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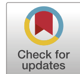

Specialized structure and function of the apical extracellular matrix at sense organs

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ABSTRACT

Apical extracellular matrix (aECM) covers every surface of the body and exhibits tissue-specific structures that carry out specialized functions. This is particularly striking at sense organs, where aECM forms the interface between sensory neurons and the environment, and thus plays critical roles in how sensory stimuli are received. Here, we review the extraordinary adaptations of aECM across sense organs and discuss how differences in protein composition and matrix structure assist in sensing mechanical forces (tactile hairs, campaniform sensilla, and the tectorial membrane of the cochlea); tastes and smells (uniporous gustatory sensilla and multiporous olfactory sensilla in insects, and salivary and olfactory mucus in vertebrates); and light (cuticle-derived lenses in arthropods and mollusks). We summarize the power of using *C. elegans,* in which defined sense organs associate with distinct aECM, as a model for understanding the tissue-specific structural and functional specializations of aECM. Finally, we synthesize results from recent studies in *C. elegans* and *Drosophila* into a conceptual framework for aECM patterning, including mechanisms that involve transient cellular or matrix scaffolds, mechanical pulling or pushing forces, and localized secretion or endocytosis.

1. Introduction

Sense organs are the doors and windows to the outside world. They grant the remarkable ability to gather information through different sensory modalities so that the brain can construct a complex, detailed representation of the world. As described by Aristotle in his book *De Anima (On the Soul)* in 350 BCE, each sense organ holds a unique *"power of receiving"* a distinct characteristic of an object (Aristotle, 1931: Book II, Part 12). He designated the eyes, nose, tongue, skin, and ears as the five major sense organs of animals that distinguish objects based on their visible features, odors, flavors, tangible characteristics, and sounds, respectively. Centuries of investigation have since expanded this list to include sense organs that detect other characteristics including temperature, pain, proprioception, and balance (Bárány, 1914; Hensel, 1973; Sherrington, 1903, 1907), and in some fish and bird species, electric and magnetic fields (Murray, 1960; Wiltschko and Wiltschko, 1972). The ability to sense and process visual, chemical, and mechanical cues is essential, as it allows animals to thoroughly assess and appropriately respond to the immediate environment. Sense organs are therefore critical structures, and how physical properties of the world are transduced into cellular signals is a fundamental question in biology.

Several components of the sensory transduction apparatus are well-studied and understood, but others remain largely mysterious. For example, it is well established that the endings of sensory cells contain sensory organelles called cilia that house important molecules for detecting environmental cues (Christensen et al., 2007). The molecules that capture sensory stimuli, such as photons and odors, and many of the ion channels and effector proteins that convert sensory information to electrical signals have been identified and characterized (Arshavsky et al., 2002; Boll, 1877; Buck and Axel, 1991; Firestein, 2001). However, between the sensory cells and the environment lies a key interface that facilitates how environmental signals reach the cells, yet which remains poorly understood: the apical extracellular matrix (aECM).

The aECM is a complex network of secreted macromolecules that covers all outward-facing cellular surfaces. The conventional view has been that aECM serves as a protective structural barrier. This comes from evidence that aECM lining the lumen of internal tube-shaped organs, such as the gut and vasculature, plays critical roles in preventing pathogen entry and in maintaining tube integrity during morphogenesis (Gaudette et al., 2020; Johansson et al., 2013). However, aECM can also

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play key roles at sense organs, where it is intimately associated with the endings of sensory cells and influences their ability to detect environmental cues from the outside world. For example, gel-like aECM, such as the mucus of the nasal cavity, is viscous and contains proteins that capture odors and present them to cilia (Getchell et al., 1984a; Pelosi, 1996). Pores in the aECM of insect and nematode sense organs provide physical openings that give cells direct access to chemical cues in the external environment (Shields, 2008; Sulston et al., 1980; Ward et al., 1975). Meanwhile, a resonating matrix in the inner ear transmits physical forces to the underlying cells by bending their sensory endings (Goodyear and Richardson, 2018; Gueta et al., 2006; Legan et al., 2000; Sellon et al., 2019). Several molecular components – including zona pellucida (ZP) domain proteins, glycoproteins, collagens, and chitin – are shared across aECM, but they are distributed and organized in distinct manners (Getchell et al., 1993; Legan et al., 1997; Muthukrishnan et al., 2022; Page and Johnstone, 2007; Richardson et al., 1987; Sundaram and Pujol, 2024; Thalmann et al., 1986, 1987; Witt, 1996). Thus, the structure and composition of sensory aECM is tailored to the function of each sense organ.

Here, we will review the diverse array of aECM associated with sense organs across the animal kingdom and describe how the specific composition and form of the aECM contributes to function. Then, we will briefly summarize our recent work showing how the cuticle aECM of *C. elegans* can be used as a model for specialized aECM patterning at sense organs. Finally, we will discuss some potential underlying mechanisms by which specialized aECM structures at sense organs may be patterned.

2. Diverse structural and functional aECM at sense organs

A vast array of distinct aECM shapes and forms associated with sense organs are important for detecting mechanical, olfactory, gustatory, and visual cues. A majority of the aECM described comes from studies on mammals and arthropods. Based on their structural and functional properties, the aECM can be grouped into five categories: aporous force-transmitting structures, resonating matrix, nanoscale pores, gel-like and viscous mucus, and optical lenses (Fig. 1).

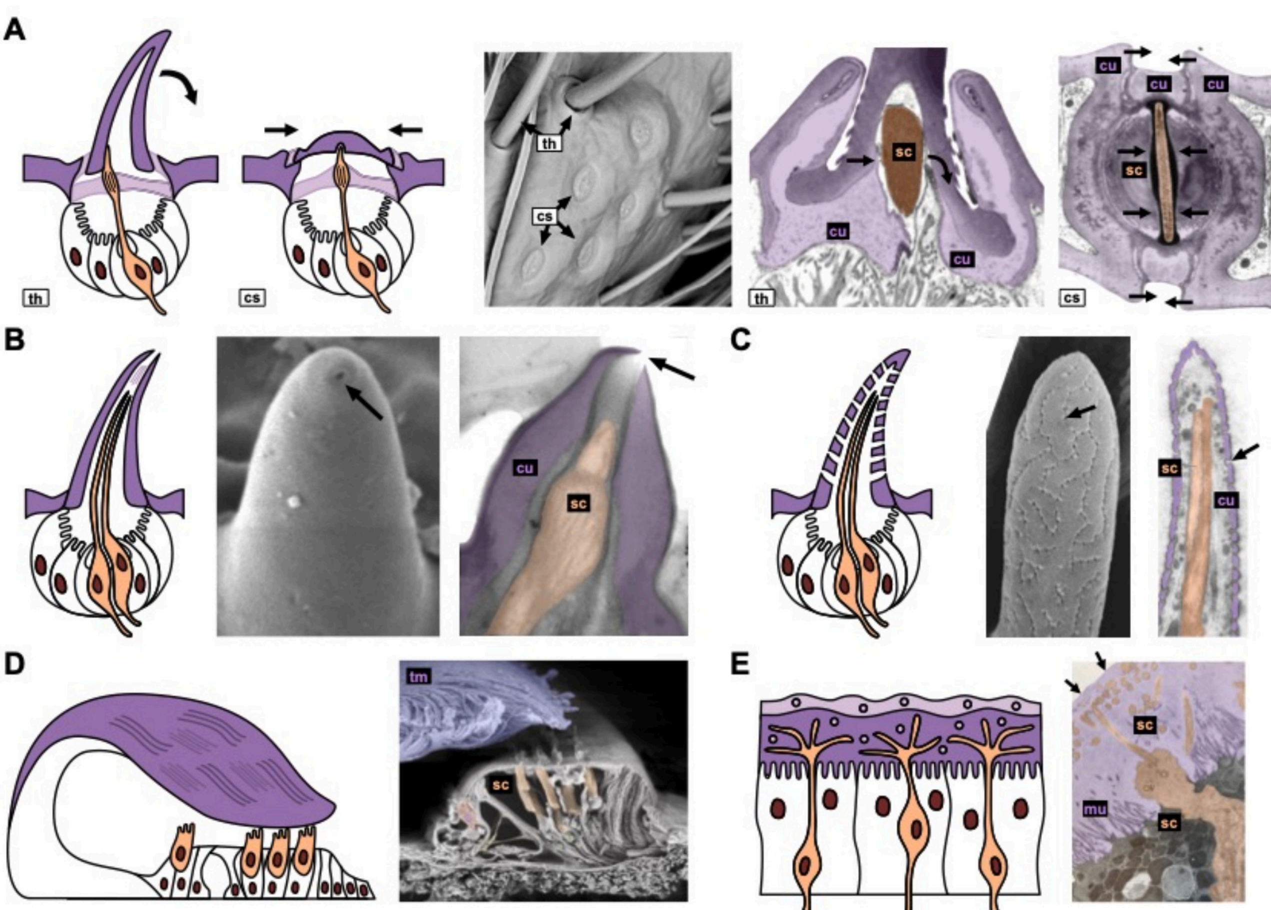


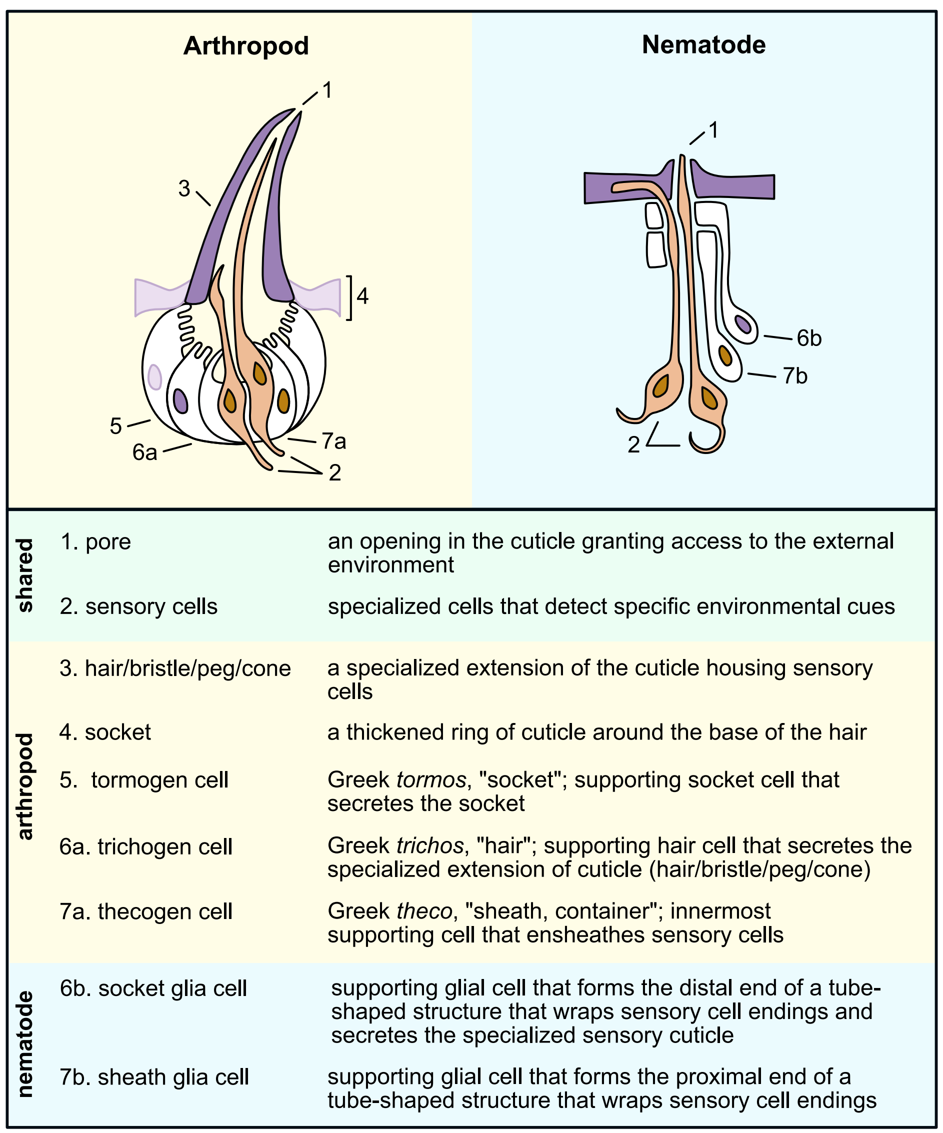
Fig. 1. Adaptations of aECM across sense organs.
Structural and functional specializations of aECM in sense organs of (A-C) insects and (D,E) mammals and amphibians. Purple, aECM; orange, sensory cells. (A) In tactile hairs (th) and campaniform sensilla (cs), respectively, sensory neurons associate with aECM shaped like a hair that is deflected to sense environmental forces or a dome that is pinched to sense compression forces from joint flexure. In both cases, the composition of the aECM (including additional subcuticle structures that attach the cuticular socket to the mechanosensory neuron and cuticular hair or dome; light purple) can vary to tune the sensitivity regime of the neuron. (B, C) In gustatory and olfactory sensilla, respectively, sensory cells (sc) associate with cuticle (cu) aECM housing that (B) terminates in a single pore for exposure to tastants or (C) is perforated by numerous nanopores for exposure to odorants. Some gustatory sensilla contain a fibrillar plug that is thought to confer selectivity (light purple). (D) In the mammalian cochlea, sensory hair cells associate with the tectorial membrane (tm), a large free-standing aECM that facilitates detection of sound waves. (E) In the olfactory epithelium of mammals and amphibians, multiciliated sensory cells (sc) are embedded in mucosal aECM (mu) that affects the permeation of odorants. Images adapted from (A) Taszakowski et al. (2023), Keil (1997); (B) Xu et al. (2017), Shanbhag et al. (1999); (C) Glueckert et al. (2005); and (E) Porter and Bonneville (1968). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.1. Aporous force-transmitting structures

Many aporous aECM structures have evolved in arthropods, including insects, arachnids, and crustaceans, to transmit mechanical forces that distort the exoskeleton, or cuticle, covering the animal to the underlying sensory neurons. The arthropod cuticle is an aECM layer primarily composed of chitin, a polysaccharide that organizes into highly stable crystalline aggregates and associates with proteins to build a stiff, rigid protective armor (Hackman, 1987; Muthukrishnan et al., 2022; Neville et al., 1976). However, the cuticle is not a homogeneous, uniformly stiff aECM layer. Instead, the composition and structure of the cuticle is modified at mechanosensory organs to provide both stiffness and elasticity (Vincent and Wegst, 2004) – properties important for withstanding and detecting external forces that are strong enough to be damaging or that are as weak as air currents generated by nearby prey. To meet these needs, evolution has fashioned intricate cuticle structures for two major classes of mechanosensory organs in arthropods: tactile hairs and campaniform sensilla (Fig. 1A).

Tactile hairs, also called bristles in *Drosophila*, are widely distributed across the whole surface of the animal and are designed to detect touch, contact with external objects, and movement in the air (Barth and Höller, 1999; Murphey, 1985; Theiß, 1979; Tobias and Murphey, 1979; Tuthill and Wilson, 2016). Each tactile hair is inserted into a cuticular socket and is associated with a single mechanosensory neuron, whose

Box 1
Glossary of key terms.



shared	1. pore	an opening in the cuticle granting access to the external environment
	2. sensory cells	specialized cells that detect specific environmental cues
arthropod	3. hair/bristle/peg/cone	a specialized extension of the cuticle housing sensory cells
	4. socket	a thickened ring of cuticle around the base of the hair
	5. tormogen cell	Greek *tormos*, "socket"; supporting socket cell that secretes the socket
	6a. trichogen cell	Greek *trichos*, "hair"; supporting hair cell that secretes the specialized extension of cuticle (hair/bristle/peg/cone)
	7a. thecogen cell	Greek *theco*, "sheath, container"; innermost supporting cell that ensheathes sensory cells
nematode	6b. socket glia cell	supporting glial cell that forms the distal end of a tube-shaped structure that wraps sensory cell endings and secretes the specialized sensory cuticle
	7b. sheath glia cell	supporting glial cell that forms the proximal end of a tube-shaped structure that wraps sensory cell endings

Simplified schematics comparing arthropod and nematode sense organs. Each sense organ is drawn containing both a mechanosensory and chemosensory cell (left and right, respectively). Note that "socket" refers to a cuticle structure in arthropods but to a glia cell in nematodes. Numbering of the trichogen/socket glia cell and thecogen/sheath glia cell reflects their comparable positioning in the sense organ. Some *C. elegans* sense organs associate with a secondary socket glia cell (PHso2) or a specialized skin cell (hyp3) that may be comparable to the tormogen cell.

sensory cilium contacts the base of the hair (Fig. 1A, Box 1). When the hair is deflected by a mechanical force, the base of the hair acts as a lever that bends and pushes against the cilium – this effectively transmits the applied force to the neuron and triggers an electrical impulse (Barth et al., 2004; Gaffal et al., 1975; Keil, 1997). Hairs that are specialized for detecting airflow are less stiff and more flexible than hairs that solely respond to touch (Barth and Dechant, 2003; Fratzl and Barth, 2009; Politi et al., 2021). The increased sensitivity of airflow sensors to weaker forces is thought to be attributed to increased water content and the presence of elastomeric proteins, such as resilin, in the cuticular socket and cuticular membrane that attaches the hair to the socket (Fratzl and Barth, 2009; Keil, 1997; Michels and Gorb, 2012; Politi et al., 2021; Weis-Fogh, 1960). Resilin is a rubber-like protein in insects that provides materials the ability to deform more easily and reversibly, similar to elastin in vertebrates, which provides elasticity to cartilage and stretchiness to arteries (Andersen and Weis-Fogh, 1964; Gosline et al., 2002). Tactile hairs of arthropods can therefore detect different magnitudes of force depending on their elastic and mechanical properties. The mammalian counterparts of arthropod tactile hairs are mechanosensory whiskers or vibrissae that also detect touch and airflow but are primarily composed of a different aECM protein, keratin. It is intriguing that, using different aECM components, both mammals and arthropods have developed similar hair-like structures throughout evolution to sense tactile cues.

In contrast, campaniform sensilla are commonly found near joints and are built to detect strains and deformations in the surrounding cuticle (Pringle, 1938; Snodgrass, 1935; Tuthill and Wilson, 2016). A distinct structural design is used to achieve this function. Each sensillum contains a round or oval-shaped cuticular dome, called the cap membrane, that is associated with a cuticular socket and a single mechanosensory neuron (Fig. 1A). The tip of the neuronal cilium is directly attached to the underside of the cap membrane (Keil, 1997; Moran et al., 1971). When the animal initiates body movements and the cuticle is strained, the cap membrane is displaced and transmits force to the underlying neuron by compressing or pinching its sensory cilium (Chapman et al., 1973; Keil, 1997; Thurm, 1964). Sensitivity of the sensillum can be influenced by the stiffness and elasticity of the socket, which affects the extent to which the cap is displaced in response to an applied force (Dinges et al., 2022; Skordos et al., 2002). As in the cuticular socket of tactile hairs, those of the campaniform sensilla also appear to contain resilin (Michels and Gorb, 2012; Weis-Fogh, 1960). Thus, by taking on different structural designs, aECM built from similar proteins can promote distinct mechanosensory functions.

2.2. Resonating matrix

A resonating matrix called the tectorial membrane overlies the mechanosensory outer hair cells in the inner ear of mammals and is known to play critical roles in hearing (Fig. 1D). Each hair cell contains a bundle of sensory protrusions called stereocilia, and the tallest stereocilium of each cell is directly embedded in the tectorial membrane (Kimura, 1966; Lim, 1972). When sound vibrations move fluid in the inner ear, the stereocilia are pushed up against this aECM layer and deflected, resulting in a mechanical shearing force between neighboring stereocilia that is converted into electrical signals. This process is central for sound amplification and hearing sensitivity and requires the tectorial membrane to provide a rigid surface for the stereocilia to act upon (Goodyear and Richardson, 2018; Gueta et al., 2006; Legan et al., 2000; Sellon et al., 2019).

The mechanical properties of the tectorial membrane arise from its composition and structure. The core of the tectorial membrane is composed of radially oriented collagen fibrils that are surrounded by striated sheets of matrix mainly composed of non-collagenous tectorin proteins (Hasko and Richardson, 1988; Killick and Richardson, 1997; Legan et al., 1997; Richardson et al., 1987; Thalmann et al., 1986, 1987). The fibrils contain type II, IX, and XI collagens – proteins that are often found in cartilage and that provide tissues with the stiffness and tensile strength for absorbing stress (Muiznieks and Keeley, 2013; Richardson et al., 1987; Slepecky et al., 1992). In the region of the tectorial membrane directly overlying the outer hair cells, the collagen fibrils become more densely packed along the apical to basal end. This change in fibril density is thought to contribute to the ten-fold increase in stiffness of the tectorial membrane along its length – a property that tunes the apical and basal ends to low and high frequency sounds, respectively (Gueta et al., 2006). The radial orientation of the collagen fibrils is also important as mutant mice with disorganized collagen fibrils exhibit defects in hearing sensitivity (Asamura et al., 2005; Gueta et al., 2011; Legan et al., 2000; Masaki et al., 2009). Precise arrangement of the fibrils not only requires the different collagen types (Asamura et al., 2005; McGuirt et al., 1999) but also depends on the presence of the tectorin-based matrix that surrounds the fibrils (Goodyear et al., 2017; Gueta et al., 2011; Legan et al., 2000). The two tectorin proteins, TECTA and TECTB, are unique to the mammalian inner ear but share ZP and zonadhesion domains with proteins from the sperm-egg adhesion system, which enable the tectorins to polymerize and bind to one another (Legan et al., 1997). Loss of TECTA and TECTB results in disorganized collagen fibrils and the complete detachment of the tectorial membrane from the outer hair cells, demonstrating the importance of tectorins in establishing aECM structure and intimate contacts with sensory endings (Legan et al., 2000). The tectorial membrane is therefore a clear example of how the precise composition and arrangement of aECM proteins is essential for establishing biomechanical properties that promote sensory function.

2.3. Nanoscale pores

Environmental access is essential for olfactory and gustatory neurons that need to capture chemical compounds from the outside world. In mammals, these sensory neurons gain access through breaks in the epithelium (Frisch, 1967; Kinnamon and Yang, 2008; Moran et al., 1982; Witt, 2019). Meanwhile, a problem arises in insects and other invertebrates, which are surrounded by a thick, impenetrable cuticle aECM that forms a physical barrier between the neurons and the outside world. To overcome this problem, the aECM of insect olfactory and gustatory sense organs contain pores that provide access to the external environment (Fig. 1B-C, Box 1). These nanoscale aECM structures are precisely built, and remarkably, each sense organ contains a distinctive number and pattern of pores.

2.3.1. Multiporous olfactory sensilla

The antennae and mouthparts, or maxillary palps, of insects are fully covered by olfactory sensilla that appear as hair-like or peg-like cuticular protrusions (Steinbrecht, 1969). Unlike mechanosensory organs, these sensilla are multiporous – each cuticular protrusion is perforated by hundreds to thousands of pores (Richards, 1952; Shanbhag et al., 1999; Shields, 2008) (Fig. 1C). However, aECM pore patterning and cuticular networks for transporting odorants are specialized and differ between three distinct morphological subtypes of sensilla: trichodea, basiconica, and coeloconica (Shanbhag et al., 1999; Shields, 2008; Steinbrecht, 1969, 1997).

Both sensilla trichodea and basiconica contain a single wall of cuticle that surrounds the olfactory sensory neuron endings. The cuticle wall contains pores that each widen to a circular chamber, or pore kettle, which bifurcates into cuticular pore tubules that extend into the fluid-filled lumen housing the sensory neuron endings (Shields, 2008; Steinbrecht, 1997). These pore tubules are thought to facilitate the inward transport of odorants into the fluid that bathes the sensory neuron endings (Shields, 2008; Steinbrecht, 1997). Although these sensilla share similar cuticular transport systems for odors, they exhibit differences in aECM pore patterning. In sensilla trichodea that mainly respond to pheromones (Chang et al., 2016; Clyne et al., 1997; Kaissling, 2014; Khallaf et al., 2021; van der Goes van Naters and Carlson, 2007), each

cuticle wall contains up to 20 pores per μm^2 in a seemingly random and irregular pattern (Shanbhag et al., 1999; Steinbrecht, 1969). Meanwhile, sensilla basiconica that respond strongly to plant odors (de Bruyne et al., 2001; Ghaninia et al., 2014; Hallem and Carlson, 2006; Lopes et al., 2002) contain pore densities as high as 100 pores per μm^2, and these pores are organized into rows along the cuticle wall (Shanbhag et al., 1999; Steinbrecht, 1969). Additionally, in flies, the pores of sensilla basiconica are four to ten times larger than those found at sensilla trichodea (Shanbhag et al., 1999). It remains unclear whether the differences in aECM pore arrangement, density, and/or size play a functional role, for example, by influencing the probability of capturing odorants and thus regulating the sensitivity of the sensilla to the odorants. However, in mutants where the cuticle of olfactory sensilla have significantly fewer pores or completely lack pores, the neurons exhibit weaker responses to a mixture of plant odors, demonstrating that pores are, at least in part, required for neuronal function (Ando et al., 2019).

Last, sensilla coeloconica contain multiple cuticular fingers that converge at the tip of the hair or peg and encase the olfactory sensory neurons, similar in appearance to a closed flower bud. Thus, two walls of cuticle – a smooth inner wall and a grooved outer wall – separate the sensory neuron endings from the external environment. Coeloconic sensilla lack pore tubules but instead contain hollow cuticular spoke channels that occur between the cuticular fingers and that extend from pores found below the grooves of the outer wall (Steinbrecht, 1969). Similar to pore tubules, these spoke channels are thought to provide a transport system for odors to the fluid-filled lumen surrounding the sensory neuron endings (Shields, 2008; Steinbrecht, 1997). It is unclear why a different structural architecture is used for odor reception, but coeloconic sensilla mainly respond to a distinct set of odors that include acids, aldehydes, and water-soluble amines like ammonia, an important cue for detecting human and animal hosts (Clyne et al., 1997; Pophof, 1997; Yao et al., 2005). Interestingly, multiporous coeloconic sensilla have also been shown to respond to humidity (Yao et al., 2005), suggesting that the structural architecture may provide a means for performing both olfactory and hygroreceptive functions.

2.3.2. Single-pore gustatory sensilla

In contrast, insect gustatory sensilla are simpler in structure – a cuticular peg or cone-like structure protrudes from the body surface and terminates with a single 10 to 200 nm pore (King and Gunathunga, 2023; Shields, 2008) (Fig. 1B). Importantly, the terminal pores are not open and permeable to all environmental compounds. The pores of gustatory sensilla near insect mouthparts contain an electron-dense plug of fibrils that overlie the sensory neuron endings (Gaffal, 1979; Shields, 1996). These fibrils are thought to confer selectivity to tastants, as they exhibit varying levels of permeability towards different metal ions (Shields, 1996). Another strategy used to regulate the permeability of the pore is observed in locusts, where the pore is physically opened and closed depending on the feeding state of the animal (Bernays and Chapman, 1972; Blaney and Chapman, 1969). When a locust finishes a meal, the foregut expands and activates stretch receptors that trigger the release of a hormone that drives closure of the pore to cease access to the environment and prevent further stimulation of the taste neurons (Bernays and Chapman, 1972). Thus, the terminal pore of insect gustatory sensilla is essential for providing access to environmental compounds through a tough, impenetrable cuticle, but entry is restricted and regulated via specialized aECM barriers.

2.4. Gel-like and viscous mucus

A viscous mucus layer in vertebrates, and a gel-like fluid called the sensillar lymph in insects, bathe the sensory neurons of olfactory and gustatory sense organs (Fig. 1E). This aECM layer is a critical interface that not only provides protection and prevents desiccation, but importantly, also actively mediates the capture and transport of odorants and tastants to the sensory cilia (Getchell et al., 1984a; Pelosi, 1996; Schmale et al., 1993). The compositions of these gel-like, mucus aECM layers have been characterized and each component contributes to specific structural and functional properties. Two major groups of proteins – mucins and odorant-binding proteins – constitute the aECM of olfactory sense organs, whereas glycoproteins and salivary proteins are found in the aECM of gustatory sense organs.

In vertebrates, mucins secreted by glial-like sustentacular cells and Bowman's glands of the olfactory epithelium contribute to the stickiness and gel-forming properties of the aECM (Foster et al., 1991; Getchell et al., 1984b; Getchell et al., 1993; Solbu and Holen, 2012). Mucins are large glycoproteins composed of a long rod-like protein backbone to which hundreds of oligosaccharide side chains are covalently attached. The carbohydrate content of these side chains and the ability of mucins to retain high volumes of water help to establish the viscosity of two distinct domains of the olfactory mucus: the thin, fluid-like top layer that overlies the neuronal cilia and the thick, gel-like bottom layer that closely surrounds them (Getchell et al., 1993; Pelosi, 1996). Meanwhile, the naked regions of the mucin glycoprotein that lack side chains crosslink with surrounding mucins via intermolecular disulfide bonds to form a stable, elastic mucus network (Getchell et al., 1993). Although the role of mucins in olfactory function has not been directly tested, the viscoelastic properties conferred by the mucins are likely to affect the rate at which odorants can diffuse through the mucus to reach the olfactory neuron endings (Cone, 2009; Getchell et al., 1984a).

Most odorants are volatile hydrophobic compounds that must cross the aqueous environment of the mucus or sensillar lymph to reach olfactory sensory neurons. To facilitate the efficient capture and transport of these hydrophobic molecules, the aECM contains odorant-binding proteins (OBPs) that act as solubilizers and carriers (Leal, 2013; Pelosi and Knoll, 2022). Vertebrate and insect OBPs include two classes of structurally distinct proteins that each contain a unique, characteristic hydrophobic binding pocket for capturing and encasing odorant molecules (Bianchet et al., 1996; Pelosi and Knoll, 2022; Sandler et al., 2000). OBPs are secreted by nasal glands in vertebrates and by support cells that wrap the sensory neuron endings in insects, and they are distributed throughout the mucus and sensillar lymph (Briand et al., 2002; Laue et al., 1994; Pevsner et al., 1986, 1988; Shanbhag et al., 2001; Steinbrecht et al., 1992). Some OBPs have been found to be exclusively expressed in a specific morphological class of olfactory insect sensilla, suggesting that OBPs may contribute to the selective detection and tuning of these sensilla to distinct sets of odorants (Laue et al., 1994; Shanbhag et al., 2001; Steinbrecht et al., 1992). After OBPs encounter and bind to an odorant, the OBP-odorant complex diffuses through the aqueous environment to reach and activate receptors on the cilia of the olfactory neurons. Electrophysiological and behavioral studies have shown that olfactory neurons in *Drosophila* and mosquitoes, respectively, no longer respond to a male-specific pheromone or an odorant found in human sweat when the corresponding OBP partner is nonfunctional or absent (Biessmann et al., 2010; Pelletier et al., 2010; Xu et al., 2005). These results indicate that insect OBPs are necessary for olfaction. In vertebrates, ligand-binding assays have demonstrated that vertebrate OBPs of the olfactory mucus are capable of binding to diverse odorants (Briand et al., 2002; Pelosi et al., 1982; Pevsner et al., 1986), but whether they are required for olfactory neuron function remains unclear (Pelosi and Knoll, 2022).

Similarly, the gustatory sense organs of mammals – the taste buds – are also filled with a viscous mucus layer of aECM that tastants must diffuse through to reach the gustatory cells for detection (de Lorenzo, 1958; Kinnamon and Yang, 2008; Schmale et al., 1993). Initial observations with gold-labeled thaumatin, a sweet tasting protein, suggested that this mucus aECM is sticky and aids in capture of tastants, as the gold-labeled protein remained bound to taste buds even after multiple washing steps (Farbman et al., 1987). The stickiness and gel-forming properties arise from the carbohydrate content of the glycoproteins that make up the mucus, which is unique across taste buds of different species, and from the stabilization and crosslinking of glycoproteins via

disulfide bonds (Witt, 1996; Witt and Reutter, 1988). They are thought to be secreted by glial-like type I cells, which contain electron-dense granules with similar carbohydrate composition and binding properties (Ohmura et al., 1989). Taste buds are also bathed in salivary proteins: proline-rich proteins and proteins from the lipocalin superfamily that bind and transport hydrophobic molecules (Glendinning, 1992; Schmale et al., 1993). The proline-rich proteins have a high affinity for tannins, which produce the bitter and astringent taste that results from eating unripe fruit (Asquith et al., 1987). Changes in the level of salivary proline-rich proteins can modulate the response of mice to tannins, where high levels decrease aversion to tannins by potentially sequestering and decreasing the concentration of free tannins that are available for capture by taste cells (Glendinning, 1992). Meanwhile, lipocalin-like proteins secreted from the von Ebner's salivary glands show high sequence similarity to secreted olfactory-binding proteins (Schmale et al., 1993). This suggests that mucus aECM surrounding both gustatory and olfactory sense organs may be functionally similar and play a role in capturing and transporting compounds to their associated sensory cells.

2.5. Optical lenses

aECM components that form the tough, hardened cuticles, exoskeletons, and shells of arthropods and mollusks are not only used to build these protective armors but, surprisingly, have been recruited to construct the lenses of primitive eyes – the visual sense organs found in several of these species. The primary function of the lens is to provide an interface that transmits and focuses light from the outside world onto the photosensitive tissue located in the back of the eye. Components used to build this structure must therefore possess critical optical properties that allow for light refraction, transparency, and minimal light scattering (Bassnett et al., 2011; Hejtmancik et al., 2015). However, the lenses of some arthropods and mollusks, such as horseshoe crabs and chitons, respectively, also require resilience and protection to withstand exposure to both air and underwater environments. These optical and protective functions are ultimately achieved by integrating cuticle and shell components into lenses and modifying their structure and organization (Li et al., 2015; Spaeker et al., 2022). For example, in the lateral pair of eyes on the horseshoe crab, an array of lenses forms inward-projecting cone structures that are composed of cuticular chitin-protein fibers. The chitin-protein fibers are organized in a helicoid arrangement around the cone and create a lamellated appearance, where the center of the cone contains lower volumes of chitin and water compared to the surrounding edges. This change in composition is thought to contribute to the refractive index gradient of the lens, which is highest in the center and decreases at the edges and serves to improve the focusing of light onto sensory cells (Spaeker et al., 2022). Meanwhile, the hundreds of eyes that dot the shell surface of chiton contain lenses built from the mineral aragonite (Speiser et al., 2011). Compared to the arrangement of the aragonite crystals in the surrounding non-sensory regions of the shell, the aragonite in the lens is highly aligned and uniform and, as a result, minimizes light scattering. Although the modification of aragonite structure and the presence of soft photosensitive tissues supports visual function, there is a trade-off – the lens is less durable and cannot withstand as much mechanical force as the surrounding shell (Li et al., 2015). Additional studies have shown that cuticular proteins are also abundantly expressed in the eyes of mosquitoes and *Drosophila*, although their functions remain largely unclear (Kim et al., 2008; Komori et al., 1992; Stahl et al., 2017; Zhou et al., 2016). However, a recent study revealed that a ZP domain protein, Dusky-like, is transiently expressed on the apical surface of lens-secreting cells in *Drosophila* to maintain cell surface area and to organize chitin and other secreted aECM proteins – all of which are required for forming the distinct curved shape of each lens (Ghosh and Treisman, 2024). Overall, these examples demonstrate that by simply modifying their organization and composition, the same aECM components can be used to build sensory structures that have entirely different functional properties specialized for detecting mechanical versus visual cues.

2.6. Summary

The diversity of aECM found across sense organs in the animal kingdom illustrates that aECM is integral to the structure and function of sense organs. To fulfill these roles, aECM is intricately patterned to provide an appropriate interface between a specific sensory cell type and the outside world (Fig. 1) – a connection that is central to sensory function. Such intricate patterning must involve a highly regulated process that controls the precise proportion of aECM components used and how these aECM components are deposited. While the cellular and molecular mechanisms that pattern these specialized aECM structures remain elusive, these examples show the power of using sense organs as a model to study specialized aECM patterning.

3. *C. elegans* cuticle as a model for sense organ aECM patterning

The cuticle of the nematode *C. elegans* provides a genetically powerful model for aECM patterning (Cohen and Sundaram, 2020; Sundaram and Pujol, 2024). It is a highly structured, flexible aECM layer that serves as a barrier between the animal and the outside world and is important for maintaining body shape and supporting locomotion. The cuticle is primarily secreted by the underlying epithelium, which includes hypodermal and seam cells (Page and Johnstone, 2007). The first cuticle is synthesized during late embryogenesis, and as animals enter each subsequent larval stage, they undergo a molting process: the old cuticle is shed off and replaced with a new, specialized cuticle whose composition and structure is unique to that developmental stage (Cox et al., 1981b). This process is repeated a total of four times as animals transition through each of four larval stages (L1, L2, L3, and L4) and eventually reach the adult stage. Prior to each molt, a distinct set of genes that are required for the assembly of the new cuticle is transiently switched on. The synthesis of aECM components therefore oscillates with the molting cycle, although the relative abundance and temporal expression of each gene is different and changes across life stages (Frand et al., 2005; Hendriks et al., 2014; Johnstone, 2000; Johnstone and Barry, 1996). These aECM components mainly include collagens and non-collagenous cuticlins (Cox et al., 1981a; Johnstone, 2000; Sapio et al., 2005; Sebastiano et al., 1991). Whereas collagens represent over 80% of the protein content of cuticle, cuticlins are the heavily cross-linked, insoluble proteins that remain after treatment of nematode cuticles with strong detergents and reducing agents (Cox et al., 1981a; Fujimoto and Kanaya, 1973; Page and Johnstone, 2007). Most studies have focused on characterizing aECM genes that are required for maintaining cuticle integrity and that contribute to the formation of prominent circumferential and longitudinal ridges found along the surface of the animal (annuli and alae, respectively) (Adams et al., 2023; Forman-Rubinsky et al., 2017; Katz et al., 2022; McMahon et al., 2003; Page and Johnstone, 2007; Sandhu et al., 2021; Sapio et al., 2005; Sundaram and Pujol, 2024; Thein et al., 2003). However, less is known about the cuticle aECM that is associated with sense organs of *C. elegans* and how these regions differ from the surrounding non-sensory cuticle.

Importantly, similar to the aECM of sense organs in other animals, the *C. elegans* cuticle is closely associated with the endings of neurons and glia found in sense organs of the head, midbody, and tail. This includes the 2 amphid (AM), 4 cephalic (CEP), 6 inner labial (IL), and 6 outer labial (OL) sense organs in the head; 2 anterior and 2 posterior deirids (ADE/PDE) in the midbody; and 2 phasmid (PH) sense organs in the tail (Altun and Hall, 2010; Ward et al., 1975). There are additional male-specific sense organs in the adult male tail, including 9 bilateral pairs of rays, a sensory hook, a pair of spicules, and a pair of post-cloacal sensilla (Lints and Hall, 2009; Sulston et al., 1980). Each sense organ typically contains one or more neurons associated with two supporting glial cells, called the sheath and socket glia cells (Box 1). Each neuron extends its ciliated sensory ending towards the surface of the animal,

where the cilium makes direct contact with the overlying cuticle aECM. The sheath and socket glia also extend their processes towards the surface, where they form a cellular tube that wraps around the distal portion of the neuronal sensory ending(s). It is clear from electron microscopy images that the cuticle overlying each of these neurons and glial cells is different (Fig. 2). Intriguingly, the annuli ridges that span the length of the animal abruptly cease at the nose tip, where sensory neurons of the head contact the cuticle (Fig. 2B).

In the region immediately overlying a sense organ, the cuticle is specifically tailored to its sensory function (Sulston et al., 1980; Ward et al., 1975; Ware et al., 1975) and can take on three different forms: closed sheet, narrow pore, and wide pore. Sense organs with a single ciliated mechanosensory neuron (OL, ADE/PDE, male post-cloacal sensilla, hermaphrodite CEP) are associated with a closed sheet of cuticle, with the entire cilium of the mechanosensory neuron directly embedded in the cuticle to sense external forces (Altun and Hall, 2010; Ward et al., 1975) (Fig. 2G-K). By contrast, sense organs that have a mechanosensory and chemosensory neuron pair (IL, male rays and hook, male CEP) also form a closed sheet of cuticle around the cilium of the mechanosensory neuron; however, the chemosensory neuron protrudes through a narrow pore in the cuticle that opens to the external environment and provides access to environmental cues (Sulston et al., 1980; Ward et al., 1975). These nanopores consistently appear in the same position in the cuticle across animals and are lined by a layer of electron-dense material that is distinct from the surrounding aECM (Fig. 2C, E-F). Finally, sense organs that contain two or more chemosensory neurons (AM, PH, male spicules) are associated with a large pore that multiple neurons protrude through to access the environment. For example, the wide cuticle pore of the amphids – the major and most well-studied sense organ of *C. elegans* – accommodates ten cilia of eight chemosensory neurons that are involved in olfaction and gustation (Ward et al., 1975) and, unlike the narrow pores, it is not lined by a layer of electron-dense material (Fig. 2B). Despite these ultrastructural differences in the cuticle across sense organ types, little to no information is known about their protein composition and how they are patterned.

A wealth of genetic tools and techniques can be used to investigate aECM patterning in *C. elegans*. First, existing cell-type-specific promoters enable visualizing and targeting neurons and glia of specific sense organs (Fung et al., 2023; Taylor et al., 2021). Second, single-cell RNA sequencing datasets for different cell types across multiple life stages of *C. elegans* are publicly available (Cao et al., 2017; Packer et al., 2019; Purice et al., 2023; Taylor et al., 2021). These datasets can be mined to identify genes that are differentially expressed by neurons and glia associated with a closed sheet versus a narrow or wide pore and that are likely to influence cuticle patterning, such as aECM proteins (i.e. collagens, cuticlins) and enzymes that break down aECM (i.e. matrix metalloproteinases). Notably, some evidence suggests that the glial cells express and secrete matrix materials (Altun and Hall, 2010; Bacaj et al., 2008; Perens and Shaham, 2005; Ward et al., 1975), but it remains unclear whether and, if so, how these glial secretions affect the overlying cuticle. Finally, *C. elegans* has facile genetics, enabling large-scale unbiased screens to identify mutants that disrupt any aspect of aECM protein expression or localization. In a typical approach, a fluorescent reporter strain is subjected to random chemical mutagenesis and grown for two generations to produce mixed pools with thousands of animals, each bearing unique homozygous mutations. Then, these progeny are visually screened to isolate mutants that exhibit altered appearance of the fluorescent reporter. Such screens can be performed at genomic scale by a single investigator in a matter of weeks.

Recently, we used these approaches to identify a transcriptional program in a single glial cell that is necessary and sufficient to pattern the cuticle aECM of a specific sense organ into a nanoscale pore (see Box 2).

4. Potential mechanisms for patterning aECM at sense organs

As described in the examples above, aECM structures associated with sense organs are assembled with remarkable precision: specialized aECM structures are formed at specific positions on the animal surface, contain macromolecules that are directionally oriented or organized into a distinct arrangement, have a particular proportion of protein and water content to achieve specific viscoelastic properties, and form precise contacts with sensory neuron endings. How are such specialized and intricate structures formed? While aECM patterning at sense organs remains largely unexplored, concepts from studies of the luminal aECM of tube-shaped organs and ECM that localizes to the basal (inward-facing) surface of an epithelium provide a framework for possible mechanisms (Fig. 3).

4.1. Transient matrix

As aECM is a heterogeneous, multi-layered network of macromolecules, the prevailing model is that aECM components are secreted in a stepwise manner, where components that are secreted first become the top outermost layer. This model assumes that secreted aECM components are permanently incorporated into the final mature structure. However, there is growing evidence that a transient matrix is secreted prior to synthesis of the stable, mature aECM (Cohen and Sundaram, 2020; Sundaram and Pujol, 2024). Several ZP domain proteins, lipocalin-related proteins, and the polysaccharide chitin have been observed to transiently appear on the luminal surfaces of tube-shaped organs, including the excretory tube and vulva of *C. elegans* and the trachea of *Drosophila*, during early stages of morphogenesis. However, these matrix components are subsequently degraded and not incorporated into the final mature aECM that lines these tube-shaped organs once morphogenesis is complete (Cohen et al., 2020; Cohen and Sundaram, 2020; Gill et al., 2016; Sundaram and Pujol, 2024; Tonning et al., 2005). Despite its short-lived expression, the transient matrix is important for proper organ development and has been proposed to serve as a temporary scaffold or template for assembling the newly synthesized aECM (Cohen et al., 2020; Gill et al., 2016). This is driven by observations that loss of individual proteins results in misshapen or fragmented tubes and disrupts the localization and clearance of other transient aECM proteins (Cohen et al., 2020; Cohen and Sundaram, 2020; Gill et al., 2016; Sundaram and Pujol, 2024; Tonning et al., 2005). Furthermore, a transient matrix not only plays important roles in tube-shaped organs but also appears between the old and new cuticle of *C. elegans* during the L4 to adult molt to pattern the longitudinal alae ridges that run along the lateral surface of the animal (Katz et al., 2022). Likewise in the wing cuticle and lens of *Drosophila*, ZP domain proteins form templates or scaffolds that organize and instruct the deposition of newly secreted proteins and subsequent aECM layers, as their absence leads to cuticle defects that result in misshapen wings and lenses (Ghosh and Treisman, 2024; Sobala and Adler, 2016). It is therefore likely that the assembly of aECM at sense organs also depends on a transient matrix. This hypothesis is supported by our observations of GRL-18 (Box 2). At sense organs, a transient matrix could potentially anchor and restrict proteins to a designated region so that specialized aECM structures are built in the correct position, build temporary placeholders that prevent protein deposition in valleys or pores of the future aECM, and/or hold the newly synthesized aECM in place as stable attachments are formed between the aECM and sensory neuron endings.

4.2. Physical scaffolds

Precise aECM shapes can be sculpted by cellular protrusions and proteins that act as temporary physical scaffolds or placeholders (Fig. 3, left). For example, studies have shown that cellular protrusions are used to sculpt the hair-like sensilla of insects that house chemosensory and mechanosensory neurons. During development of these sensilla, an

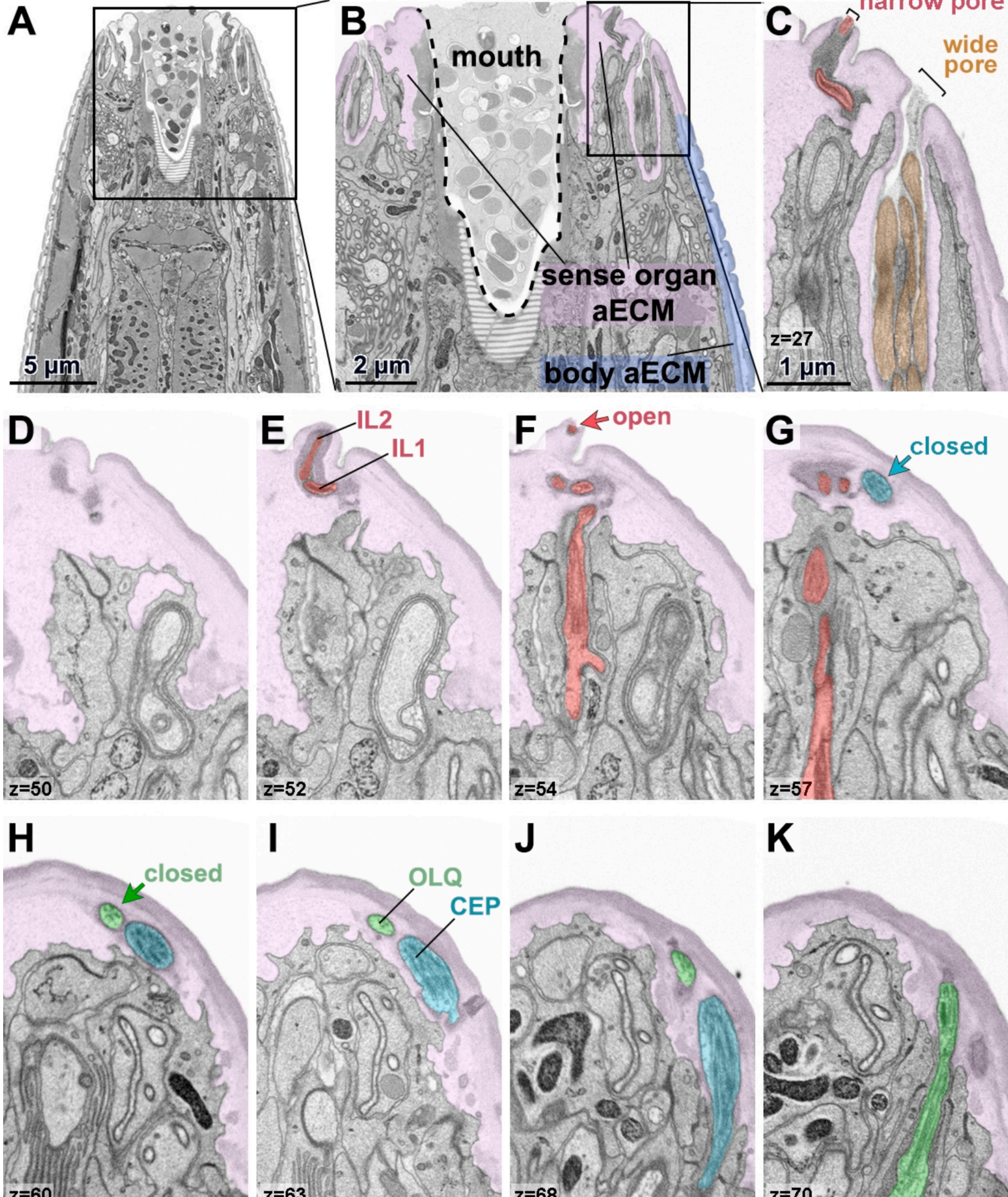


Fig. 2. *C. elegans* sensory neurons associate with distinct aECM structures.
Longitudinal serial thin sections of an adult hermaphrodite *C. elegans* prepared by array tomography scanning electron microscopy (AT-SEM) as described in Fung et al. (2023). (A) Overview of head. Nose is up. (B) Magnification of boxed area in (A). Mouth opening is filled with bacterial food. Body cuticle aECM (pseudocolored blue) forms circumferential rings, or annuli, that appear as evenly spaced ridges when sectioned. A distinct sense organ cuticle aECM (pseudocolored purple) lacking annuli covers the nose tip. (C) Magnification of boxed area in (B). Ciliated endings of three chemosensory neurons (orange) of the amphid sense organ protrude through a tube formed by the amphid socket glial cell that is lined with cuticle aECM and terminates in a relatively wide pore (~500 nm) in the cuticle aECM. By contrast the ciliated ending of a single IL2 chemosensory neuron (red) of the inner labial sense organ protrudes through a narrower pore (~200 nm) in the cuticle aECM. (D-K) Selected serial sections at the indicated z-planes, with each z-plane corresponding to a section of ~80 nm thickness. Scale is the same as (C). The ciliated dendritic endings of the IL1 and IL2 sensory neurons (*E*-G, red) associate with a distinctive aECM cuticle dome that terminates in an open pore (F) through which the IL2 cilium protrudes directly into the external environment. By contrast, the mechanosensory ciliated dendritic endings of the OLQ (H-K, green) and CEP (G-J, blue) neurons are embedded within a closed sheet of cuticle aECM that presumably transduces external force. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Box 2
Using *C. elegans* to identify determinants of sense organ aECM patterning.

C. elegans offers a powerful system with which to identify mechanisms that confer distinct aECM composition and patterning to different sense organs. We focused on the CEP sense organ, because its overlying cuticle aECM undergoes an interesting sex-specific switch: in hermaphrodites, the aECM is patterned into a solid sheet, whereas in males it forms a nanoscale pore that allows the sensory cilium of a male-specific neuron to protrude directly into the external environment. To identify mechanisms that control sense organ aECM patterning, we took advantage of experimental strengths of *C. elegans*. These steps provide a general workflow for characterizing aECM in *C. elegans*.

Use of gene expression databases to identify sense organ-specific aECM proteins

Drawing on the extensive literature on *C. elegans* gene expression and recent comprehensive single-cell transcriptomic studies (Cao et al., 2017; Hao et al., 2006; Packer et al., 2019), we noted that socket glia express many sense organ-specific putative aECM proteins. We identified the secreted protein GRL-18 and the collagens COL-53 and COL-177 as highly specific to socket glia of the IL sense organs (Fung et al., 2020, 2023), suggesting they may contribute to sense organ-specific aECM. The aECM of the IL sense organs is patterned as a nanoscale pore in both sexes. We found that adult males initiate GRL-18 expression in additional glia: the CEP socket glia and male-specific tail glia (Fung et al., 2023). Intriguingly, aECM of these male sense organs also forms a nanoscale pore, suggesting GRL-18 may be part of a gene expression module that patterns the aECM into a nanoscale pore.

Tagging and visualization of aECM proteins in vivo

Next, we took advantage of rapid genome editing available in *C. elegans* and its amenability to live microscopy as a small transparent animal. We engineered a fluorescent tag into the endogenous GRL-18 protein and visualized the localization of this protein in males as the new cuticle is patterned. We found that GRL-18 localized to transient rings near the glial endings, appearing as the new cuticle is synthesized, and then quickly disassembling as the new cuticle matures. These distinctive structures that were specific to the time and location where aECM pores form suggested that GRL-18 may play a direct role in aECM patterning, likely together with other factors.

Genetic manipulation of aECM patterning

Finally, we took advantage of the large collection of publicly available *C. elegans* mutant strains, the power to quickly isolate new mutants, and the ability to manipulate the sex identity of individual cells as parallel approaches to alter the sex-specific expression of GRL-18 in CEP socket glia. We found that we could prevent GRL-18 expression in CEP socket glia in males or force its expression in hermaphrodites, either by mis-expressing the known sex identity genes *tra-2* and *fem-3* (Lee and Portman, 2007; Mehra et al., 1999; Mowrey et al., 2014; Sammut et al., 2015; White et al., 2007) or by using any of several mutants that disrupt glial sex identity that we identified in candidate and unbiased genetic screens: *mab-3* and *jmjd-3.1,* which prevent GRL-18 expression in male CEP socket glia; and *nfya-1* and *bed-3,* which force male-like expression in hermaphrodites. Electron microscopy of these mutants revealed that male-specific gene expression in the CEP socket glial cell is necessary and sufficient to pattern the overlying aECM into a nanoscale pore.

epidermal cell known as the trichogen cell extends a single microtubule- and actin-based process and secretes cuticle. This trichogen process acts as a mold for the newly secreted cuticle – similar to how molds are used for casting metals and plaster – and once the cuticle hardens and stabilizes, the process retracts and leaves behind a fluid-filled hair-like structure that neuronal sensory endings subsequently occupy (Ando et al., 2019; Keil, 1997; McIver, 1975; Steinbrecht, 1997). The multiple cuticular fingers found in coeloconic chemosensory sensilla are similarly formed, except the trichogen cell extends multiple processes instead of one (Steinbrecht, 1997). Cellular protrusions can therefore act as physical scaffolds for shaping aECM structures that are several microns in length.

But, what about nanoscale aECM structures that are 1000 times smaller? Cuticle pores found in chemosensory organs of insects and *C. elegans*, for example, have diameters ranging from 10 to 200 nm (Shields, 2008; Ward et al., 1975). However, cellular protrusions are built from multiple actin filaments and microtubules that are each approximately 7 nm and 25 nm in diameter, respectively (Cooper, 2000a, 2000b). It is therefore unclear whether nanoscale pores – especially ones as small as 10 nm in diameter – can be formed from actin- or microtubule-based cellular protrusions. A potential alternative is to use transient aECM proteins to form a physical plug that holds the space open for a future aECM pore as the new cuticle is being synthesized. Once the cuticle stabilizes, the transient aECM proteins will be degraded and will leave behind an open pore structure in the cuticle (Fung et al., 2023).

4.3. Mechanical pulling and pushing forces

aECM is known to play important mechanical roles during the morphogenesis of sense organs (Chong et al., 2021; Chung et al., 2001; Heiman and Shaham, 2009; Low et al., 2019). Likewise, the assembly of the developing aECM itself can also be regulated by mechanical forces exerted by underlying support cells (Fig. 3, center). Pulling forces can be transmitted to the aECM via connections between the actin cytoskeletal networks of the underlying cells and components of the aECM. For example, the current model for patterning the alae ridges of the adult *C. elegans* cuticle involves connections between longitudinal actin filaments of the epidermal seam cell and the aECM. When the seam cell sinks downward, this generates a pulling force that tugs on the matrix components overlying the longitudinal actin filaments and leads to the formation of downward valleys (Katz et al., 2022). The size, shape, and number of actin filaments present may also affect aECM patterning, as studies on *Drosophila* sensory bristles revealed that increasing the number and size of actin filament bundles results in stiffer, straighter bristles, whereas flattening and decreasing the size of actin filament bundles results in flaccid, twisted bristles (Overton, 1967; Tilney et al., 1995, 2004). Another noteworthy example is observed in the ECM of connective tissues, where specialized fibroblast cells secrete fibronectin glycoproteins and assemble them into filaments via contractile forces. Secreted fibronectin proteins are bound to receptors on the apical surface of fibroblasts, which are coupled to intracellular proteins that connect the fibronectin to actomyosin networks. When contractile forces are generated, fibronectin is stretched and unfolded, revealing binding

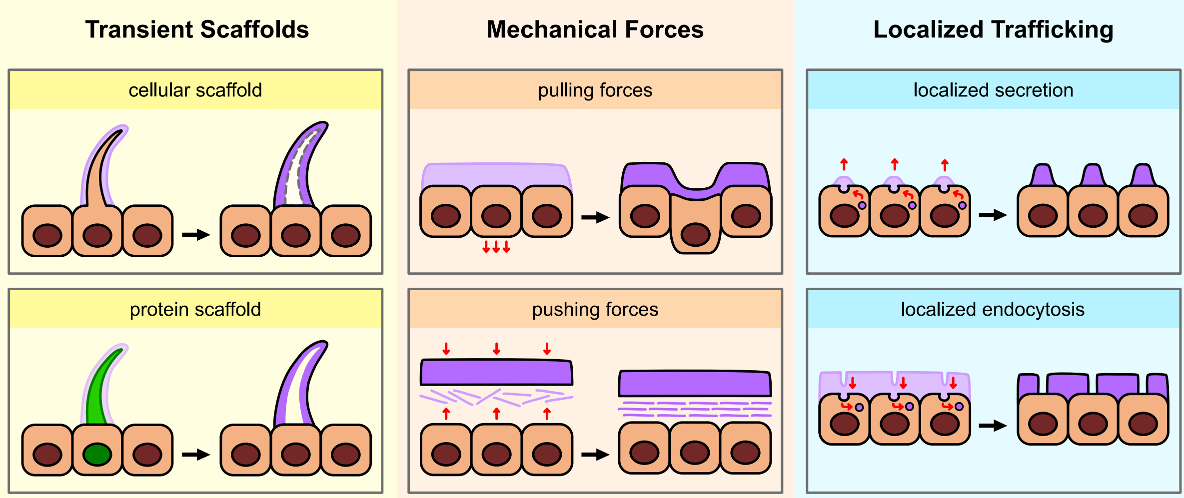


Fig. 3. Mechanisms of aECM patterning.
Studies of *C. elegans* and *Drosophila* aECM patterning suggest three general mechanisms that may act in various combinations to sculpt matrix structures. First, transient scaffolds formed by cellular protrusions or matrix proteins can serve as physical placeholders to pattern the deposition of stable matrix components but are themselves removed once aECM synthesis is complete. Second, mechanical forces from actomyosin-mediated contraction of the cell surface (pulling) or from external tissues (pushing) can organize aECM components during synthesis. Third, localized secretion or endocytosis of matrix components can build up or remove aECM in a spatially organized pattern.

sites that facilitate interactions between fibronectin to drive filament assembly (Baneyx et al., 2002; Singh et al., 2010; Zhong et al., 1998). Mechanical pulling forces can therefore shape aECM by physically moving and displacing regions of the matrix or by driving conformational changes in aECM proteins. Meanwhile, pushing forces may also play a role by compressing aECM components and forcing them to organize into a given orientation. Such pushing forces may be acting in the tectorial membrane when collagens are secreted into a narrow region between epithelial cells and an overlying layer of tectorin-based matrix. Confinement and compression of the collagen fibrils by the surrounding epithelial and matrix layers can mediate their alignment into a radial orientation (Goodyear et al., 2017). Ultimately, this demonstrates that the developing aECM is malleable to both pulling and pushing forces exerted by surrounding cells.

4.4. Localized secretion and endocytosis

aECM components can be targeted to distinct regions via localized secretion by cells (Fig. 3, right). Localized secretion can be achieved via the formation of structures that serve as corrals to establish sites of aECM production and release. In the trachea of *Drosophila*, rings of actin filaments are used to concentrate chitin synthase – an enzyme that catalyzes chitin formation and facilitates its transport across the plasma membrane – to regularly spaced intervals in the apical region of epithelial cells. This leads to the deposition of chitin into stripes above the actin rings, resulting in folds in the aECM that provide mechanical strength to the trachea (Öztürk-Çolak et al., 2016). Specialized fibroblast cells of connective tissues also grow temporary cellular protrusions called fibripositors that traffic and direct collagen to extracellular channels formed by contacts between adjacent cells (Canty et al., 2004). In the mammalian inner ear, the tectorin protein TECTA is tethered and restricted to the tip of microvilli protrusions on the apical surface of epithelial cells. There, TECTA captures collagen fibrils and, upon release, crosslinks with surrounding TECTA-collagen complexes to form a highly organized, evenly spaced matrix in the developing tectorial membrane (Niazi et al., 2024). Localized secretion coupled to cell movement is also used to produce a polarized orientation of ECM fibrils in the developing *Drosophila* egg chamber (Isabella and Horne-Badovinac, 2016). In addition to localized secretion, extracellular proteins can potentially organize into structures that could selectively retain secreted aECM components in a designated region, like a corral (Cohen et al., 2021; Fung et al., 2023). Finally, cells can locally secrete enzymes, known as matrix metalloproteinases, to degrade specific parts of the matrix. This has been shown to play important roles in remodeling the surrounding ECM to allow for cell migration and shape changes (Agarwal et al., 2022; Stamenkovic, 2003; Vu and Werb, 2000). Together, these examples suggest that localized secretion of specific matrix components can be used to establish distinct structural domains in the aECM.

Instead of secreting and adding components to the aECM, cells can also do the opposite and use localized endocytosis to internalize and remove parts of the aECM (Fig. 3, right). For instance, endocytosis can be used to pinch away or carve out valleys and pores in the overlying aECM. Evidence for this mechanism has been observed in nanopore formation in the olfactory sensilla of *Drosophila*. The cuticle of each developing olfactory sensillum has a wavy appearance and these curves coincide with underlying plasma membrane structures associated with endocytic vesicles. Notably, the positioning of these vesicles resembles the highly regular spacing found between nanopores in the mature structure. It was also revealed that an endosomal protein, Gore-tex/Osiris23, is required for pore formation as *gore-tex* mutants have olfactory sensilla with a smooth cuticle that lacks pores (Ando et al., 2019; Sun et al., 2024). Localized endocytosis therefore appears to be important for establishing curvature and serves as another cell-mediated mechanism for carving out precise nanostructures in the aECM.

4.5. Self-assembly

Lastly, aECM components could in principle organize via self-assembly. Self-assembly is a cell-independent process, in which the components alone contain all the necessary information required to spontaneously assemble into a given structure (Kushner, 1969; Marshall, 2020; Misteli, 2001). This was first observed in simple spherical viruses whose shells or capsids are composed of identical protein subunits that, once synthesized, associate together in a regular pattern to build a polyhedral shape (Kushner, 1969). For aECM components, one major protein – collagen – has been shown to self-assemble into fibrils in vitro. These collagen fibrils remarkably exhibit the same cross-striated pattern as those found in connective tissues, although additional cell-mediated inputs appear to be required to specify the exact size and orientation of these fibrils in vivo (Kadler et al., 1996; Revell et al., 2021). It is thought that once collagens are synthesized, secreted into the extracellular environment, and processed by matrix metalloproteinases via proteolytic cleavage, the non-helical domains that remain after cleavage allow for intermolecular interactions between collagen molecules to drive fibril formation (Gelse et al., 2003; Revell et al., 2021). Simple structures composed of a single protein can therefore be constructed based on its intrinsic properties alone.

However, this mechanism can also be used to build more complex structures that are composed of several different proteins. This includes bacteriophage viruses, whose capsids are attached to a tail that injects genetic material into the host and tail fibers that facilitate binding to the host surface. The capsid, tail, and tail fibers are assembled independently and subsequently joined together in a precise stepwise manner (Wood, 1980). This highlights that self-assembly involves strict temporal regulation, which importantly, is also required for proper aECM patterning, as demonstrated by how cells express and secrete aECM components at distinct timepoints to build complex, highly organized matrices (Cohen et al., 2020; Fernandes et al., 2010; Goodyear and Richardson, 2018; Johnstone, 2000). Recent studies have proposed phase separation as a potential mechanism by which certain aECM components exclusively expressed during *C. elegans* cuticle patterning can self-assemble into specialized structures, including the chitinous cuticle that lines the mouth and the critical columnar structures that connect the outer and inner cuticle layers of the body (Adams et al., 2023; Kamal et al., 2022). Furthermore, the self-assembly process can also involve nonstructural accessory proteins that temporarily serve as scaffolding proteins, proteolytic cleavage enzymes, and/or stabilizing proteins, but notably, are not incorporated into the final structure (Wood, 1980). These nonstructural accessory proteins are therefore very similar to transient aECM proteins that were previously discussed. Altogether, the parallels between features of self-assembling components and aECM components suggest that self-assembly processes may help contribute to building specialized, mature aECM structures.

4.6. Summary

If sense organs are the doors and windows to the world, then aECM is the doorknob, keyhole and window shade. Precise patterning of aECM overlying each type of sensory neuron determines which stimuli will reach it, and how each stimulus will be perceived. *C. elegans* offers a powerful model for identifying the mechanisms that control aECM patterning, many of which may be shared with mechanisms of aECM patterning in other tissues, as described here. It is important to note that the potential mechanisms discussed above are not mutually exclusive. Indeed, how these mechanisms are coordinated and used to build precise nanoscale structures and distinct functional domains in the aECM surrounding sense organs represents a major unexplored frontier in sense organ biology.

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