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REVIEW

The mechanical forces that shape our senses

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ABSTRACT

Developing organs are shaped, in part, by physical interaction with their environment in the embryo. In recent years, technical advances in live-cell imaging and material science have greatly expanded our understanding of the mechanical forces driving organ formation. Here, we provide a broad overview of the types of forces generated during embryonic development and then focus on a subset of organs underlying our senses: the eyes, inner ears, nose and skin. The epithelia in these organs emerge from a common origin: the ectoderm germ layer; yet, they arrive at unique and complex forms over developmental time. We discuss exciting recent animal studies that show a crucial role for mechanical forces in, for example, the thickening of sensory placodes, the coiling of the cochlea and the lengthening of hair. Finally, we discuss how microfabricated organoid systems can now provide unprecedented insights into the physical principles of human development.

KEY WORDS: Mechanobiology, Sensory development, Neural crest, Placodes, Stem cells, Organoids

Introduction

Embryonic tissues undergo drastic size and shape transitions to create organs with specialized forms and functions. The morphing of tissue shapes and the movement of cells are inherently physical; however, molecular and biochemical mechanisms dominate our understanding of these processes. An emerging body of research from the past decade has demonstrated that mechanical forces play essential roles in shaping developing tissues in addition to biochemical signaling. The forces instruct tissue composition and cell behavior, thus molding organ structure over developmental time. In particular, the peripheral sensory organs undergo dramatic morphological changes during development, such as the coiling of the inner ear's cochlea, in which physical forces play an integral role. Recent advances in state-of-the-art microscopy and physical manipulation techniques have resulted in several seminal studies addressing how mechanical forces, coupled with biochemical mechanisms, explain how sensory organs achieve their distinctive shapes and forms.

Several thorough reviews have discussed models exploring mechanical forces in development (Heisenberg and Bellaïche, 2013; Irvine and Shraiman, 2017; Stooke-Vaughan and Campàs, 2018). Here, we begin with an overview of the basic mechanics of living systems with examples from various species. We then discuss how specific mechanical forces shape tissues during sensory organ development, focusing on the lens, nose (olfactory system), inner ear and skin – organs that have been the subject of notable publications on mechanoregulatory mechanisms. Lastly, we explain why understanding these principles is essential for designing artificial systems and how organoid models can help provide further information on these processes to bridge the gap between model organisms and human biology.

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The mechanics governing morphogenesis

Mechanical forces can influence tissue morphogenesis at all scales. Motor, cytoskeletal and mechanosensing proteins enable cells to sense (Table 1) and generate (Table 2) forces that can propagate across the tissue and enact changes in movement, shape and organization. We briefly describe these mechanisms below and provide a glossary of terms in Box 1.

The force-generating machinery of the cell

Cells generate forces through contractile (see Glossary, Box 1) actomyosin networks. The versatile cross-linking regulation of the actin cytoskeleton network can give rise to different subcellular structures (such as supracellular cables, lamellipodia and filopodia). Cells use these structures to generate contraction, pushing or pulling forces in the pN to nN range and to bear tension. A single actin filament generates a force of up to 2 pN (Footer et al., 2007). Interconnected actin cables that generate forces between cells are collectively termed 'supracellular cables', which can both generate forces or bear tension (see Glossary, Box 1). For example, supracellular cables can form circumferential structures termed purse-strings (Schwayer et al., 2016), which can bear tensile stresses in the range of 100 Pa (Brugués et al., 2014; Le et al., 2021). Lamellipodia are broad, sheet-like membrane extensions with submicron thickness. They are enriched with branched actin filaments and devoid of actomyosin stress fibers. Filopodia are finger-like membrane extensions consisting of linear, polymerized actin fibers. Lamellipodia at the front of migrating cells can generate ∼20 pN pushing forces (equivalent to stresses of 50-100 Pa) (Brugués et al., 2014; Le et al., 2021), and filopodia can generate a force of ∼3 pN (Cojoc et al., 2007). Intermediate filaments and microtubules can also contribute to force generation in some cell types (Lim et al., 2020; Vleugel et al., 2016), but the mechanisms are not generalizable across all cell types.

Mechanical properties of cells and tissues

Anisotropy (see Glossary, Box 1) in the distribution of contractile actomyosin networks provides cells with mechanical properties; the most typical examples are cell stiffness, which is regulated by the actin cortex (Chugh and Paluch, 2018), or the fluctuation of myosin contractile tension, which determines cell shape and polarity (Heisenberg and Bellaïche, 2013; Lecuit et al., 2011). Therefore, whole tissues can behave as a material with intrinsic physical properties (Stooke-Vaughan and Campàs, 2018), influenced by the cell's cortical actomyosin contractility, cell adhesion, extracellular

Table 1. Force-sensing mechanisms

	Cell scale			Tissue scale			
Stage of morphogenesis	Cell-cell adhesion	Cortical contractility and junctional tension	Cell shape (strain)	Physical constraint (e.g. neighboring tissue, extracellular matrix)	Environment stiffness	Hydraulic pressure	Internal stress
Formation of placode compartments	Breau and Schneider-Maunoury, 2014; Theveneau et al., 2013	N.A.	N.A.	N.A.	Monnot et al., 2021	N.A.	N.A.
Neural crest cell migration	Theveneau et al., 2013	Theveneau et al., 2013	N.A.	Bajanca et al., 2019; Perissinotto et al., 2000; Szabó et al., 2016	Barriga et al., 2018	N.A.	N.A.
Invagination to form lens pit and optic vesicles	Houssin et al., 2020; Lang et al., 2014	Houssin et al., 2020; Lang et al., 2014	N.A.	Hosseini et al., 2014; Huang et al., 2011; Oltean et al., 2016	Hosseini et al., 2014; Oltean et al., 2016	N.A.	N.A.
Invagination to form nasal pits	N.A.	Jidigam et al., 2015	N.A.	N.A.	N.A.	N.A.	N.A.
Invagination to form otic vesicles	N.A.	Sai and Ladher, 2008; Sai et al., 2014	Sai and Ladher, 2008; Sai et al., 2014	N.A.	N.A.	N.A.	N.A.
Otic vesicle growth	N.A.	Mosaliganti et al., 2019	Mosaliganti et al., 2019	N.A.	N.A.	Mosaliganti et al., 2019	N.A.
Formation of vestibular systems	N.A.	N.A.	N.A.	Gnedeva et al., 2017; Munjal et al., 2021	N.A.	Munjal et al., 2021	Gnedeva et al., 2017
Formation of cochlea	Chacon-Heszele et al., 2012	Cohen et al., 2020; Yamamoto et al., 2009	Yamamoto et al., 2009	Ishii et al., 2021	N.A.	N.A.	N.A.
Spacing of hair cells	Cohen et al., 2020	Cohen et al., 2020	N.A.	N.A.	N.A.	Cohen et al., 2020	N.A.
Formation of hair placode	N.A.	Shyer et al., 2017	N.A.	N.A.	Harn et al., 2021; Shyer et al., 2017	N.A.	N.A.
Formation of hair follicle	Ning et al., 2021	Ning et al., 2021	Ning et al., 2021	N.A.	N.A.	N.A.	N.A.
Epidermis stratification	Ning et al., 2021; Schlegelmilch et al., 2011	Ning et al., 2021	Box et al., 2019; Ning et al., 2021	N.A.	N.A.	N.A.	Box et al., 2019
Formation of pseudostratified retina	Miroshnikova et al., 2018	Miroshnikova et al., 2018	Miroshnikova et al., 2018; Yanakieva et al., 2019	N.A.	N.A.	N.A.	N.A.
Spacing of photoreceptors in retina	Nunley et al., 2020	N.A.	N.A.	N.A.	N.A.		Nunley et al., 2020

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Table 2. Force-generating mechanisms

	Cell scale			Tissue scale			
Stage of morphogenesis	Cortical contraction	Membrane structures (e.g. lamellipodia, filopodia)	Supracellular actomyosin cables	Cell density changes (by proliferation, apoptosis)	Collective migration	Single-cell movements	Result on tissue morphology
Formation of placode compartments	N.A.	Theveneau et al., 2013	N.A.	N.A.	Streit, 2002; Theveneau et al., 2013; Torres-Paz and Whitlock, 2014	N.A.	N.A.
Neural crest cell migration	N.A.	Shellard et al., 2018; Theveneau et al., 2013	Shellard et al., 2018	N.A.	Barriga et al., 2018; Shellard et al., 2018; Theveneau et al., 2013	N.A.	Tissue segregation, cell fate commitment
Formation of lens pit and optic vesicles	Lang et al., 2014	Chauhan et al., 2009; Sidhaye and Norden, 2017	Houssin et al., 2020	Oltean and Taber, 2018	Sidhaye and Norden, 2017	N.A.	Bending and curvature formation
	N.A.	N.A.	N.A.	N.A.	Monnot et al., 2021	N.A.	Forces transmitted to form olfactory placode
Formation of nasal pits	Jidigam et al., 2015	N.A.	N.A.	N.A.	N.A.	N.A.	Bending and curvature formation
Formation of otic vesicles	Sai and Ladher, 2008; Sai et al., 2014	N.A.	N.A.	Lang et al., 2000	N.A.	N.A.	Bending and curvature formation
Otic vesicle growth	N.A.	N.A.	N.A.	Mosaliganti et al., 2019	N.A.	N.A.	Growth in size
Formation of vestibular systems	Munjal et al., 2021	N.A.	Munjal et al., 2021	N.A.	N.A.	N.A.	Budding and elongation of semicircular canals
	N.A.	N.A.	N.A.	Gnedeva et al., 2017	N.A.	N.A.	Growth of utricular macular
Formation of cochlea	Ishii et al., 2021; Yamamoto et al., 2009	Driver et al., 2017	N.A.	Ishii et al., 2021	Driver et al., 2017; Ishii et al., 2021; Yamamoto et al., 2009	N.A.	Bending and elongation
	N.A.	N.A.	N.A.	Cohen et al., 2020	Cohen et al., 2020	N.A.	Pattern formation
Formation of hair placode	N.A.	Ahtiainen et al., 2014	N.A.	N.A.	Ahtiainen et al., 2014; Cetera et al., 2018	N.A.	Bending and curvature formation
Formation of hair follicle	Ning et al., 2021	N.A.	N.A.	N.A.	Cetera et al., 2018	N.A.	Elongation, curvature formation, compartmentalization
Epidermis stratification	Ning et al., 2021	N.A.	N.A.	Box et al., 2019; Miroshnikova et al., 2018	N.A.	Ning et al., 2021	Growth in size, multilayer formation
Formation of pseudostratified retina	Norden et al., 2009	N.A.	N.A.	Azizi et al., 2020	N.A.	N.A.	Growth in cell packing

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Box 1. Glossary

Anisotropy. The property of material that allows it to change or assume its properties in any direction, as opposed to isotropy. Anisotropy can be applied to any physical property of material, such as strength, refractive index, etc.

Cell competition. A process whereby a cell deemed 'less fit' is removed via apoptotic extrusion in epithelia from a population of more 'healthy' cells.

Cell delamination. A process whereby a cell or a group of cells is physically separated from their original group. Some examples of cell delamination are cell extrusion from epithelia, or NCCs migrating away from the neural tube.

Cell intercalation. A process by which cells exchange neighbors.

Contractile. Able to generate contraction, which results in shortening in terms of length. In living systems, actomyosin networks are inherently contractile. This is because actin filaments can be organized into bundles with myosin as crosslinkers in an anti-parallel organization. The coordinated translocation of myosin along actin filaments makes the filaments slide past each other and generates contractile forces.

Convergent-extension. A morphogenesis process whereby the tissue extends in one direction while simultaneously narrowing in the direction perpendicular to the extension axis. This process is typically driven by collective migration and cell intercalation.

Elasticity. The measure of how material returns to its original shape after being deformed by external forces. Elasticity is measured by Young's modulus, which describes the relationship between stress and strain. For a material that is linearly elastic, strain is linearly proportional to stress. The actomyosin network is often modeled as an elastic material.

Hydraulic pressure. Pressure applied by fluids.

Hydrodynamic forces. The forces applied by the movement of fluids on a solid body immersed in the fluids. Some examples are hydraulic pressure and shear forces created by the shear flow.

Lumen. The inside space of a tubular structure that is filled with fluids. It is typically surrounded by epithelial cells with tight junctions.

Luminogenesis (or lumen formation). The creation of fluid-filled spaces. This involves three basic steps: the polarization of the cells to determine where space is formed, the addition of new membrane surrounding the spaces (apical membrane), and the maturation of structures to stabilize the lumen.

Pressure. The force per unit area.

Pseudostratified epithelium. Columnar epithelium that only has one layer of cells attached to the basement membrane, but the cells are highly packed such that the nuclei of those cells are located at different depths and make the epithelium look like stratified epithelium.

Strain. A measure of the deformation of material from a reference shape. The deformation of the object typically results from external stress applied to it. Strain is measured by fractional changes in length, area or volume.

Stratification. The process whereby a single layer of cells (usually epithelia) becomes multilayered. In stratified epithelia, only one layer, termed the basal layer, contacts the basement membrane. Some examples of stratified epithelia include skin, mammary glands of the breast, alveoli.

Stress. The measure of internal force per unit area exerted within a material in response to an external force. Tensile stress results in the stretch of an object, whereas compressive stress results in compaction.

Tension. Force that is applied along the length of a string (or a similar object) when both ends of the string are pulled.

Viscoelasticity. The property of a material that has both elastic and viscous characteristics upon being deformed.

Viscosity. The measure of how a fluid resists deformation at a given rate. Higher viscosity means higher resistance to flow. Cells or tissues are considered to behave like viscoelastic materials.

matrix (ECM) components and cell density. As such, mechanical forces represented by stress (see Glossary, Box 1) can be correlated with tissue deformation represented by strain (see Glossary, Box 1) (Irvine and Shraiman, 2017). These quantities can be measured and quantified *in vivo*, allowing inferences of how mechanical forces regulate tissue growth and changes. For example, quantifying cell geometry, tissue shape and junctional length can be used to infer tissue stresses and junctional tension (Roffay et al., 2021). The use of magnetic droplets or atomic force microscopy can be used to measure tissue rigidity and mechanical state *in vivo* and correlate it with tissue morphology and growth (Serwane et al., 2017). The spatiotemporal distribution of tissue material properties defines the collective movements of cells [e.g. intercalation, invagination, convergent-extension (see Glossary, Box 1), etc.], which subsequently sculpt the tissue shape.

Communication of mechanical forces

Tissues sense extrinsic forces from the embryonic environment, such as the stiffness of surrounding tissues, shear stress from blood flow or hydrostatic pressure from luminal growth. These forces are integrated by the mechanosensitive proteins of a cell and can be transmitted to local or distant neighboring cells to influence tissue-scale shape changes.

Cells probe and react to the environment using the cytoskeleton and cell-adhesion complexes, including cell-cell adhesions and cell adhesions with the ECM. The cytoskeleton filaments connect to mechanosensitive adaptors in cell-adhesion complexes, to which they apply forces. Depending on the force magnitude, the conformation of the proteins in adhesion complexes can be modulated to recruit more actin or disassemble the complexes (Ladoux and Mège, 2017). In focal adhesions, integrins connect the ECM with actomyosin networks via force-sensitive adaptors, such as talin or vinculin. In cell-cell adhesion, the force-sensing complex is usually an adherens junction with vinculin, α-catenin or β-catenin acting as mechanosensitive adaptors (Ladoux and Mège, 2017). The contractile forces applied by actomyosin filaments result in assembly or disassembly of the focal adhesion. Such force-sensing mechanisms are linked to integrin-related growth signaling pathways and regulate cell survival (Sheetz, 2019).

Local dynamics, such as cell migration, cell rounding during division or delamination, can alter local junctional tension, which can be sensed by the neighboring cells. These mechanical cues are communicated within the tissue by force propagation through the supracellular actin cables, which link to the cell-cell adhesions via mechanosensitive molecules, such as α-catenin and β-catenin or vinculin (Ladoux and Mège, 2017). The fluctuation of tension modulates actin organization and cell shape, which ultimately alters tissue movements, growth and morphogenesis. The contractility-junctional tension feedback on global tissue movement is well-recognized in developmental processes, such as the epithelial invagination of *Drosophila* mesoderm and endoderm, germband extension and vertebrate neural tube closure (Heisenberg and Bellaïche, 2013). In addition, external forces applied to the tissue can trigger changes in cell behaviors (e.g. growth). For example, it has been shown *in vitro* that large-scale stretching can directly induce cell proliferation, whereas compression limits cell growth (Eisenhoffer et al., 2012; Gudipaty et al., 2017; Yu et al., 2016).

In living systems, hydraulic pressure (see Glossary, Box 1) can be integrated by tissue on a large scale to coordinate cell function and tissue morphogenesis. Hydraulic pressure during luminogenesis (see Glossary, Box 1) can be translated into cortical tension changes, triggering long-range transmission of contractile forces and tissue response. For example, in embryonic day (E) 3.5-E5 mouse embryos, the hydraulic pressure by luminal growth in the blastocyst leads to increased cortical tension of trophectoderm

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cells and, in turn, activates actomyosin contractions and tight junctions to maintain shape (Chan et al., 2019). The same mechanism has also been observed in the size control of inner ear development of the zebrafish (Mosaliganti et al., 2019) (discussed below). During *Drosophila* dorsal closure, osmosis changes driven by apoptosis reduce the volume of amnioserosa cells, triggering an imbalance of intracellular pressure within the tissue and, subsequently, large-scale tissue contraction (Saias et al., 2015). This process is independent of junctional tension-actomyosin contractility.

Crosstalk between biochemical and mechanical signaling

Mechanical forces can integrate with biochemical pathways to control growth; YAP/Hippo signaling (reviewed by Irvine and Shraiman, 2017; Panciera et al., 2017) and MAPK/ERK signaling (Kinoshita et al., 2020) are the most-studied examples. YAP is a transcriptional co-regulator that translocates from the cytoplasm into the nucleus to upregulate genes necessary for proliferation, cell survival and cell fate commitment (Panciera et al., 2017). YAP relocalization can be triggered by a diverse set of mechanical cues, such as redistribution of adherens-junction tension (Irvine and Shraiman, 2017), tissue relaxation (Gnedeva et al., 2020) or cell stretching (Saw et al., 2017). Conversely, the MAPK/ERK signaling pathway is activated by directional migration (Hino et al., 2020; Ishii et al., 2021; Ogura et al., 2018) or stretching forces (Kinoshita et al., 2020; Moreno et al., 2019). Mechanical forces generated by these processes are coupled with the propagation of ERK activation (ERK waves), which can both activate cell proliferation responses (Kinoshita et al., 2020) and feed back to the cell's contractile network (Ogura et al., 2018), creating a positive-feedback loop.

In short, cells can sense external mechanical stimuli and respond by generating forces. Large-scale force transmission facilitated by cell-cell interaction allows tissues to coordinate their responses to external forces. By integrating force-sensing, internal mechanical properties with biochemical signaling, a tissue can regulate cell survival, movement and fate commitment. In the next section, we briefly review key features of sensory organ development before looking more closely at specific examples of mechanical mechanisms employed during the genesis of these organs.

Sensory organ specification

The sensory systems require three basic cellular 'building blocks': an epithelium (i.e. the sensor of mechanical or chemical stimuli), neurons and glia (i.e. the conduit of information to the central nervous system), and mesenchyme (i.e. the supportive tissue). The epithelial components of sensory organs, including the eyes, ears, skin, olfactory and gustatory (taste) systems in mammals are derived from the ectoderm. Following gastrulation, Wnt, fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) signaling pattern the ectoderm into surface ectoderm (non-neural ectoderm), laterally, or the neuroectoderm (also known as the neural plate), medially (Fig. 1A). The non-neural ectoderm region lying between the neural plate and the future epidermis is called the neural plate border. During neural tube formation, cells at the neural plate border, which have attenuated BMP and high FGF signaling, differentiate into neural crest cells (NCCs) and pre-placode cells – key cell types contributing to the sensory organ development (Singh and Groves, 2016). Cells from the neural plate border receive strong Wnt signaling activators and commit to NCCs, and the domains with inhibited Wnt become pre-placode cells expressing genes of the Six and Eya families (Singh and Groves, 2016).

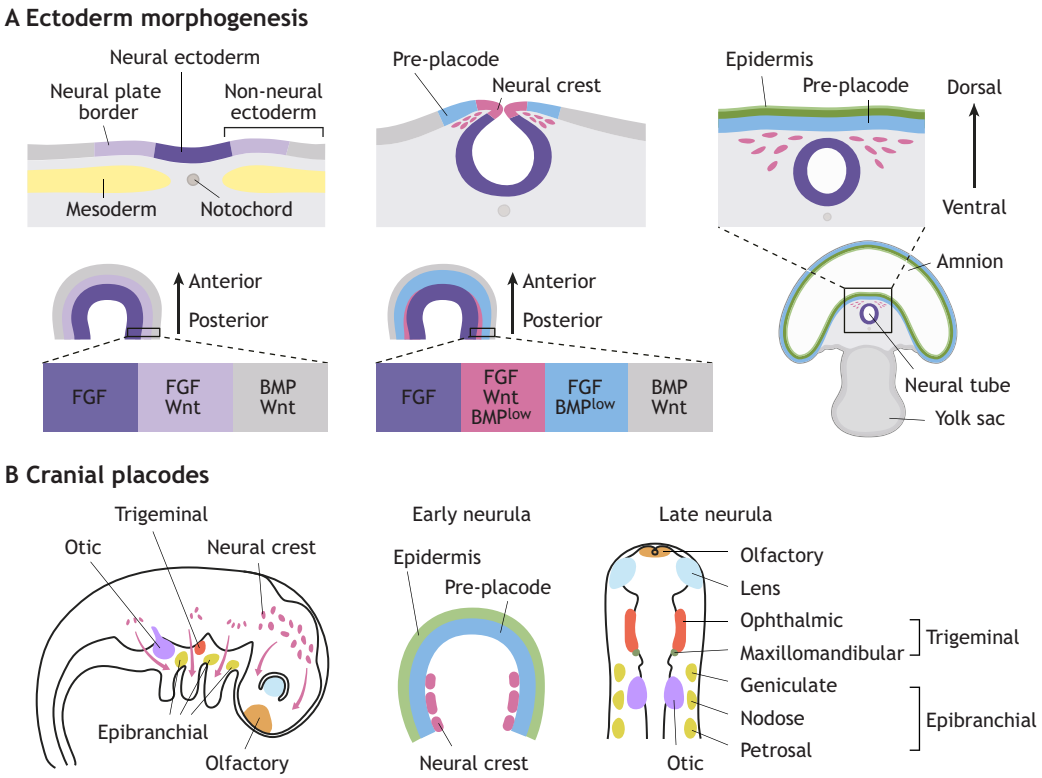

Fig. 1. Placode formation. (A) The early morphogenesis of ectoderm, which is patterned by FGF, WNT and BMP signaling to form the neural plate border, neural ectoderm and non-neural ectoderm. Subsequent morphogen signaling patterns the non-neuronal ectoderm to form epidermis, pre-placodes and neural crest regions. (B) Development and positioning of cranial placodes in the embryo (chick embryo illustrated as an example).

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Cranial placode-derived sensory organs

Around week 4 of human development or E7.5-E8 of mouse development, the ectoderm containing placode progenitors undergoes thickening and invagination to form segregated domains (Fig. 1B). These domains are olfactory, adenohypophyseal, lens, trigeminal, epibranchial and otic placodes. The olfactory, lens and otic placodes contribute to the formation of olfactory, visual and inner ear systems, whereas the trigeminal and epibranchial placodes contribute to sensory neuron development. Around the same time, the embryo forms pharyngeal arches that consist of ectoderm, endoderm, NCCs and mesoderm (Graham, 2003). The tongue and its gustatory placodes are derived from the pharyngeal ectoderm (Cobourne et al., 2019).

By employing epithelial dynamics, such as thickening, stratification, invagination, lumen formation, rosette formation, collective migration, cell intercalation and delamination (see Glossary, Box 1), these placodes form high-ordered structures with more complex architectures. Between weeks 4 and 5 in humans, the lens placode cells invaginate and further pinch off from the surface ectoderm to become lens pits. At the same time, the optic vesicle also emerges from an invagination of neural tube epithelium that contacts the lens placode, which later develops into the bilayered optic cup with pigmented retinal epithelium and neural retina (Heavner and Pevny, 2012). The invaginations of lens placodes and optic vesicles are synchronized. Invagination of epithelium also induces the formation of nasal pits from olfactory placodes and otic vesicles from otic placodes. The nasal pits further invaginate and deepen to form nostrils and more complex nasal cavities. At about week 5 in humans or E11 in mice, the otic vesicles undergo topological changes to form canals. Subsequently, the epithelium at the anterior-ventral end elongates to form the cochlea (Driver et al., 2017). The higher-order structures are further developed through the shaping and bending of tissues (for more detail, see Driver et al., 2017; Heavner and Pevny, 2012; Sarnat and Flores-Sarnat, 2019). Many of these processes are orchestrated by mechanical forces at different scales.

Skin and skin appendages

The skin can be included among the sensory systems that are derived from the surface ectoderm (Fig. 1A). The adult skin is innervated by NCC-derived mechanoreceptor and nociceptor neurons that mediate touch and pain perception, respectively (Jenkins and Lumpkin, 2017; Meltzer et al., 2021). The early epidermis of the skin consists of a single layer of multipotent cells that express keratin 8/18 (K8/K18). Once committed to epidermis fate, starting from week 4 in humans, the basal layer expresses K5/K14 and stratifies into a multilayered epithelium (Fig. 2A). The first layer becomes the basal layer, which attaches to the basement membrane by hemidesmosomes and integrin focal adhesion. Basal cells undergo asymmetric division to establish the intermediate and periderm layers (Lechler and Fuchs, 2005); the stratification happens throughout embryonic development. Postnatally, the complete human epidermis consists of a multilayer of cells with the cornified envelope on the surface. The dermis is formed by mesenchyme cells derived from the NCCs in the face and paraxial mesoderm elsewhere in the body. A variety of immune cells and blood vessels derived from the lateral plate mesoderm also populate the tissue. Skin appendages, such as hair follicles (Fig. 2B) and sweat glands, develop continuously from week 12 to 22 in humans; first hair follicles and later sweat glands. Skin appendage morphogenesis, beginning with placode formation, depends heavily on epithelial-mesenchymal interaction between the epidermis and the dermis.

Now we can appreciate epithelial placodes and NCCs as the early contributors to the complexity of sensory organs. We next consider

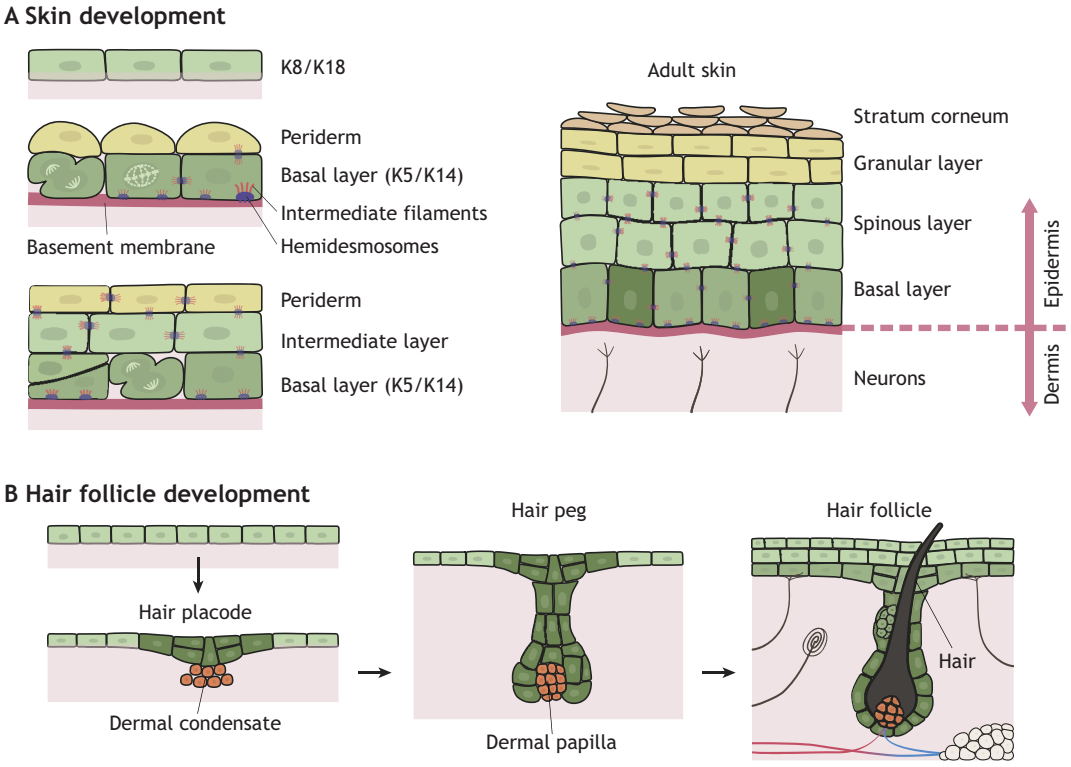

Fig. 2. Skin and hair follicle development. (A) Skin development from an epithelial monolayer to a stratified epithelium via orientated cell division to form the adult skin. (B) The stages of hair follicle development from a hair placode.

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how mechanical cues instruct early placode formation, including how cranial placodes are compartmentalized and further shaped by invagination. We also discuss how placodes develop into higher-ordered structures, which are governed by the collective migration of NCCs and epithelial cells. We address how large-scale mechanical signals, such as pressure and stress, can be integrated by cells at the local scale to regulate tissue size and growth. Finally, we review the mechanosensing mechanisms used to regulate spatial patterns in tissues.

Compartmentalization of sensory placodes

The differential adhesion model can explain how the early placodes are compartmentalized into specific domains while committing to their fates (Breau and Schneider-Maunoury, 2014). According to this model, placode cells, through random movements, can adhere and aggregate with other cells expressing similar adhesion proteins. As a result, initially intermingling placode cells in pre-placode regions can be sorted into compartments of specific cell identities based on different adherent strengths. The model is supported by studies showing segregation of lens and olfactory pre-placodes in the chick (Bhattacharyya et al., 2004), sorting of lens placode in mice (Collinson et al., 2001), and sorting of otic and epibranchial placodes in zebrafish (McCarroll et al., 2012). How the cranial placode cells coordinate their fate commitments with terminal localization is still controversial (Breau and Schneider-Maunoury, 2014). Specifically, the unresolvable timings between placode fate commitment and their regional segregation suggested that differential adhesion sorting may not be sufficient. The movement of pre-placode cells to their positions may additionally depend on directional cues. Cell competition (see Glossary, Box 1) could also regulate the segregation of placodes (Collinson et al., 2001). There could be a combination of multiple processes when directional migration is necessary to form domains of cells at transitional fates, and differential adhesion can sharpen the border of adjacent placode domains. Experiments using live imaging of cells expressing multiple reporter genes for specific placode fates could further clarify these issues. Although the differential adhesion model supports the role of adhesion strength, there has not yet been any direct measurement of adhesive and repulsive forces. Characterizing different placode cells' adhesion strength using physical measurement methods (Roffay et al., 2021) could also help deduce when the differential adhesion model comes into play or lead to generation of a better model.

Epithelial invagination during sensory placode formation

Epithelial invagination refers to the bending of an epithelial sheet to form pits or vesicles, a process shared between lens, otic and olfactory placode morphogenesis (Fig. 3).

Apical constriction

Epithelial invagination to form placodes typically initiates through apical constriction of ectodermal cells. As the ectodermal layers thicken, the actomyosin network is enriched at the apical domains of the cells. In general, such polarized enrichment results in the contractile forces that constrict the apical surfaces. The engagement between actin cables and cell-cell junctions enables these forces to coordinate apical constriction of multiple cells (Christophorou et al., 2010; Houssin et al., 2020; Lang et al., 2014; Martin and Goldstein, 2014; Pontoriero et al., 2009). Although the general mechanical principle is shared between lens, olfactory and otic placode, how actomyosin is recruited to the apical domains depends on specific molecular components.

In the lens placode, the Shroom family of actin-binding factors are instrumental for the recruitment of actin filaments to the apical domains of pre-placodal cells in *Xenopus*, chick and mice (Chauhan et al., 2015; Lee et al., 2009; Plageman et al., 2010). Pax6, a crucial transcription factor in eye development (Nishina et al., 1999; Smith et al., 2009), induces expression of Shroom3 (Plageman et al., 2010). Shroom3 is also important for the recruitment of the actin-polymerizing factors Vasp and Rock1/2, which promote myosin contractility essential for inducing the contraction of the apical surface (Plageman et al., 2010; Roffers-Agarwal et al., 2008) (Fig. 3A).

In the otic placode, apical constriction is preceded by basal relaxation (Sai and Ladher, 2008). FGF signaling induces phosphorylation of myosin at the basal side, thus depolymerizing

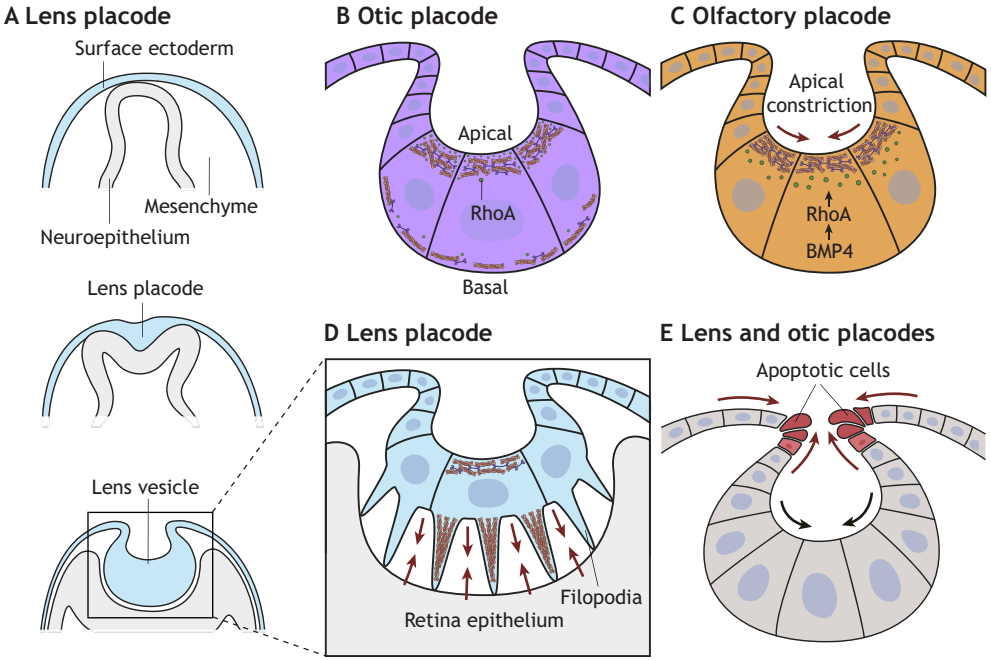

Fig. 3. Mechanisms of epithelial placode invagination. (A-C) Apical constriction mechanisms in the lens placode (A), otic placode (B) and olfactory placode (C). (D) Lens placode epithelial invagination driven by pulling forces of filopodia on retina epithelium. (E) Additional forces necessary for formation of otic or lens vesicles. Apoptotic cells (red) provide additional tension (associated red arrows) that facilitates vesicle closure. In addition, cell height changes generate strains (black arrows), which increase curvature of the epithelium and facilitate closure of the vesicles.

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the actin filaments and relaxing the basal side (Sai and Ladher, 2008). Subsequently, apical constriction is induced by the polarized localization of RhoA (Sai et al., 2014), which activates myosin contractility (Fig. 3B). The precise mechanism to reconcile the fact that myosin contractility occurs at both basal and apical domains, but only the apical domain contracts, remains elusive.

Conversely, in olfactory placodes, BMP signaling is important for recruiting RhoA and F-actin for apical constriction (Jidigam et al., 2015) (Fig. 3C). How BMP signals can crosstalk with actomyosin-mediated apical constriction has not been shown directly in chick experiments. However, in *Drosophila*, Dpp (a BMP homolog) upregulates integrin expression. Integrin further stabilizes microtubules and thus orients the distribution of F-actin and activated myosin II to the apical side (Fernandes et al., 2014). Similar mechanisms could be employed in vertebrates.

Despite differences in molecular regulators, the placodes still achieve polarized contraction at the apical domain. The distinct environment for each organ, however, imposes a slightly different physical barrier, such that apical constriction force alone may not be sufficient for complete invagination. As we discuss below, some organs employ additional mechanisms.

Interaction between the lens placode and retina

The lens placode interacts with the presumptive retina to coordinate their invaginations. A rich pool of ECM proteins exists between lens placode and retinal epithelium, and is essential for invagination (Huang et al., 2011). Computational modeling has shown that ECM can form stiffness gradients and serve as the constraint to direct the growth of optic cups (Oltean et al., 2016), implying a similar function is applied to the lens placode. Lens placode cells also use filopodia as the pulling force to enhance the bending process. In lens placode, actin filaments bundle into filopodia structures at the basal sides (Fig. 3D). These filopodia protrude and tether the lens placode to the retina epithelium (Chauhan et al., 2009) and transmit forces for coordinated bending of lens placode and retinal epithelium. In reverse, the presumptive lens placode area in surface ectoderm also serves as a mechanical constraint to control the invagination of the optic vesicles and, subsequently, retina epithelium (Hosseini et al., 2014).

Apoptotic extrusion in lens and otic placodes

Apoptotic extrusion, an active process to remove dead cells from the tissue, can provide additional forces to induce bending of epithelia during invagination of the lens and otic placodes, as suggested by observations in chick embryos. Apoptotic extrusion engages both cell-cell adhesion and cell-substrate adhesion to generate forces that induce large-scale tissue dynamics (Le et al., 2021; Saw et al., 2017; Toyama et al., 2008). Apoptotic extrusion generates forces that can overcome the large compressive stresses distributed along the circumferential axis to complete epithelial invagination in the lens placode (Oltean and Taber, 2018). Apoptosis occurs heavily at the transitional regions between the forming pits and the flat epithelium during otic vesicle closure (Fig. 3E), suggesting the presence of apoptotic forces during otic placode invagination (Alvarez and Navascués, 1990; Lang et al., 2000). Further combined live microscopy and computational modeling experiments could provide a clearer estimation of mechanical forces exerted by apoptotic extrusion in the completion of the invagination process to form otic and lens vesicles. Nevertheless, apoptosis does not seem to be involved in olfactory placode invagination (Jidigam et al., 2015).

Other mechanisms

Epithelial invagination may not be restricted to these published mechanisms. As shown in *in silico* models of gastrulation in *Drosophila* and sea urchin, processes such as cell rounding induced by mitosis (Kondo and Hayashi, 2013) or hydrodynamic forces (see Glossary, Box 1) (Pouille and Farge, 2008) could result in changes in cell strain, which bend the tissue and lead to invagination. These observations could be helpful for *in vitro* organoid design of sensory organs, for which external manipulation could feasibly be used for inducing more accurate replication of placode development.

Collective migration

Collective migration is a process by which groups of cells migrate together but still retain their cell-cell contacts. The generalized mechanism of collective migration is that the cohort of cells undergoes epithelial-mesenchymal transition (EMT), or partial EMT for the case of epithelial cell sheets (Ladoux and Mège, 2017; Szabó and Mayor, 2018) (Fig. 4). The cells at the front establish front-back polarization, where they can form protrusions, generating forces that can be transmitted by actomyosin structures via the cell-cell junctions to trigger movement in the cells at the rear (Fig. 4A) (Ladoux and Mège, 2017). Collective migration is the hallmark of several embryonic development events, many of which are important for sensory organ morphogenesis, such as NCC migration (Shellard et al., 2018; Szabó and Mayor, 2018), placode formation (Cetera et al., 2018; Streit, 2002), the convergent-extension of epithelia during the formation of the cochlea's spiral shape (Ishii et al., 2021), and migration of epithelia to form the optic cup in teleosts (Kwan et al., 2012; Sidhaye and Norden, 2017). It is noteworthy that heterotypic interactions between epithelia and mesenchyme exist throughout organogenesis, so the collective behaviors of these tissues are interdependent.

NCC collective migration

How the mechanical environment instructs collective migration during sensory system development can be best illustrated by the migration of cranial NCCs from the neuroectoderm and their effects on placode formation. In most vertebrate models, pre-migratory NCCs switch the adhesion from E-cadherin (cadherin 1) to N-cadherin (cadherin 2), and upregulate mesenchymal markers, such as Snail/Slug, Foxd3 and Sox9/10 (Szabó and Mayor, 2018), making the cells less adhesive and more motile. The NCCs exhibit collective migration with extensive use of supracellular actin cables (Shellard et al., 2018). Collective migration of NCCs from the border plate contributes to the formation of mesenchyme populations, regulates the development of sensory neuron aggregations, and the innervation in the sensory system (Steventon et al., 2014).

Substrate interactions: stiffness and the ECM

The mechanical stiffness of the mesoderm, which exhibits a dorsal-to-ventral gradient of tissue stiffness by increased cell density, could guide the migration of the NCCs in *Xenopus* (Barriga et al., 2018; Shellard and Mayor, 2021) (Fig. 4B). NCCs migrate from softer to stiffer regions according to this stiffness gradient (Shellard and Mayor, 2021). Furthermore, the mesoderm provides a heterogeneous distribution of ECM proteins, which favor migration in the direction that allows better cell-matrix adhesion (Bajanca et al., 2019). The 'molecular clutch' model (Elosegui-Artola et al., 2016) can be used to explain this phenomenon: when engaging in a stiffer or more adhesive substrate, cells exert more force on the focal adhesion complexes, which further expose binding sites of mechanosensitive

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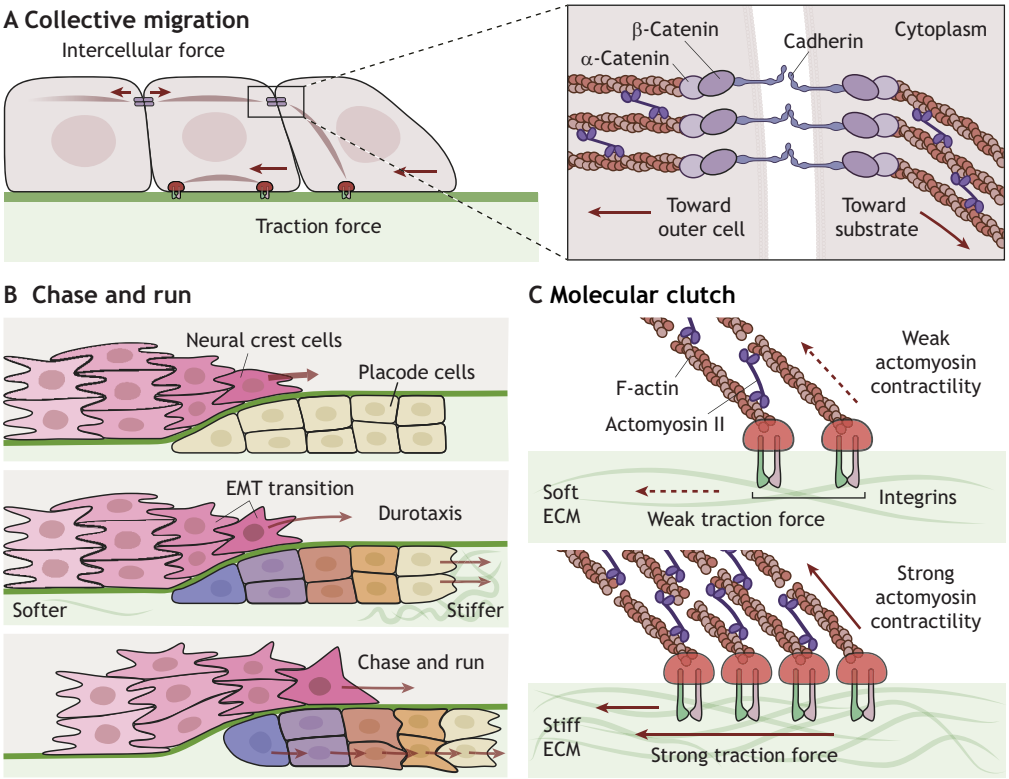

Fig. 4. Collective migration in sensory organ development. (A,B) Collective migration can be used by both epithelia (A) and mesenchyme (B), whereby forces (red arrows) are transmitted along the intercellular actin cables connecting the cell-cell adhesions (e.g. cadherins) and cell-substrate adhesions. (B) Neural crest cells collectively migrate according to the tissue stiffness (top and middle). This results in the 'chase-and-run' mechanism between NCCs and placode cells (bottom). (C) Molecular clutch model. Stiffer matrices facilitate more mature focal adhesions and thus stronger actomyosin contractility and traction force, which enables the tissue to move forwards. Illustration modified from Ladoux and Mège (2017) and Shellard and Mayor (2021).

proteins, such as talin, leading to further recruitment of actin to the focal adhesion. This reinforcement of focal adhesion complexes enables the cells to exert more forces on the substrate to move forwards and favors migration into stiffer areas (Fig. 4C).

NCC interaction with placodes

Surrounding tissues can provide spatial confinement to the path of NCC migration by the expression of adhesion repellants, such as semaphorins (Bajanca et al., 2019) in *Xenopus* and versican (Szabó et al., 2016) in *Xenopus* and chick embryos (Perissinotto et al., 2000). The confinement not only provides biochemical signals to direct migration, but also helps the NCCs to maintain their directional persistence and efficiency. As such, the NCCs can integrate all these physical cues with the non-homogeneous distribution of biochemical signaling to direct themselves during collective migration.

The migration of the NCCs helps shape placode formation via epithelial-mesenchymal interaction (Steventon et al., 2014). The spatial proximity and coincidental timings of neural crest streams and placode regions supports the suggestion that the NCC migration impacts placode formation and the morphology of sensory organs (Steventon et al., 2014; Szabó and Mayor, 2018; Torres-Paz and Whitlock, 2014). During the development of the epibranchial placodes that generate cranial nerves in *Xenopus*, the NCCs contact epibranchial pre-placodal cells via SDF1/CXCR4 interaction. This binding enables redistribution of forces and loss of focal adhesion near the cell-cell contact site (Theveneau et al., 2013). Consequently, the epibranchial cells migrate towards the direction opposite to the interaction. The mechanism has been termed 'chase-and-run', inhibition of which results in defects in placode formation and in the migratory patterns of the associated NCCs (Fig. 4B).

Although NCC transcriptional expression and migratory patterns are conserved among vertebrates (Kulesa et al., 2004), how robust and conserved mechanical cues impact NCC and placodal formation in humans remains an interesting question. Organoids derived from human cells that contain both placode cells and NCCs, which can be perturbed, are useful models to address such questions (Koehler et al., 2013; Lee et al., 2018). Even though the genetic fingerprints differ, the physical principles of collective migration seem to be conserved. What is left to be explored is how these physical principles can be integrated with the specific biochemical signaling pathways or with more large-scale extrinsic forces from the embryonic environment to specify the development of each sensory organ. The next section delves into the two most well-studied examples of how mechanical forces crosstalk with biochemical signaling to shape the tissue: the formation of the cochlea in the inner ear and hair placode in the skin.

Epithelial migration for tissue shaping

Convergent-extension in the inner ear

The formation of the spiral-shaped cochlea – the sensory hearing organ of the inner ear – epitomizes how force transmission during collective migration interregulated with biochemical signaling could lead to special structures.

Starting from E11 in mice, the epithelium at the anterior-ventral region of otic vesicles elongates into cochlea epithelium, a ductal structure that extends in length and coils in the lateral-medial direction (Fig. 5A). Sensory hair cell progenitors (prosensory cells) and sensory cells emerging from this epithelium undergo collective migration, which is characterized by myosin II contractility, directed protrusion formation and active cell movements (Driver et al., 2017; Mu et al., 1997; Yamamoto et al., 2009). Such migration results in large-scale tissue extension along the base-to-apex axis of the cochlea and concomitant thinning of the cells in the perpendicular axis (convergent-extension). Interestingly, when molecular components essential for force transmission, such as myosin or p120-catenin (Ctnnd1), is interrupted, the convergent-extension

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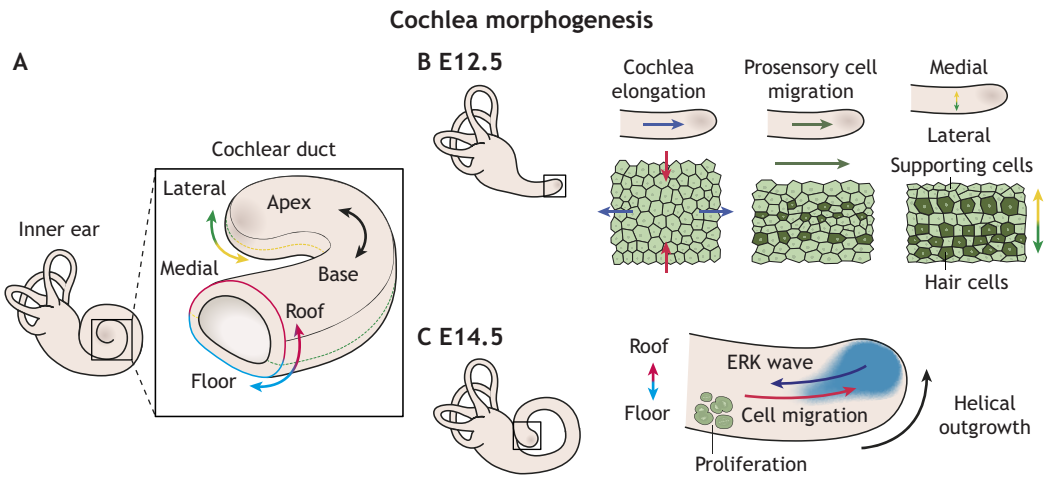

Fig. 5. Collective cell migration of the epithelia is used by sensory cells and their progenitors for formation of the coiled-shape cochlea in mice. (A) Overview of the inner ear with cochlear duct. (B) Collective migrations of the hair cells to induce convergent-extension of the cochlear duct. (C) Collective migration is coupled with an ERK-wave induced by differential cell proliferation to form helical coiling (for more details, see Driver et al., 2017; Ishii et al., 2021). Arrow colors relate to the directions indicated in A.

process is disrupted (Chacon-Heszele et al., 2012; Yamamoto et al., 2009).

However, convergent-extension alone can only explain the elongation of the cochlea duct, not the helical coiling morphology. A recent study has attempted to explain how cell migrations are translated into the coiling of the cochlear epithelium by observing cell flow coupled with retrograde ERK waves (Ishii et al., 2021) (Fig. 5B). Differential cell proliferation results in ERK activation, which promotes actomyosin contraction and cell migration from base to apex at the lateral side of the cochlear duct. ERK activation occurs in a wave-like manner from the roof to the floor of the lateral epithelium, thereby resulting in an asymmetric migratory path, leading to the helical bending of the epithelium. The study proposed an asymmetric mode of migration that is complementary to the convergent-extension mode. The process by which ERK waves are triggered and maintained is unclear; however, confinement may be a crucial factor. ERK wave-dependent mechanochemical coupling has been observed in various epithelia, such as in Madin–Darby canine kidney (MDCK) cells (Hino et al., 2020) and *Drosophila* tracheal placode (Ogura et al., 2018), and confinement appears to be crucial in these models. For the developing cochlea, the principal confining tissues that may regulate duct coiling are the peri-otic mesenchyme, the hindbrain or the skull base. Future work should probe these tissues to define better the role of confinement in cochlear ERK-wave formation.

Different modes of collective migration in hair placode formation

Collective migration could result in special morphology changes in the tissue, which dictate the tissue architecture and cell fates. Live imaging and 3D cell tracking in mammalian skin have shown that hair placode cells exhibit directed, collective cell migration with protrusive structures and actomyosin enrichment from the peripheral to central direction at about E14 in mice (Ahtiainen et al., 2014). Comprehensive cell tracking during hair placode cell migration at E15.5 has shown that, shortly after the hair placode region is established, the hair placode cells adopt counter-rotational migration (Cetera et al., 2018). The counter-rotation cell flow repositions centrally positioned cells towards the leading edges and the peripheral-positioned cells towards the center (Cetera et al., 2018). This behavior is coupled with changes in gene expression, such that cells at the leading edges express sonic hedgehog (Shh), which later become the hair matrix, and those trailing behind express Sox9, which later become the hair follicle stem cells. The flow displaces the dermal condensates (a cluster of mesenchymal cells from the dermis and enclosed by hair placode cells; Fig. 3B), which further controls epithelial cell fate and generates the asymmetric pattern of hair follicles from the – initially symmetrical – hair placode.

Summary and discussion

By engaging in changing cell shapes (in the case of placode invagination) or active migrations (in the cases of NCCs, inner ear prosensory cells or hair placode cells), epithelial placode cells actively use their cytoskeleton to generate forces to drive morphogenesis. Even though the discussion has been focusing on this view, mechanical forces could be derived from other sources, such as adjacent tissue movements or interactions with the ECM. For example, a recent report in zebrafish has shown that movement of the neurogenic cells in the olfactory circuit at the medial-lateral axis is non-autonomous and depends on the movements of optic cup tissues, not on the NCCs. Active movements of optic cup cells result in the traction forces, transmitted via ECM interactions and instruct the migration of the adjacent cells in the olfactory placode (Monnot et al., 2021). The ECM, in addition to serving as a constraint, can also generate osmotic forces. A recent report on the formation of semicircular canals in zebrafish has shown that hyaluronan can actively drive the budding of the otic epithelia, whereas actomyosin contractility only serves as a guiding cue (Munjal et al., 2021). A study using intestinal organoids has also shown the role of hydraulic pressure in driving the complex morphology (Tallapragada et al., 2021). As hydraulic forces are ubiquitous in the embryo environment, future research could focus on these forces to study the development of sensory organs. In the next section, we discuss some of the exciting progress that has been made towards illustrating the role of hydraulic forces in tissue growth.

How are mechanical forces integrated to control organ growth and size?

Combined with experimental measurements, mathematical and physical models have shown that mechanical stress provides

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regulatory feedback to cell proliferation/cell death and controls tissue growth (Irvine and Shraiman, 2017). Owing to the complexity of the organ and tissues, the integration of forces differs from tissue to tissue.

Growth regulation by pressure in inner ear development

Pressure is present throughout development: from hydrostatic pressure in the early blastocyst to formation of the fluid-filled channels in the inner ear in later development. Studies of zebrafish and mouse inner ear development have delineated how global tissue pressure can be translated to lower-scale cell proliferation in controlling tissue size and growth.

In the zebrafish inner ear, the growth of otic vesicles is controlled by the integration of hydraulic pressure from the lumen, the flux of fluids via the epithelium and the viscoelasticity of the epithelium regulated by the cell shape and actomyosin contractility (Mosaliganti et al., 2019). The epithelium allows the flux of fluids and induces lumen formation, which leads to rapid initial growth; the lumen expansion results in hydrostatic pressure that deforms the epithelium. The hydrostatic stress of the epithelium also generates tissue tension that induces epithelial cell proliferation to counterbalance the tension. Once the luminal pressure reaches a certain threshold, inward flux is halted, so the size of the vesicle is controlled. Interestingly, cell proliferation is a consequence of these processes, rather than the factor that controls organ growth. The same principles of integrated luminal pressure and cell stretching are also seen in blastocyst size and cell fate commitments (Chan et al., 2019). Therefore, the same framework using pressure probes, live imaging, and mathematical models could be used to revisit the role of hydrostatic pressure in the development of other organs. One example is the role of intraocular stress in the development of the eyes, which has been documented but the mechanism is still obscure (Coulombre, 1957).

Although hydraulic pressure could induce tissue growth, compressive stress from surrounding constraints could inhibit tissue expansion. The growth of the utricular macula (Gnedeva et al., 2017) and the organ of Corti (Gnedeva et al., 2020) in the mouse cochlea are restricted by pressure applied by surrounding tissues. In the utricular macula, the surrounding tissues exert a confinement effect that, once relieved, leads to nuclear translocation of YAP and triggers cell proliferation. The evidence that tissue relaxation can lead to YAP translocation seems to be a general phenomenon and has been demonstrated in various other epithelia (reviewed by Panciera et al., 2017). Therefore, it is highly probable that size control for the organ of Corti is also under the influence of stress-relaxation processes.

Growth regulation by cell and nucleus migration in stratified and pseudostratified epithelia

In stratified or pseudostratified epithelia (see Glossary, Box 1), tissue growth control is controlled by cells sensing tension distribution from their neighbors and using the actomyosin cytoskeleton as the active force. As a result, cells undergo cell division, delamination or nucleus migration to control tissue size.

The growth control of mammalian skin in late development is mainly regulated by epidermal stratification and asymmetric cell division (Fig. 2A). The crowding state of the basal layer induces stratification (Miroshnikova et al., 2018) when basal cells can undergo asymmetric cell division (Lechler and Fuchs, 2005) or delamination (Miroshnikova et al., 2018) to contribute to the suprabasal layer. Basal cells orient their mitotic spindles oblique or perpendicular to the basal lamina, which results in one of the daughter cells being positioned to the suprabasal layer (Fig. 2A). The asymmetric division is mechanically regulated by the cell geometry induced by crowding in early epidermis development (Box et al., 2019). The compactness of the tissue results in narrowing shapes of basal cells, favoring asymmetric division. By contrast, the basal cells tend to be more elongated and divide more planarly when tissues are stretched as a result of improper neural tube closure. The resulting daughter cells can also exhibit hypercontractility, which induces basal cell proliferation and inhibits them from committing to hair follicle fate (Ning et al., 2021). The relay of contractility to the basal cell's proliferation is possibly through E-cadherin- or α-catenin-dependent YAP1 translocation (Ning et al., 2021; Schlegelmilch et al., 2011). The crowding of the basal layer also induces low cortical tension and increased cell adhesion in a stochastic manner, enabling these cells to differentiate and delaminate. These delaminated cells activate E-cadherin expression as they reach the upper layer, stabilizing them at the suprabasal layer. It is also interesting to note that, during stratification, differentiated cells are in proximity with mitotic cells. The process is reminiscent of *in vitro* studies in which cell division is coupled to cell extrusion to maintain epithelial homeostasis (Eisenhoffer et al., 2012; Kawaue et al., 2021 preprint): this process is tightly regulated by the anisotropic distribution of tissue stress and intercellular tension (Saw et al., 2017).

During the formation of pseudostratified epithelia (PSE), a type of tissue forming the retina and part of the inner ear, the growth of the tissue is constrained laterally. Cell proliferation, in this case, has to be coordinated so that the overall tissue morphology of elongated cells is maintained. Our understanding of how mechanical forces can regulate these processes in the context of sensory tissue formation is limited. However, general physical principles could be learned from results of other PSE (Strzyz et al., 2016); for example, in the *Drosophila* wing disc, mechanical stretch can trigger cell proliferation of the PSE. In the vertebrate retina, the nuclei of retinal cells migrate to the apical side before undergoing mitosis and diffusing back to the basal side (Azizi et al., 2020). During apical nucleus migration, the retinal cells of zebrafish use actomyosin contractility (Azizi et al., 2020; Norden et al., 2009; Yanakieva et al., 2019). Such actomyosin-driven nucleus migration depends heavily on sensing tissue morphology, cell shape-related parameters, as well as overall tissue density (Yanakieva et al., 2019). As such, the growth of the PSE tissue seems to depend on tension sensing, which is analogous to the mechanisms of the growth of stratified epithelia. However, how tissue growth is controlled concerning unique characteristics of different PSEs remains to be dissected. For example, the spatiotemporal relationship between cell proliferation and cell elongation is also an interesting question to be tackled.

Mechanical cues regulate organ patterning

The final prenatal sensory organs have specialized cells ordered in precise arrays, a phenomenon seen across organs: the hair follicle in the epidermis (Shyer et al., 2017), the checker-board hair cell pattern in the inner ear (Cohen et al., 2020), and the cone photoreceptors in the outer retinal layer (Nunley et al., 2020). Periodic pattern formation has been conventionally explained by the reaction-diffusion model: the pattern is determined by the interplay of biochemical inhibitors and activators with different reaction and diffusion rates (Turing, 1952; Gierer and Meinhartd, 1972; Jacobo and Hudspeth, 2014; Sick et al., 2006). Recent evidence suggests the role of mechanical forces from developmental processes, which can be incorporated into the model for pattern formation.

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Global stress distribution and cell-cell repulsive forces seem to be the central mechanisms regulating periodic pattern formation. In the inner ear, hair cells (HCs) redistribute from a disordered manner to a checkerboard-like pattern as hexagonal vertices interspersed with support cells. Shear forces induced by lateral tissues movements and reduction of junctional tension were observed during this transition (Cohen et al., 2020). Live imaging and 2D vertex modeling have shown that the integration of global shear forces and local junctional tension reduction via HC-HC repulsion are crucial regulators of this pattern formation. A similar junctional tension reduction via cell-cell repulsion has also been observed in the mosaic distribution of photoreceptors in zebrafish retinae (Nunley et al., 2020).

How global cell motions and local cell-cell interactions regulate the periodic patterning of photoreceptor cells in the retina might be explained by modeling the whole tissue as liquid crystals that form topological defects as a result of its curvature (Nunley et al., 2020). In short, in a liquid crystal monolayer, the particle motions and shape can induce defects that accumulate local isotropic stress. The model shows that photoreceptor cells are preferentially distributed at these defects, where cell-cell repulsion regulates the distances between these positions.

In the skin, hair placode patterning seems to be induced from forces exerted by the dermis. In avian skin, the aggregation of mesenchymal cells applies compression stresses, causing buckling of the basal membrane and epidermis (Shyer et al., 2017). Furthermore, the regularity and size of the hair follicle aggregate can be tuned by varying substrate stiffness and cellular contractility. *Ex vivo* culture of avian feather skin has shown that a very soft substrate (<0.1 kPa) makes the tissue over-contractile and unable to form patterns. A progressive increase in stiffness substrate increases the size and regularity of the hair follicle patterns. However, extremely stiff substrates (>40 kPa) overstretch the tissue, preventing pattern formation. Pharmacological perturbation of contractility leads to the same results. The stiffness-dependent formation of hair placodes has also been observed in wounded mouse experiments (Harn et al., 2021). Mechanical forces control spatial patterning and commit to hair follicle differentiation by activating the β-catenin nuclear localization pathway (Shyer et al., 2017). The exact mechanism of how mechanical cues are translated into periodical spatial distribution needs further clarification, perhaps with models incorporating mechanical stresses and reaction-diffusion of biochemical signals (Hiscock and Megason, 2015).

Applying mechanobiological principles to study human development and disease

Our insights into mechanical force mechanisms have been made using model organisms, so it is unclear how well these mechanisms are conserved in humans. Yet, understanding the mechanobiology of developing and adult human organs could lead to novel therapies. Dysfunction of mechanical processes have been implicated in some congenital disorders. For example, dysregulation of intraocular pressure and optic cup closure could result in anophthalmia and microphthalmia eye abnormalities in infants (Verma and FitzPatrick, 2007). Some neural tube defects have been linked to a failure of NCC migration (Morriss-Kay et al., 1994). The malfunction of fluid regulation to maintain homeostatic pressure is also implicated in age-related diseases, such as glaucoma of the eye

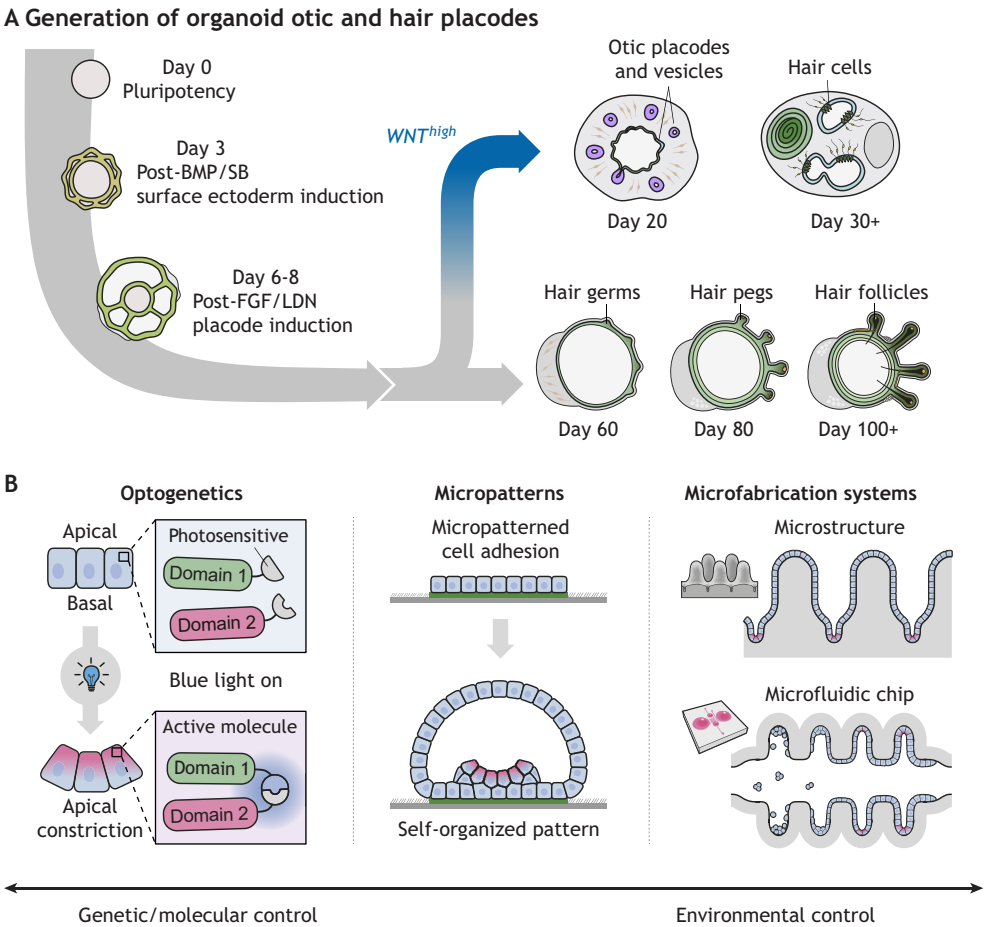

Fig. 6. Strategies for studying mechanical forces in sensory organ development *in vitro*. (A) Representative organoid platform that can be used to generate otic or hair placode, as described by Koehler et al. (2017) and Lee et al. (2020). LDN, LDN-193189 (BMP inhibitor); SB, SB-431542 [transforming growth factor beta (TGFβ) inhibitor]. (B) Recent examples of genetic, molecular and environmental techniques being employed to augment cell and tissue mechanical properties and morphology. From left to right: light-induced OptoShroom3 control of apical constriction (Martínez-Ara et al., 2021 preprint), 2D and 3D micropatterned stem cell cultures (Haremaki et al., 2019; Karzbrun et al., 2021; Warmflash et al., 2014) and microfabricated culture systems with biomimetic architecture (Gjorevski et al., 2022; Nikolaev et al., 2020).

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or Ménière's disease, an inner ear disorder leading to dizziness, hearing loss and a congested feeling in the ear. However, direct experimental evidence linking mechanical dysregulation to human disease is lacking, perhaps because fetal specimens are difficult to obtain for research. Thus, the emergence in recent years of new human cell-based models has led to exciting advances in our ability to study human mechanobiology.

Techniques for force manipulation and measurements are well-established in culture models using cell lines in simple 2D cultures. But these 2D systems cannot recapitulate every aspect of *in vivo* morphogenesis. Organoids, which contain one or more cell lineages derived from pluripotent stem cells, could improve our understanding of mechanical force generation between cell layers. For example, early work deriving optic cup organoids derived from embryonic stem cells has demonstrated that epithelial buckling during eye development can be recapitulated and studied *in vitro* (Eiraku et al., 2011). This work has also suggested that retinal morphogenesis, albeit dependent on ECM interactions, can occur independently of the lens. More complete models incorporating mesenchyme components could be used to study epithelial-mesenchymal interactions during sensory organ development, such as our recently developed models that recapitulate the inner ear (Koehler et al., 2013) and skin (Lee et al., 2020) (Fig. 6A). Notably, these organoid models recreate sensory and hair placode morphogenesis. However, despite their recent popularity, there are drawbacks to self-organized organoids, such as organoid-to-organoid variability and inconsistent results across cell lines.

A major research focus today is to produce more controllable and manipulable *in vitro* organoid systems, using both cell-intrinsic and -extrinsic approaches. Cell intrinsic methods, such as genetic engineering, can allow us to perform non-invasive manipulations of forces. For example, recent optogenetic tools have been devised that target cell-cell adhesions (Ollech et al., 2020) or actomyosin contractility (Martínez-Ara et al., 2021 preprint). One notable example is the development of light-inducible Shroom3 (OptoShroom3), which can trigger apical constriction and deform 3D epithelia in neural organoids (Martínez-Ara et al., 2021 preprint) (Fig. 6B). As apical constriction has versatile roles in the development of placodes and lumen formation, OptoShroom3 is a promising tool for wide applications in the study of forces in organoid models.

For extrinsic manipulation of cell cultures, researchers have been leveraging mechanistic understandings from animal models to constrain cells and tissues. Recent work using micropatterning has demonstrated that a combination of geometric confinement and small molecule induction can steer the development of human stem cell sheets from 2D to 3D neural tubes (Karzbrun et al., 2018, 2021; Warmflash et al., 2014) (Fig. 6B). Interestingly, titration of BMP4 in these micropattern cultures has also been shown to induce formation of germ layers and anterior-posterior axes (Simunovic et al., 2019). In other studies, researchers have designed substrates with specific stiffnesses to direct mesenchyme differentiation (Guimarães et al., 2020), or applied shear flows to improve organoid vascularization (Homan et al., 2019). Finally, in several recent groundbreaking studies, introducing geometry into 3D microfabrication has helped to control formation of intestinal crypts (stem-cell zone) in a deterministic manner (Gjorevski et al., 2022; Nikolaev et al., 2020) (Fig. 6B). Such an approach, combined with microfluidics, could replicate the healthy homeostasis of the growing gut and enable the study of interacting dynamics during development. It will be exciting to see how generalizable these approaches will be and whether they could be applied to pattern and sculpt a sensory organ's epithelial, mesenchymal and neuronal cells.

Conclusions and outlook

In this Review, we have surveyed our current understanding of how mechanical cues guide the development of sensory organs, ranging from placode formation to the specialization of sensory epithelia. The physical principles governing the initial stages of cranial placode development, and hair placodes in the skin, share similarities in epithelial dynamics, collective cell migration and force-induced epithelial folding. As the organs become more specified, the integration of forces depends on tissue-tissue interactions that grow in complexity over developmental time. Further study is required to understand how sensory organs integrate mechanical and biochemical cues at later, more functionally mature developmental stages. Microphysiological systems will be essential tools to address the monumental challenge of building tissues with the form and function to see, smell, hear and feel like native organs.

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Competing interests

K.R.K. is an inventor on several patents and patent applications pertaining to the skin and inner organoid models discussed in this Review. The other authors have no competing interests.

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