig. 6), which is based on the differences and similarities between lysins of different abalone species. We believe this model applies to all species of the molluscan Order archeogastropoda. The model does not apply to other animal species. There are two steps to the proposed mechanism. (1) Species-specific recognition occurs between a VERL repeat and a lysin dimer. This recognition is mediated by lysin's deep clefts, the variable residues around the clefts, and lysin's electrostatic surface signature, all of which are complimentary to VERL's surface characteristics (Fig. 6B). This first step destabilizes the lysin dimer in the proximity of VERL. (2) The weakly associated monomeric subunits of the lysin dimer dissociate and bind tightly to VERL repeats via their positively charged residues (Fig. 6C). The conserved, positively charged basic track amino acids in each lysin monomer (24 residues, 18% of the sequence) competitively displace the intermolecular hydrogen bonds holding VERL repeats on apposing VERL molecules together. In other words, the positively charged arginine and lysine residues of lysin's basic tracks form hydrogen bonds with the negatively charged glutamic and aspartic acid residues of VERL repeats. As is true for lysin's basic tracks, the negatively charged amino acids of VERL repeats are conserved in position between species.[38] The fibrous VERL molecules then lose cohesion to each other and splay apart, exposing other VERL repeats to similar interactions with lysin dimers, and eventually forming the hole in the VE for sperm passage (Fig. 6D).

Between-species mixtures of lysins and VEs show that the major event in species recognition is in the initiation of VERL molecules unraveling; it takes more heterospecific lysin to initiate the unraveling reaction than to continue it.[6,18] However, once initiated, the unraveling (VE dissolution) will go to completion with lysin from a different species. Another way of saying this is that VE dissolution by lysin exhibits "positive cooperativity", meaning that it is more difficult to initiate the reaction than it is to sustain it once it has begun. This phenomenon can be explained by our model because the initial interaction of a lysin dimer with a VERL repeat loosens its bonds to other VERL repeats and exposes the repeats to interactions with lysin (Fig. 6D).

Alternative models for lysin's mode of action could involve the binding of both the basic track and hydrophobic patch amino acids to sites on VERL repeats. Lysin could also sever the long, thin VERL molecules in a way similar to proteins that sever actin microfilaments.

The future

This article has recounted what we have learned about the molecular mechanism of sperm–egg interaction in a major

group of marine mollusks. Combining the techniques of biochemistry, X-ray crystallography, and cellular, molecular and evolutionary biology has led to a relatively deep understanding of lysin and VERL. Future research will be directed towards attempting to grow X-ray diffraction quality crystals of the complex of VERL repeats and lysins. If the structure of this complex can be solved, it will define the atomic contacts between lysin and VERL and solve the long-standing problem of the molecular basis of species-specific fertilization in at least one animal group. Such information may prove pertinent to the study of other examples of intercellular recognition in developmental and disease processes. It may also be important to a deeper understanding of how reproductive isolation evolves as new species arise.

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