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Physiology, Biomechanics, and Biomimetics of Hagfish Slime

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Abstract

Hagfishes thwart attacks by fish predators by producing liters of defensive slime. The slime is produced when slime gland exudate is released into the predator's mouth, where it deploys in a fraction of a second and clogs the gills. Slime exudate is composed of two cell types, gland mucous cells and gland thread cells, which produce the mucous and fibrous components of the slime, respectively. Here, we review what is known about the composition of the slime, morphology of the slime gland, and physiology of the cells that produce the slime. We also discuss several of the mechanisms involved in the deployment of both mucous and thread cells during the transition from thick glandular exudate to ultradilute material. We review biomechanical aspects of the slime, along with recent efforts to produce biomimetic slime thread analogs, and end with a discussion of how hagfish slime may have evolved.

Contents

INTRODUCTION

Hagfishes (Craniata: Myxini) are an ancient group of benthic marine chordates that are best known for their ability to produce large volumes of defensive slime when they are attacked (1, 2). Hagfish slime differs from other mucous secretions in its composition, mode and speed of release, and function. One unique characteristic of hagfish slime is the presence of thousands of silklike protein threads (1, 3, 4), which lend the slime a surprising mechanical coherence. Although many biological functions have been proposed for the slime, the most obvious and best studied is its ability to deter attacks from fish predators by its capacity to adhere to and clog gills (**Figure 1**) (2, 5, 6). Biologists have been fascinated by hagfish slime for centuries (7), yet the literature on this subject is sparse. Although there are currently only 32 peer-reviewed publications on hagfish slime, advances in molecular and cellular biology and a surging interest in biomimetics have fueled a recent spike in research on the subject. In this review, we highlight the most recent research in this area and discuss efforts to create biomimetic analogs of hagfish slime threads.

HAGFISH SLIME GLANDS

Hagfish slime exudate is produced within numerous specialized epidermal slime glands, which exist in segmental pairs down both sides of a hagfish's body (**Figure 2**). The glands have a typical diameter of 2 to 3 mm and contain two kinds of secretory cells, gland mucous cells (GMCs) and gland thread cells (GTCs), which produce the mucous and fibrous components of the slime, respectively (**Figure 2**) (8). GMCs and GTCs arise from a basal epithelial cell layer and move toward the center of the gland as they grow and mature (**Figure 3**). The gland epithelium abuts a thin collagenous capsule, which is itself surrounded by a thin layer of striated muscle, the musculus decussatus (9). Contraction of this muscle layer increases the pressure within the gland and forces mature GMCs and GTCs through the narrow gland duct and pore into the surrounding seawater. During holocrine secretion through the duct, both kinds of cells lose their plasma membranes;

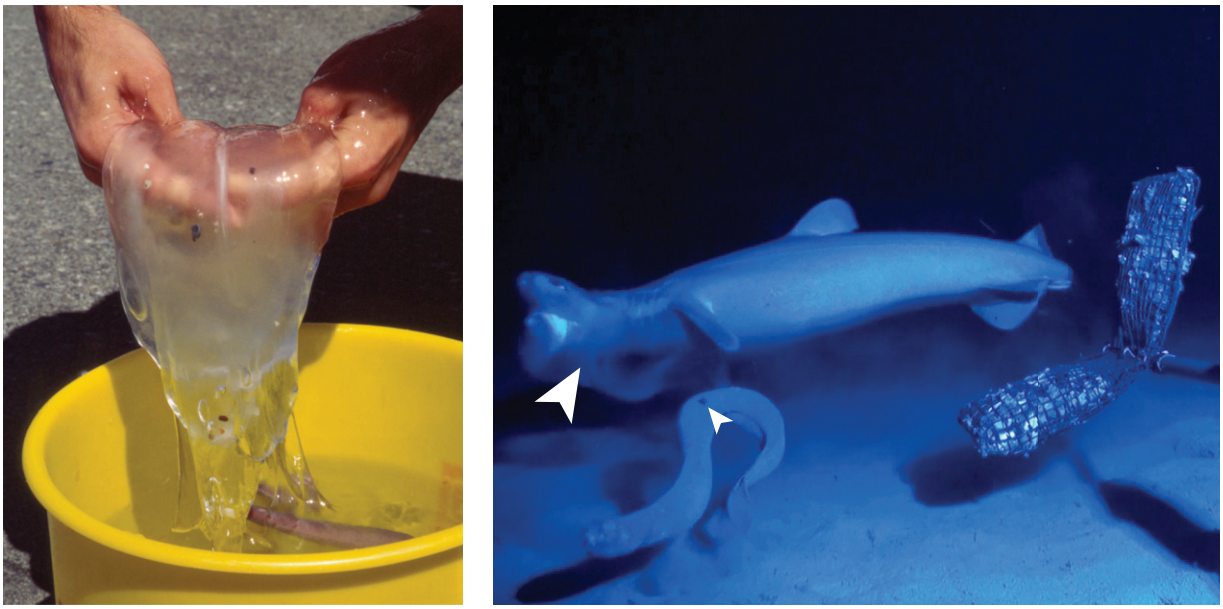

Figure 1

Hagfish slime as a defense against gill-breathing predators. (*a*) Hagfishes can produce large volumes of slime in under 100 ms (1, 2). (*b*) Fish predators that attack hagfishes, such as this seal shark (*Dalatias licha*), end up with large volumes of slime clinging to their gills (*large arrowhead*) and abort further attacks (6). Hagfishes are rarely injured in these encounters, although this animal shows evidence of some bleeding (*small arrowhead*) (6).

GMCs release several thousand mucin vesicles, and each GTC releases a single coiled slime thread known as a skein (4, 5). These two components mix with seawater, which triggers swelling of the vesicles (10–12) and unraveling of the thread skeins. It is currently not known how long it takes for a slime gland to refill after it has discharged its slime exudate, but it is most likely in the range of days to weeks, and it also likely varies among species (9).

GLAND MUCOUS CELLS

GMCs produce numerous disc-shaped mucous vesicles that are formed in the Golgi apparatus (10). The behavior of the mucin vesicles in seawater is discussed in detail in the sections on deployment, below.

GMCs likely originate from stem cells in the gland epithelium, and increase dramatically in size as their cytoplasm fills with mucin vesicles, reaching diameters of 150 μm when mature. Salo et al. (13) analyzed the chemical composition of mucus from the slime glands and found it to consist of 77% protein, 12% carbohydrate, 5% lipid, and 6% sulfate by dry weight. These data suggest that the mucus is likely made up of glycoproteins in which the dominant carbohydrates are sulfated mucopolysaccharides. However, the sequence of the proteins is not known, nor is whether they are related to mucin proteins of the MUC family (14). Incidentally, a carbohydrate content of only 12% is unusual for mucins, which typically consist of greater than 85% carbohydrate. Subramanian et al. (15) recently showed that hagfish slime also contains substantial levels of alkaline phosphatase, lysozyme, and cathepsin B, which are involved in innate immunity in many aquatic chordates.

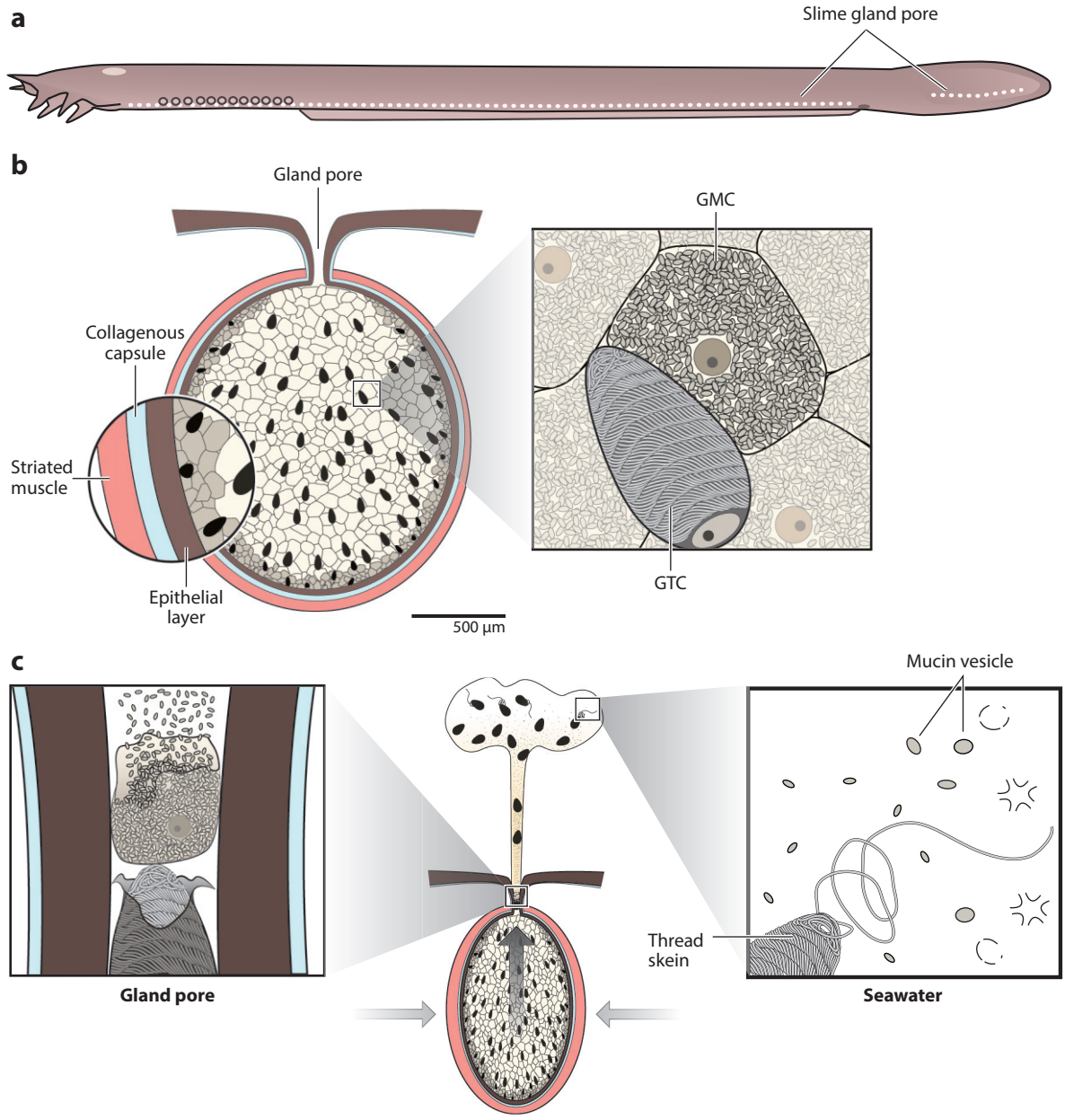

Figure 2

Anatomy and morphology of hagfish slime glands. (*a*) Hagfishes have a large series of segmentally paired slime glands and associated gland pores that run down both ventrolateral surfaces of the body. (*b*) Slime glands produce two main cell types, gland thread cells (GTCs) and gland mucous cells (GMCs), which make the fibrous and mucous components of the slime, respectively. (*c*) Upon stimulation and contraction of the striated muscle layer surrounding the gland, both cell types are forced through the narrow gland pore, shearing off their plasma membrane, thereby allowing them to quickly interact with the surrounding seawater (11).

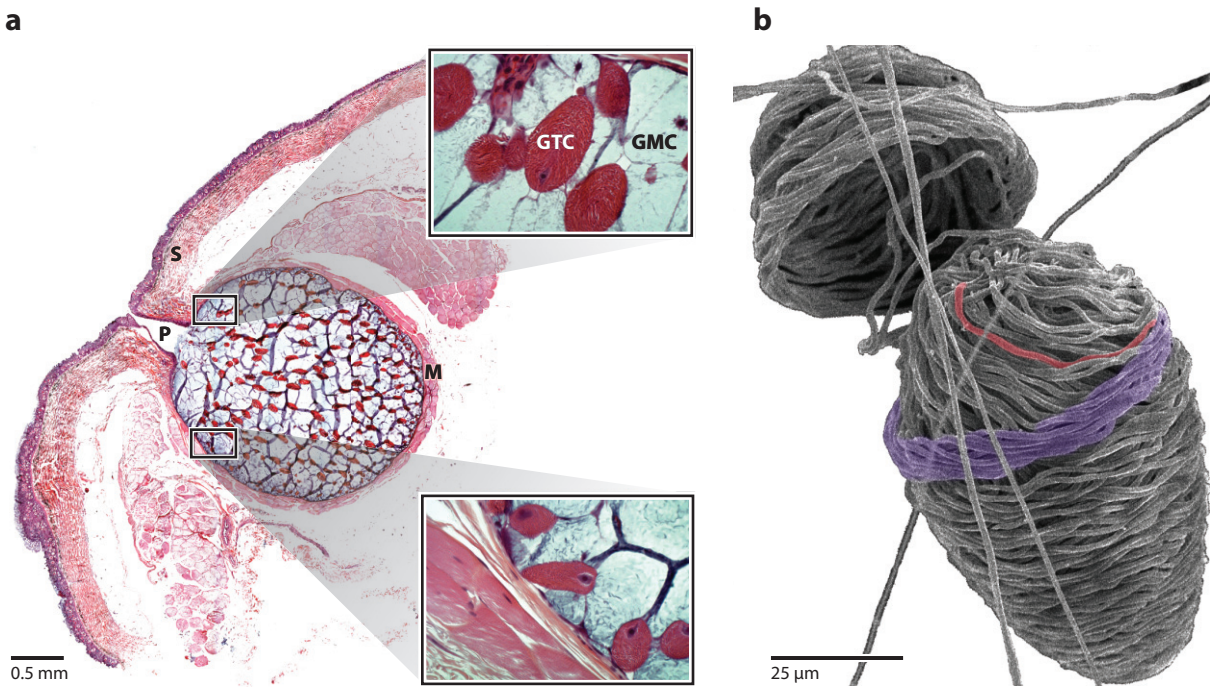

Figure 3

Slime gland histology and skein structure. (*a*) Gland histology reveals eosinophilic staining gland thread cells (GTCs), basophilic staining gland mucous cells (GMCs), the gland pore (P), striated muscle around gland (M), and skin (S). Immature GTCs are clustered around the periphery of the gland, and mature GTCs are in the center of the gland lumen. (*b*) Scanning electron micrograph of a broken open skein reveals the organization of staggered thread loops (*partially highlighted in red*), which form layers of conical loop arrangements that spiral around the skein (*one layer is highlighted in purple*) (22).

GLAND THREAD CELLS

GTCs produce the fibrous component of hagfish slime and, similar to GMCs, likely originate from stem cells within the gland epithelium. They too undergo a massive increase in size as they move from the gland periphery toward the center of the gland, with cell volume increasing by a factor of roughly 4,500. Gland histology (**Figure 3**) reveals that the youngest GTCs are oriented with their long axis parallel to the gland epithelium, but as they grow and mature, their orientation shifts by approximately 90° so that their apical ends point toward the gland pore, with their long axis roughly perpendicular to the epithelium. This change in orientation likely facilitates the orderly ejection of GTCs and GMCs through the narrow gland duct during deployment of slime exudate and minimizes premature unraveling of the thread skein. GTCs produce an elaborately coiled thread in their cytoplasm that in mature cells is roughly 150 mm long and 1–3 μm in diameter (1). How a cell builds and organizes a protein thread that is 1,000 times longer than itself is a question that has puzzled biologists for decades; however, recent developments have started to illuminate this remarkable process.

Thread Proteins

The slime thread produced by GTCs consists mainly of proteins belonging to the intermediate filament (IF) family (see the sidebar titled Intermediate Filaments). Early work on thread proteins

INTERMEDIATE FILAMENTS

Intermediate filament (IF) proteins are an important superfamily of proteins that self-assemble in cells into 10-nm fibers that make up an important component of the cytoskeleton in most animal cells (48). Several human diseases have been attributed to defects and mutations in IFs (49). IF proteins are be classified into six types:

- Types I and II. The many isoforms of type I (acidic) and type II (basic) keratins can be further divided into two groups: the epithelial keratins and the trichocytic or hair keratins (50).
- Type III. These proteins are divided into four groups: desmin (structural component of sarcomeres in muscle cells) (51), glial fibrillary acidic protein (GFAP; expressed in astrocytes and other glia) (52), peripherin (found in peripheral neurons) (53), and vimentin (most widely distributed IF; found in fibroblasts, leukocytes, and blood vessel endothelial cells) (54).
- Type IV. These neurofilaments are found in high concentrations along developing axons of the central nervous system (55).
- Type V. Nuclear lamins A, B, and C form a supportive structural meshwork beneath the nuclear membrane (56).
- Type VI. Often expressed in proliferating nerve cells, nestin is associated with the radial growth of axons (57).

from hagfish slime revealed the presence of three abundant IF proteins (α, β, and γ) that could be separated with anion exchange chromatography (16). Subsequent work discovered that β protein is most likely a posttranslationally modified version of γ protein (17). The nature and purpose of this modification are not known, although the high threonine content of γ protein (13%) and changes in thread ultrastructure that occur over GTC maturation are consistent with phosphorylation of this protein. Both α and γ proteins are classified as IF proteins on the basis of several diagnostic features, including a central rod domain containing heptad repeats of apolar residues, rod subdomain structures (1A, 1B, 2A, and 2B) shared with other IF proteins, a so-called stutter of the heptad repeat pattern in subdomain 2B, conserved sequences at both ends of the rod domain, and nonhelical N- and C-terminal domains that flank the central rod domain (18, 19). Because these proteins exhibit low sequence identity with other IF proteins, previous researchers struggled to classify them, but settled on calling them keratin-like due to the presence of keratin-like features in the N and C termini (18, 19). More recently, Schaffeld & Schultess (20) described a group of "thread keratin" genes, similar to α and γ proteins, that are present in lamprey, teleost, and amphibian genomes. This work confirmed that α protein is a type II keratin homolog and that γ protein shares some features with the type I keratins, but intriguingly also bears some structural similarities to the type III IFs, which include vimentin, desmin, and glial fibrillary acidic protein (GFAP). This finding is consistent with the hypothesis that the keratin IF proteins evolved from an ancient type III protein (20, 21).

Thread Production and Coiling

Most epithelial cells contain a rich network of IFs, but GTCs differ in that they package huge numbers of IFs and IF proteins into a single, continuous, unbranched slime thread that in mature cells occupies the vast majority of the cell volume. Although the mechanisms involved in the production and coiling of the thread are not fully understood, detailed descriptions of the structure of the thread skein are available, and these provide some important insights (4, 5). The smallest organizational unit within the skein is the thread loop, which has a curvilinear base near

the skein periphery, and a narrower apex that points toward the apical end of the cell. Subsequent thread loops lie staggered on top of preceding loops, with the pattern of staggered loops spiraling around the long axis of the cell. A complete 360° spiral of loops forms a higher-order structure known as a conical loop arrangement; approximately 20 of these continuous structures nest together to make up a complete skein (**Figure 4**).

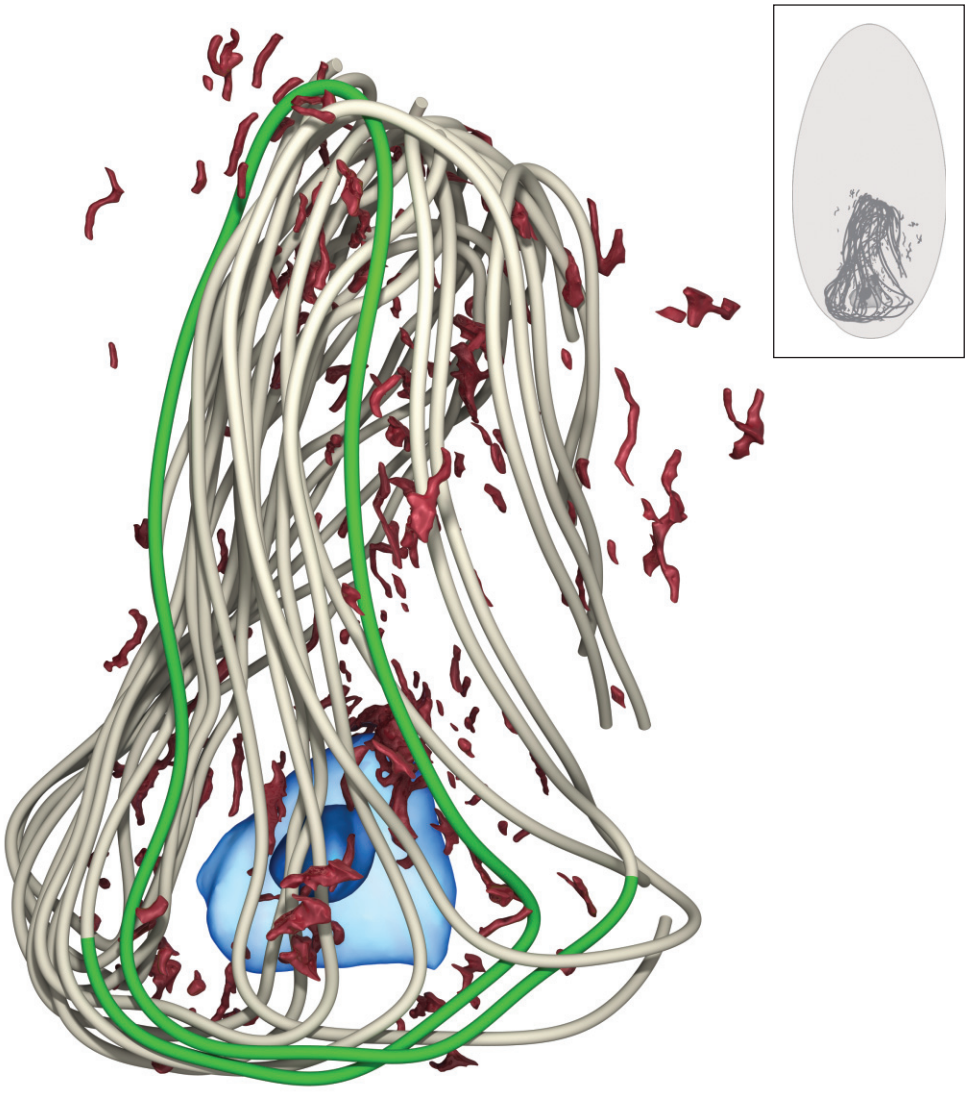

Figure 4

Three-dimensional reconstruction of staggered thread loop formation in a gland thread cell (GTC). Isolation of a dozen continuous loops within a developing GTC reveals the precise pattern of thread coiling (*single loop shown in green*). Part of the nucleus (*light blue*), the nucleolus (*dark blue*), and many mitochondria (*maroon*) are also shown. The loops depicted are not the most recent ones laid down on the nucleus, but they do reflect nuclear shape at the time of synthesis. (*Inset*) The position of the rendered structures within the whole cell (22).

Recent work elucidating the structure of thread loops and conical loop arrangements emphasized the importance of the nucleus in shaping these structures (22). Slime gland histology and transmission electron microscopy (TEM) of GTCs at various stages of maturation have revealed that the GTC nucleus undergoes dramatic changes in size, shape, and position over the course of GTC development. In a young GTC, the nucleus is roughly spherical and takes up a large fraction of the cell volume. As the cell matures, the nucleus becomes more conical, eventually taking the form of an elongate spindle with a wide base. At later stages, the spindle recedes to the basal end of the cell, where it exists as a small hemispherical cap. These changes in nuclear morphology correspond with changes in the shape of conical loop arrangements from the apical to the basal end of GTCs (**Figure 4**). This correspondence likely reflects the manner in which the thread elongates and is laid down as the cell grows, with new loops of thread laid down on the apical and lateral surfaces of the nucleus (**Figure 5**).

Thread Maturation

The nuclear template hypothesis explains the particular shape of the loops and conical loop arrangements, but how exactly the thread assembles, elongates, and coils is currently not understood. The cytoplasm on the apical side of the nucleus is rich in both mitochondria and polysomes (**Figure 6**) (23) and, therefore, is the mostly likely site of IF protein production and assembly. Three independent studies have investigated the ultrastructure of GTCs at various stages of development (22–24), but none of them were able to capture an image of the growing end of the thread, which will undoubtedly provide important clues about how the cell assembles IFs into a coherent thread. Detailed examinations of thread ultrastructure throughout the GTC (**Figure 7**) provide some hints about the mechanisms of thread production, and raise additional questions. The smallest (and therefore youngest) portions of the thread appear as a simple bundle of only a handful of IFs. Thread diameter increases via the addition of more IFs to the thread, as well as microtubules (MTs). Threads containing MTs also appear to be wrapped by a 12-nm-diameter filament that either spirals around the thread or exists as separate rings, but the function of this wrapping filament is not apparent (22, 23). The next stage of thread development involves condensation of discrete IFs into a single IF superstructure (22–24). MTs remain visible in threads after this condensation step, and the surface of the thread takes on a distinctive fluffy appearance, which may involve the direct addition of IF subunits to the growing thread. In the latest stages of thread maturation, the spaces occupied by MTs are filled in and the fluffy rind on the thread surface disappears (**Figure 7**). These changes to the thread appear to occur globally (i.e., in all parts of the thread) and, therefore, are likely to be mediated by biochemical signals throughout the cytoplasm. In addition, thread length and diameter appear to increase concomitantly in early stages of GTC development, but after the condensation step, it is likely that thread length is constant, and further growth involves only increases in thread girth. The biochemistry involved in IF condensation is currently not known, but it likely involves the posttranslational modification of γ protein, described above. Understanding this process could be important for current biomimetic efforts to make IF-based protein fibers that are as strong and tough as slime threads (25, 26).

POLYHEDRAL CELLS

Newby (27) was the first to point out the existence of a distinct population of small cells near the base of the slime gland duct, which he named polyhedral cells because of their shape. Newby also pointed out the similarity between these cells and the squamous epidermal cells with which they are continuous (**Figure 8**). Polyhedral cells stain positively using pan-keratin and tubulin antibodies (28). The same author suggested that polyhedral cells represent a store of undifferentiated cells

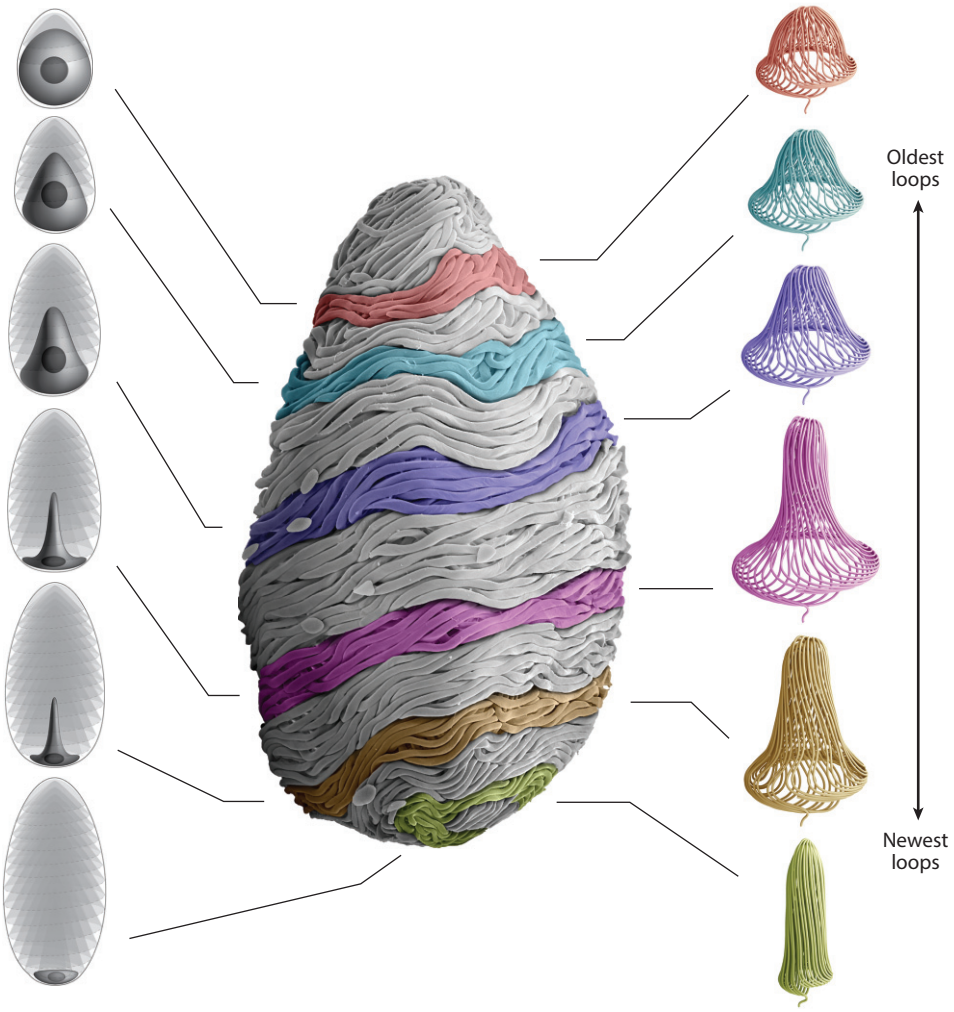

Figure 5

Spatiotemporal model of thread development and organization in gland thread cells (GTCs). (*Left*) The GTC nucleus changes in size and shape as the cell grows and develops. Newly formed loops of thread form on the apical and lateral surfaces of the nucleus. (*Right*) The successive arrangements of loops reflect nuclear morphology at the time they were laid down. (*Center*) In a mature thread skein, conical loop arrangements are nested together in a manner that facilitates rapid unraveling of the slime thread in seawater (22).

that ultimately give rise to the GTCs and GMCs, but subsequent studies support an alternative hypothesis that small stem cells in the gland epithelium are the source of GTCs and GMCs. One possible function of polyhedral cells is the formation of a mechanical plug that allows the pressure within the gland to increase when the muscle surrounding the gland contracts, with the plug rupturing once a certain threshold pressure is achieved (28). Observations of exudate release by electrostimulated slime glands are consistent with this hypothesis, in that the initial ejection of exudate from a gland is often far more difficult to elicit but also far more forceful than subsequent ejections from the same gland.

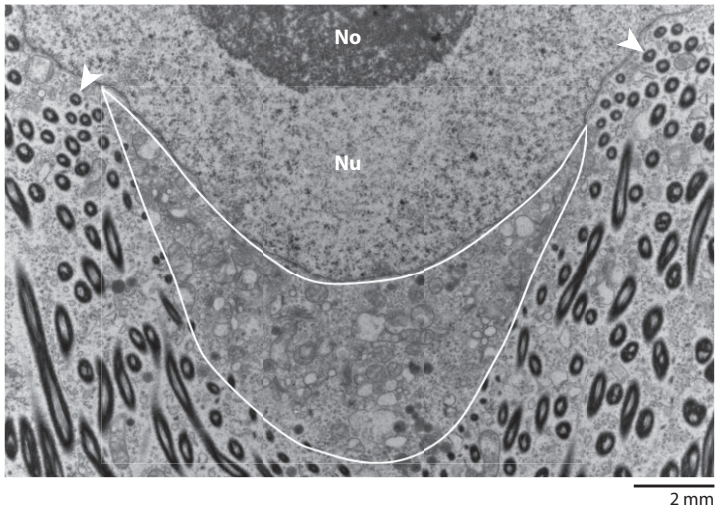

Figure 6

Mitochondria-rich zone (MRZ) apical to the gland thread cell (GTC) nucleus. The GTC nucleus (Nu) contains a large prominent nucleolus (No), and the cytoplasm immediately apical to the nucleus is rich in polysomes and mitochondria, a region of the cell known as the MRZ (*outlined in white*). The MRZ appears to be where intermediate filament (IF) proteins are synthesized and assembled into mature IFs. Arrowheads indicate cross sections through the developing thread.

GLAND INTERSTITIAL CELLS

Gland interstitial cells (GICs) were recently discovered via fluorescent labeling of paraffin-embedded sections of slime glands, which revealed the presence of cell nuclei in the interstices between the two main secretory cell types, GTCs and GMCs (28). Newby (27) describes numerous so-called trabeculae penetrating the gland between GTCs and GMCs, but he interpreted these as being associated with a connective tissue reticulum within the gland. Modern histology, light microscopy, and electron microscopy have revealed that Newby's trabeculae are in fact a single layer of GICs that occupy the spaces between adjacent GTCs and GMCs (28). Other researchers likely overlooked the presence of GICs because of their very small cell volume relative to that of their GTC and GMC neighbors. GICs stain positively for keratin IFs using a pan-keratin antibody (28). TEM has also revealed that each GIC has a large, often irregularly shaped nucleus, and cytoplasm that is rich in mitochondria, Golgi bodies, and cytoplasmic vesicles. Long and thin processes extend from the cell body and occupy the narrow spaces between the much larger GTCs and GMCs. The function of GICs is unknown, but possibilities include shepherding of developing GTCs and GMCs from their site of origin toward the gland lumen, acting as nurse cells for developing GTCs and GMCs, and acting as a mechanical restraint that prevents the release of immature GTCs and GMCs during expulsion of exudate from the gland. The nurse cell hypothesis is consistent with TEM observations of vesicles from GICs fusing with adjacent GTCs and GMCs (28). TEM studies have also suggested that each GTC and GMC contacts one to three GICs, which would make GICs the most abundant cell type in the slime gland lumen.

SLIME STABILIZATION AND DEPLOYMENT

A remarkable feature of hagfish slime is its ability to transform from a thick glandular exudate into an ultradilute slime permeated by 150-mm-long protein fibers in a fraction of a second. The

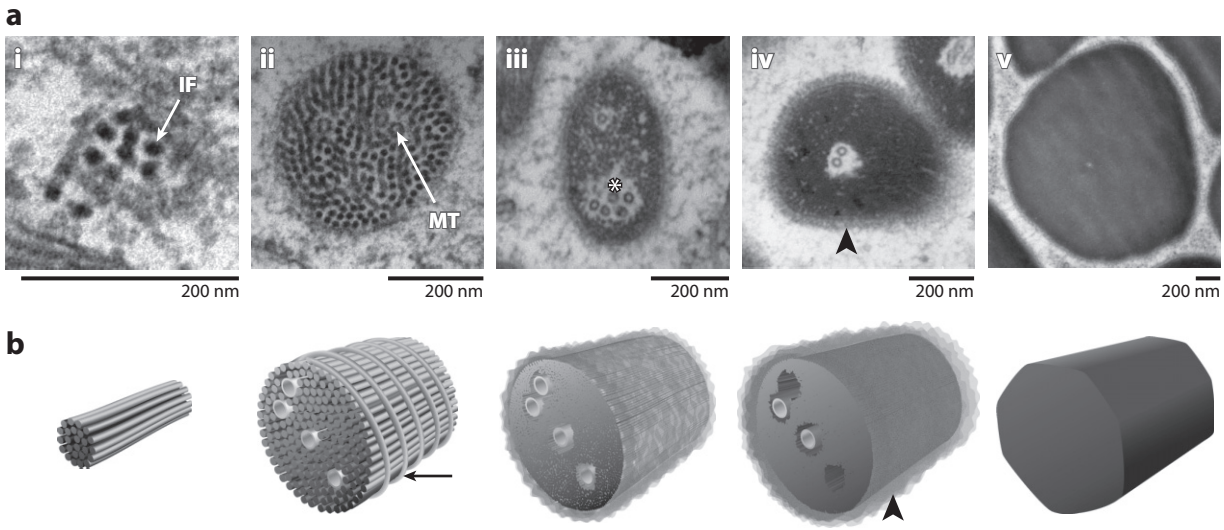

Figure 7

Developmental series of thread ultrastructure. (*a*) Transmission electron microscopy (TEM) sections depicting the development of slime threads from very immature to fully mature gland thread cells (GTCs). Threads initially consist of only a handful of 12-nm-diameter intermediate filaments (IFs) (*i*), but slowly increase in girth by the addition of more IFs, and eventually by the addition of microtubules (MTs) (*ii*). IFs become more tightly packed, creating electron lucent halos (*asterisk*) around the MTs (*iii*). With further compaction of the IFs, a fluffy rind (*arrowheads*) appears on the thread surface (*iv*), which likely corresponds to the direct addition of IF subunits or proteins to the thread. In a fully mature thread (*v*), IF proteins are further compacted, the MTs and fluffy rind disappear, and the spaces once occupied by MTs are filled in. (*b*) Models of thread ultrastructure illustrate the development and condensation of the thread as it matures. The appearance of a 12-nm-diameter filament wrapping around the thread (*arrow*) and the development of a fluffy rind on the thread surface (*arrowhead*) are clearly depicted (22).

volume of slime produced is also remarkable; a 150-g Pacific hagfish can produce 900 mL of slime from only a handful of its 158 slime glands (1). A full understanding of this process requires knowledge both about how the slime is stabilized within the gland and about the chemical and physical factors in seawater that trigger this transformation. High-speed video footage of hagfishes releasing slime in aquaria demonstrate that this transformation can happen in as little as 100 ms (2), and field observations are consistent with this conclusion (6). Observations of simulated attacks in aquaria also revealed that the slime is forcefully ejected from the glands, which likely aids in its deployment and increases the probability that it will find its target (the predator's gills) and not simply enshroud the hagfish in slime (2). Chemical analyses of the fluid component of slime gland exudate (obtained by centrifuging freshly collected exudate) have revealed a high concentration of organic osmolytes, with three methylamines—betaine, trimethyl amine oxide (TMAO), and dimethyl glycine (DMG)—making up a combined concentration of approximately 390 mM (**Table 1**) (12). Hagfishes are osmoconformers, with plasma and tissue osmolarities that are close to those of seawater. Unlike osmoconforming vertebrates like sharks and rays, however, most of the osmotically active particles (approximately 97%) in hagfish plasma are inorganic ions (29, 30). In slime gland fluid, the concentration of K^+ is greater than that found in the plasma, whereas Na^+, Cl^-, and Ca^{2+} are less concentrated (**Table 2**). These ions may be less concentrated in the fluid component of the exudate due to the abundance of organic osmolytes, which are absent in the plasma (12). These data raise the question of why slime gland fluid is so rich in methylamines. Herr et al. (12) tested the idea that betaine and TMAO are used to stabilize mucin

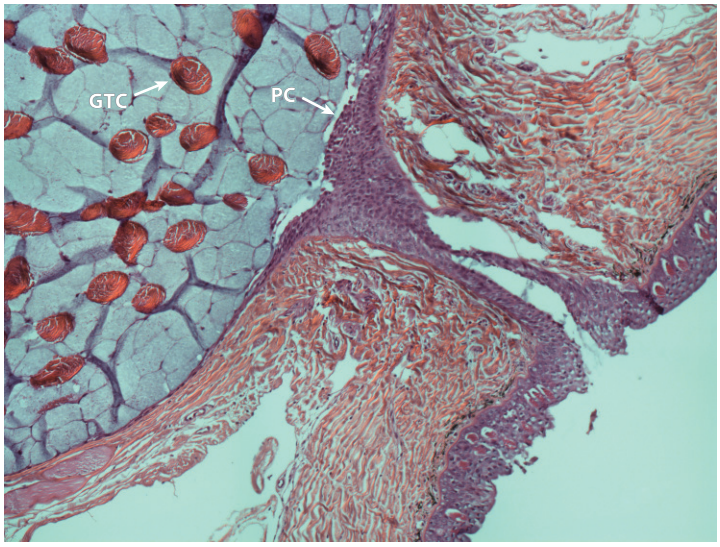

Figure 8

Polyhedral cells (PCs) of the slime gland. First described by Newby (27) and found near the base of the slime gland duct, these cells appear continuous with the gland epithelial lining and share morphological similarities with the squamous epidermal cells. PCs may form a mechanical plug near the gland pore opening and facilitate forceful ejection of slime from the gland. Abbreviation: GTC, gland thread cell.

vesicles in the gland and found that these compounds are not especially good at stabilizing the vesicles. Further investigation showed that the fluid component of slime gland exudate is also not able to stabilize all of the vesicles, suggesting that GMC cytoplasm differs from this fluid in important ways. Subsequent research on stabilization and deployment of the thread skeins suggests that methylamines may be involved in stabilizing the putative protein adhesive that holds the skein together in the gland (31), but this hypothesis requires further investigation.

Table 1 Concentrations of organic osmolytes in the supernatant of hagfish slime exudate[a]

Organic osmolyte[b]	Concentration in supernatant (mmol/L)[c]
Glucose	1.23 ± 0.22
Inositol	2.30 ± 0.68
Taurine	2.13 ± 0.42
Betaine	218 ± 7
Dimethyl glycine	68.6 ± 6.0
Glycine	79.9 ± 7.5
Creatine	15.0 ± 1.4
b-Alanine	2.17 ± 0.68
TMAO[b]	101.3 ± 4.8
Total	490 ± 10

[a]Data are from Reference 11.
[b]Organic osmolytes were analyzed using high-performance liquid chromatography.
[c]TMAO stands for trimethylamine *N*-oxide; analyzed using ferrous sulfate and EDTA (ethylenediaminetetraacetic acid).
[d]Values are means ± SEM (standard error of the mean); $N = 5$.

Table 2 Concentrations of inorganic ions and pH in the supernatant of hagfish slime exudate[a]

Inorganic ion[b]	Concentration in supernatant (mmol/L^1)[c]
Na^+	41.2 ± 2.6
K^+	143.0 ± 3.0
Cl^-	191.5 ± 6.6
Ca^{2+}	0.00045 ± 0.00009
Mg^{2+}	2.15 ± 0.76
Total	379
pH	7.31 ± 0.02

[a]Data are from Reference 11.
[b]Data were measured using ion-specific electrodes.
[c]Values are means ± SEM (standard error of the mean); $N = 5$.

Mucin Vesicle Deployment

Downing et al. (32) developed a variety of techniques that made it possible to collect and stabilize the components of hagfish slime exudate. Building on this work, Luchtel et al. (10) investigated the chemical conditions that stabilize or rupture mucin vesicles and concluded that the vesicles are approximately isosmotic with seawater (and hagfish tissues) and permeable to most ions except polyvalent anions. The authors reached this latter conclusion by mixing fresh exudate into solutions of varying compositions, and observing that most of the solutions tested resulted in vesicle rupture and slime formation, but salts containing high levels of sulfate, citrate, or phosphate were able to keep the vesicles in a condensed state and inhibit slime formation. Herr et al. (12) investigated vesicle function in more detail by using a flow-through rupture chamber that allowed them to observe immobilized vesicles under the microscope as they were exposed to various test solutions. These experiments revealed that the methylamines present in the glandular fluid cannot stabilize all of the vesicles, even at concentrations much higher than their native concentrations. They also revealed that the swelling kinetics of the vesicles in seawater is variable, clustering into two categories of slow and fast swellers.

A further investigation (11) demonstrated that only 40% of the vesicles rupture in hyperosmotic sodium chloride solutions, but all of the vesicles rupture if calcium ions are present at concentrations of approximately 3 mM or higher. This study also demonstrated that disruption of the vesicle membrane with a detergent homogenizes the swelling kinetics, suggesting that differences between the two vesicle types are mediated by the vesicle membrane. Aquaporins appear to be among the membrane proteins mediating this process, as vesicle swelling rates can be slowed by an order of magnitude by treating vesicles with mercuric chloride, a known aquaporin inhibitor. Two aquaporin-like genes related to AQP3 and AQP4 are expressed in hagfish slime gland tissue, but it is unclear which of these proteins might be present in the vesicle membrane (11). One of the more puzzling aspects of the behavior of mucin vesicles is that a substantial portion of them rupture in concentrated solutions of molecules like sucrose (11). The only way that a hyperosmotic sucrose solution could rupture these vesicles is if the membrane were permeable to sucrose, which raises the question of why the membrane would be permeable to such large molecules when the vesicles are destined for export into seawater, which has a very low organic molecule content. One possibility is that the transporters that let molecules like sucrose into the vesicle are there to allow a certain class of molecules out during vesicle deployment. Vesicle swelling may be driven in part by a so-called jack-in-the-box mechanism, in which the counterions responsible for stabilizing negatively charged mucins in the gland are exchanged for abundant cations like Na^+, which may

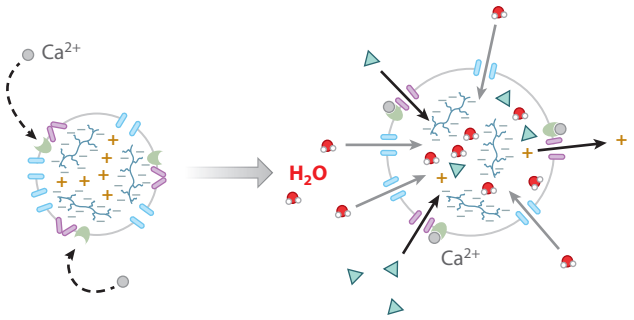

Figure 9

Model of calcium-dependent mucin vesicle rupture. Calcium present in seawater binds to putative transporters in the vesicle membrane, opening them and allowing the influx of ions from seawater. The influx of ions alters the osmotic balance and creates conditions favoring water influx via aquaporins. Triangles indicate seawater ions, and plus symbols represent positive counterions, which have yet to be identified but are presumed to be important for the condensation and stability of mucin molecules within the gland. The exchange of these counterions for positive ions from seawater may also be important in driving swelling of the mucin gel (11).

be far less effective at charge shielding than the native (likely polyvalent) counterions (33). If the native counterion is an organic molecule, this could explain the presence of pores in the vesicle membrane that are large enough to allow the passage of sucrose. **Figure 9** shows a model of Ca^{2+}-activated vesicle swelling.

Thread Deployment

How the slime thread deploys to its full length of approximately 150 mm in seawater in a fraction of a second is another remarkable phenomenon that has puzzled researchers. Early work by Newby (27) suggested that GTCs swell upon contact with seawater, which causes the plasma membrane to burst, triggering the release of internal pressure that in turn drives unraveling of the thread skein. Fernholm (5) subsequently disproved this hypothesis by showing that GTCs lose their plasma membrane during ejection from the gland and before they encounter seawater. Winegard & Fudge (34) showed that in slime skeins from *Myxine glutinosa*, unraveling is not spontaneous in seawater, but requires the presence of mucins and vigorous mixing. According to their mucin transduction hypothesis, condensed skeins are too small to be effectively pulled apart by turbulent mixing forces. However, elongated strands of mucus, which are much larger, are susceptible to turbulent mixing, and can attach to the skeins and pull them apart.

Bernards et al. (31) recently demonstrated that skein deployment in *Eptatretus stoutii* differs markedly from *M. glutinosa* in that unraveling occurs spontaneously in seawater even in the absence of mucins and hydrodynamic mixing. They also showed that skein unraveling is sensitive to both salt concentration and temperature, with maximal rates of unraveling at a sodium chloride concentration of 1.25 M and temperatures between 5°C and 15°C. The same study also explored two possible mechanisms that could power spontaneous unraveling: (*a*) swelling of the slime thread in seawater and (*b*) the release of stored strain energy triggered by the dissolution of a protein adhesive. They found no evidence for the first mechanism and made several observations consistent with the latter mechanism. Specifically, they found that unraveling could be initiated under conditions that are normally stabilizing by exposing the skeins to trypsin. Furthermore, SEM studies of skeins fixed under stabilizing and destabilizing conditions showed the presence and absence, respectively, of a gluelike substance coating the outside of the skein and bridging

adjacent loops of thread. The identity of this protein adhesive is not yet known, nor is the reason that skein deployment differs so much between the two hagfish species examined.

HAGFISH SLIME BIOMECHANICS

Many animals release noxious chemicals when they are threatened or attacked by predators, but hagfish slime appears to be primarily a mechanical defense that has evolved to clog the gills of would-be fish predators. In its mature, fully deployed state, the slime is incredibly dilute, with mucin concentrations (approximately 15 mg dry weight per liter) that are roughly three orders of magnitude lower than typical mucous secretions like gastric mucus (1). The slime threads are similarly dilute, occurring at concentrations of roughly 20 mg dry weight per liter (1). Although the slime at first appears to behave like an exceptionally good superabsorbent material, this is in fact not the case, because the slime does not bind seawater as much as it slows it down by entraining it within the spaces formed by intricate networks of threads and mucus (1). Therefore, it is possible to lift multiple kilograms of slime out of a bucket in which a hagfish has slimed (**Figure 1**), but most of the entrained water will run out of slime if held in air long enough (typically a few minutes). Ewoldt et al. (35) investigated the mechanical properties of the whole slime and concluded that hagfish slime is one of the softest biomaterials known, with an elastic modulus of 0.02 Pa, which is approximately five orders of magnitude more compliant than gelatin. The slime exhibits strain softening at large strains, with simultaneous local strain stiffening, which may correspond with the breaking of weak mucin-thread cross-links and the stretching of slime threads, respectively.

Slime Threads

As described above, slime threads consist mainly of IF proteins, yet their material properties differ radically from those of another IF-based material, mammalian α-keratin, which is found in wool, hair, nail, and related materials. Mammalian keratins are fairly stiff, even in the hydrated state, with tensile moduli in the range of 2 GPa (36). In contrast, slime threads are compliant and rubberlike in water, with an initial stiffness of 6.4 MPa and a breaking strain of 220% (37). The differences between slime threads and α-keratins are believed to be due to the highly cross-linked network of matrix proteins that surrounds the IFs in keratins and prevents them from fully rehydrating in water (36, 38). Slime threads exhibit rubberlike mechanics at strains up to 35%, allowing them to stretch and return to their original length (37). This kind of elastic behavior may be involved in the storage of strain energy in the slime thread that is released when skeins from *E. stoutii* encounter seawater (31). At strains greater than 35%, deformation is plastic and the threads do not return to their original length after unloading. The transition between elastic and plastic behavior corresponds with the disruption of α-helices within thread proteins and their reannealing into β-sheets (37). It is the formation of these stable β-sheet structures that imparts to slime threads their impressively high breaking stress (37–39). In addition to illuminating the function of hagfish slime, the study of slime thread mechanics has also led to new insights into the behavior and function of IFs in living cells (40, 41), in mammalian keratins (36, 38), and in the quest to manufacture high-performance protein materials (25, 26, 39).

Biomimetics

When slime threads are stretched in water, the α-to-β transition described above results in the formation of extensive β-sheet and β-sheet crystal content in the thread (37). These structures, along with the flexible linker domains that string them together, impart to spider dragline silk superior tensile properties (42); they are also likely to contribute to the impressive strength and

toughness of draw-processed slime threads (39), which rival those of spider silk. Over the last few decades, efforts to produce artificial spider silk in the lab have proven to be incredibly difficult (39). The discovery of slime threads as a high-performance fiber that is produced via a completely different mechanism from spider silk has inspired biomimetic research into how we might produce protein fibers in the lab with properties as good as those of slime threads. Early attempts using slime thread protein solubilized in formic acid and aggregated at an electrolyte buffer–air interface resulted in fibers that were far weaker than native slime threads (25). Subsequent efforts explored the possibility of using IF proteins (i.e., vimentin) that are easier to express and assemble than hagfish α and γ proteins (26). This study demonstrated that making fibers from vimentin protein assembled into a network of 10-nm-diameter IFs results in improved material properties, and an α-to-β transition similar to that observed in native slime threads (26). Future biomimetic work in this area will take advantage of the insights described above into how GTCs assemble IF proteins and IFs into dense threads with impressive mechanical properties.

EVOLUTION OF HAGFISH SLIME

All extant hagfishes possess slime glands, but it is not clear when this trait appeared in the evolutionary history of the lineage. The only hagfish fossil (*Myxinikela siroka*) that has been described dates from more than 300 Mya, and shows no evidence of slime gland pores or slime glands (43). If *Myxinikela* indeed lacked epidermal slime glands, then it is possible that the glands evolved in response to increasing predation pressure from gnathostomes (i.e., the jawed fishes) later in their evolutionary history. There are currently two plausible hypotheses for how the epidermal slime glands evolved from ancestors that lacked them. One hypothesis is that the slime glands evolved as modifications of the cloacal glands, which produce mucus and threads during the release of eggs and sperm. Although cloacal glands superficially resemble the epidermal slime glands, they are known to stain positively with periodic acid–Schiff (PAS) stain, whereas the epidermal slime glands are PAS negative, suggesting that cloacal gland mucins are more heavily glycosylated and therefore more similar to typical mucins (44). Thread cells are also present in the cloacal glands, but they have not been examined in detail (45). Cloacal glands might have conferred some protection against predators if the slime were released into a predator's mouth during an attack on the tail. Subsequent selection may have led to the evolution of more glands and their specialization for defense.

Another possibility is that the slime glands evolved via invagination and specialization of the skin, with the slime glands evolving first and subsequently being modified into cloacal glands. If this hypothesis is correct, then defensive sliming would have had to evolve before the appearance of the slime glands, unless unrelated selective pressures originally drove the invagination process, which is difficult to imagine. Other species use mucous secretion from the epidermis as an antipredator strategy (46), and if this were the case in early hagfishes, then invagination may have been selected for as a way to increase the surface area available for mucus production and storage, eventually leading to the ability to forcefully eject slime exudate. The invagination hypothesis is consistent with the presence of two cell types in the epidermis, large mucous cells (LMCs) and epidermal thread cells (ETCs), which resemble the two main secretory cell types in the slime glands, GMCs and GTCs (**Figure 10**) (8). Both LMCs and GMCs are large mucus-secreting cells that are packed with mucin vesicles. Both ETCs and GTCs produce a coiled protein polymer in their cytoplasm, although the slime thread in GTCs is much longer and more intricately organized. ETCs also resemble the "skein cells" that are present in lamprey epidermis (47). Although the presence of LMCs and ETCs in hagfish skin appears to favor the invagination hypothesis, it does not rule out a cloacal gland origin, especially if the tissues that give rise to the cloacal gland are found to contain

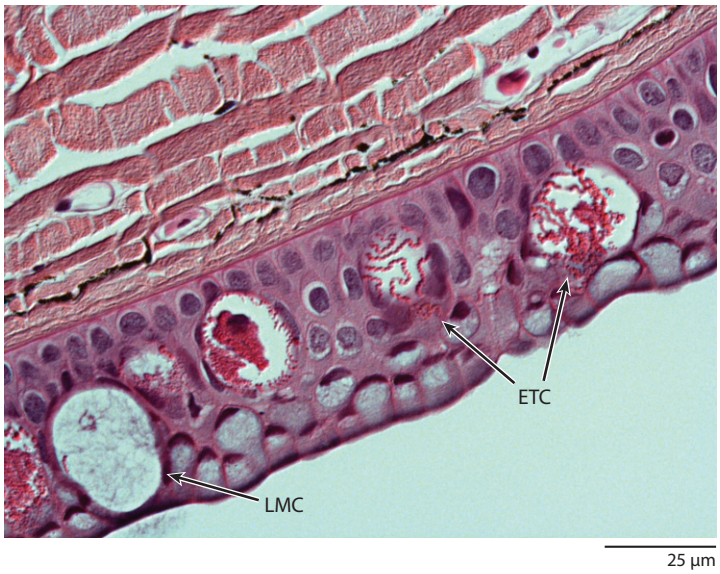

Figure 10

Mucus- and thread-producing cells in the hagfish epidermis. Histological cross sections through the hagfish epidermis reveals two large secretory cell types, the large mucous cells (LMCs) and the epidermal thread cells (ETCs). LMCs are analogous to the gland mucous cells (GMCs) in the slime gland, as both are large mucus-secreting cells packed with mucin vesicles, whereas ETCs are equivalent to the gland thread cells (GTCs), as both produce a coiled protein polymer in their cytoplasm (although the GTCs produce a much longer and more intricately organized thread).

their own kind of mucus and thread cells. Currently it is not possible to distinguish between these two hypotheses from existing evidence, but future work in hagfish embryology and the discovery of new hagfish fossils will surely shed more light on this issue.

SUMMARY POINTS

1. When provoked, hagfishes produce copious amounts of slime (approximately 1 L at a time) very quickly (in less than 100 ms) from segmentally paired slime glands, clogging the gills of would-be fish predators.
2. Hagfish slime glands secrete two main cell types: gland thread cells (GTCs) and gland mucous cells (GMCs), which produce the fibrous and mucous components of the slime, respectively. Both GTCs and GMCs lose their plasma membranes as they pass through the narrow gland duct, releasing naked thread skeins and countless mucin vesicles.
3. Thread development involves a condensation step in which intermediate filaments (IFs) merge into a super-IF structure, after which IF subunits are likely added directly to the growing thread. The GTC nucleus plays a critical role in shaping coils of slime thread as it elongates and thickens within developing GTCs.
4. Hagfish slime exudate is rich in methylamines, which may be involved in stabilizing the adhesive proteins that hold the skeins together in the gland.

5. Ca^{2+} is required for rupture of approximately 60% of the mucin vesicles, and aquaporins in the vesicle membrane accelerate the influx of water during vesicle deployment in seawater.
6. Unraveling of thread skeins is facilitated by mixing forces and the presence of mucins. In Pacific hagfish, unraveling is spontaneous in seawater and is triggered by the dissolution of a protein adhesive and the release of stored strain energy in the coiled thread.
7. Whereas hagfish slime threads have impressively high breaking stress due to the formation of stable β-sheet structures, hagfish slime as a whole is one of the softest biomaterials known, with an elastic modulus of 0.02 Pa.
8. Hagfish slime glands may have evolved either as modifications of the cloacal glands, which produce mucus and threads during the release of eggs and sperm, or via invagination and specialization of the skin.

FUTURE ISSUES

1. Functional studies point to the presence of a nonspecific transporter in the mucin vesicle membrane that allows the passage of molecules at least as large as sucrose. What kind of transporter is this, and what is its function?
2. It is clear that the swelling of mucin vesicles is determined not only by the properties of their membranes, but also by the biophysical properties of the mucin gel. Under what conditions is the gel stabilized and condensed, and what conditions cause it to swell?
3. Why are there two kinds of vesicles in slime exudate, and what are the molecular differences underlying those differences? Are the two kinds of vesicles produced within the same GMCs, or are they produced in two distinct GMC subtypes?
4. How does assembly of the slime thread in GTCs begin? What are the molecules that are required for this process? Where exactly in the GTC does thread elongation occur, and how are newly synthesized IFs recruited into the growing thread?
5. What is the function of the gland interstitial cells (GICs)?
6. What are the proteins that make up the seawater-soluble adhesive that is involved in stabilization of the skeins in the gland and their deployment in seawater?
7. How much variability exists in the slime glands and the function of the slime from the 80-plus species of extant hagfishes? How much of this variability is adaptive for the particular lifestyle of that species?
8. What regulates the condensation of IFs in the developing slime thread, and can we mimic this process in vitro to produce protein materials as strong as hagfish slime threads?

DISCLOSURE STATEMENT

D.S.F. is a coauthor of a patent (US patent 7,049,405 B2) that involves the material properties of hagfish slime threads and related materials. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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