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Current Opinion in Cell Biology

How to wrinkle a cell: Emerging mechanisms of microridge morphogenesis

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Abstract

Microridges are laterally elongated actin-based protrusions arranged in striking maze-like patterns on the apical surfaces of mucosal epithelial cells. Recent studies have begun to reveal the molecular and mechanical factors that regulate microridge morphogenesis and allow them to adopt their unique properties. Microridges form from the coalescence of short actin-filled precursor protrusions called pegs. Microridge morphogenesis requires the establishment of apicobasal polarity, cortical myosin contraction, and Arp2/3 activity. Mature microridges contain branched actin networks, keratin filaments, and plakin cytolinkers that likely connect the two cytoskeletal elements. Once formed, microridges rearrange by fission and fusion to form increasingly regular patterns. Their highly organized arrangement provides an exciting system in which to study the interplay between molecular signaling and physical forces in the formation of subcellular patterns.

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Current Opinion in Cell Biology 2022, **76:**102088

This review comes from a themed issue on **Cell Architecture**

Edited by **Adele Marston** and **Matthew J. Tyska**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.ceb.2022.102088

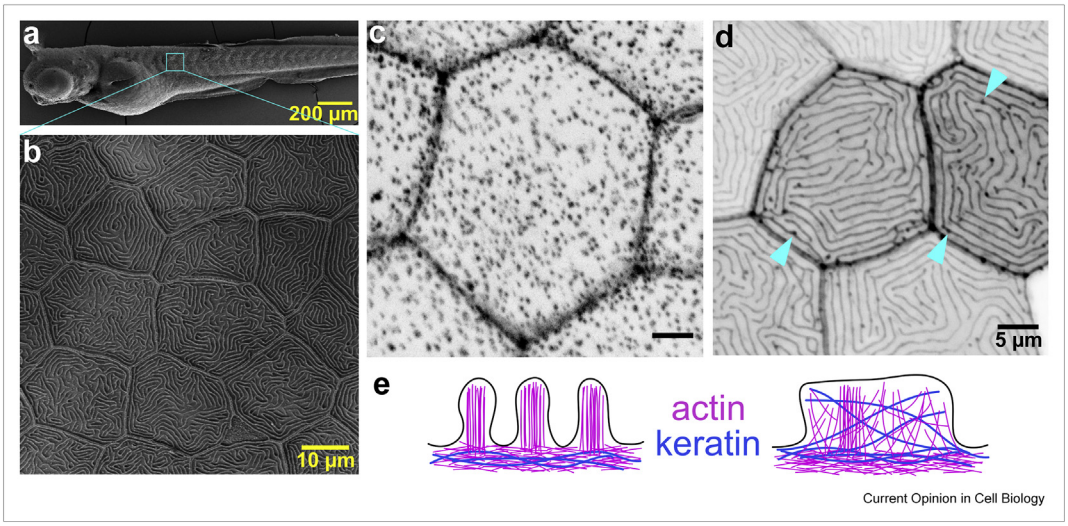
What are microridges and why study them?

Cells make diverse actin-based protrusions to interact with and move through their environments. Over the past few decades, studies of dynamic protrusions in moving cells (e.g., lamellipodia and filopodia) and more stable epithelial protrusions (e.g., microvilli and stereocilia) have uncovered biochemical and mechanical processes that allow the cytoskeleton to shape cells into myriad forms. Microridges, elongated protrusions arranged in elaborate maze-like patterns on the apical surfaces of epithelial cells (Figure 1a–b), have recently emerged as a new model to investigate the molecular basis of protrusion morphogenesis. Why study yet another kind of protrusion? Microridges have distinctive forms and dynamic properties, making them a promising system for discovering new morphogenetic mechanisms. Moreover, their labyrinthine arrangements offer an opportunity to investigate how cells form well-ordered, subcellular periodic patterns.

Microridges are widely found on the apical surfaces of mucosal epithelial cells. They were first identified as protrusions on corneal cells [1], but have since been described on a variety of human tissues, including epithelial cells lining the cornea, esophagus, and kidney [2]. Most recent studies have modeled microridge morphogenesis using zebrafish epidermal cells [3–10] since, unlike cornified mammalian skin, the entire surface of fish skin is a mucosal epithelium made up of microridge-projecting cells, called periderm cells.

Although little research has addressed the function of microridges, it has been speculated that they increase the surface area for retaining mucus on epithelial surfaces [11,12]. This idea was supported by a recent study in which zebrafish embryos were treated with small molecules that inhibit F-actin polymerization and thus disassemble microridges [4]. Surface glycan staining was reduced in cells with disrupted microridges, and persisting surface glycans preferentially coated remaining protrusions [4]. It has also been speculated that microridges serve as membrane reserves, due to their presence on esophageal epithelial cells, which experience stretching [13]. In support of this hypothesis, microridges are lost or fragmented when epithelial cells are stretched during wound healing [14–16] or when membrane homeostasis is disrupted [5]. Microridge defects have yet to be linked to human ailments but, if they indeed help retain mucus, they may be implicated in conditions characterized by vulnerable mucosal tissues, such as dry eye or mouth. Zebrafish mutants lacking mature microridges on their skin could serve as tools for investigating microridge function, but such mutant fish survive to adulthood in laboratory conditions [8], indicating that mucosal immune challenges or wounding may be required to reveal their vulnerabilities.

Figure 1



Microridge structure and development. **a**) Scanning electron micrograph of a 3-day post fertilization (dpf) zebrafish embryo. Box indicates approximate location of periderm cells in panel B. **b**) SEM of microridges on periderm cells of a 3 dpf zebrafish embryo. **c**) Representative image of a periderm cell expressing the actin reporter Lifeact-GFP in a 14 h post-fertilization (hpf) zebrafish embryo. At this stage, periderm cell surfaces are covered by pegs. Images in **c** and **d** were inverted, so high-intensity fluorescence appears black, and low intensity appears white. **d**) Representative image of a periderm cell expressing Lifeact-GFP on a 96 hpf zebrafish. At this stage, periderm cell surfaces are filled by elongated microridges. Arrowheads point to peg-like, actin-dense structures within microridges. **e**) Schematic side view of pegs and microridges.

Microridge structure

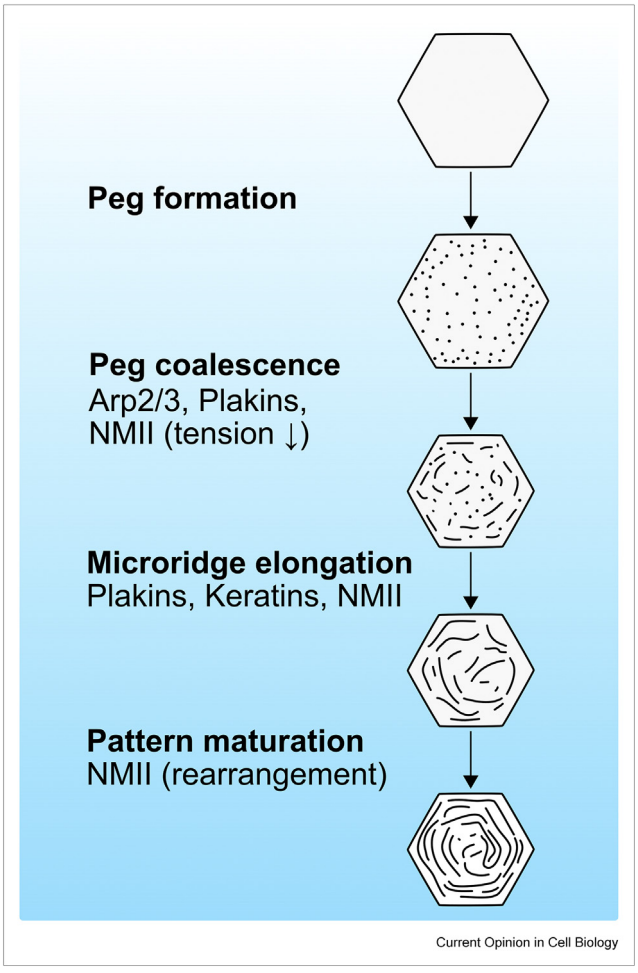
Microridges are a few hundred nanometers tall and can stretch out laterally for tens of micrometers. Like microvilli, they are uniform in height and are filled with actin filaments. Unlike microvilli, however, actin filaments in microridges form complex branching and crosslinked networks [4], rather than tight filament bundles, allowing them to extend laterally. Like in cells with microvilli, microridges project above a "terminal web" containing actin and keratin intermediate filaments [4,17]. Some early electron microscopy studies noted the presence of intermediate filaments not only below, but also within microridges [18,19], and more recent studies confirm that keratin filaments are microridge components [4,8]. Although intermediate filaments have been reported to contribute to late stages of invadopodium morphogenesis [20], the integral contribution of intermediate filaments to the cytoskeletal scaffold in microridges is a strikingly unique feature of these protrusions.

Another distinctive feature of microridges is their formation from precursor protrusions. Older scanning electron microscopy studies noted that cells with predominantly short, finger-like protrusions co-occur in the same epithelium as cells with mature microridges [21,22]. Recent live imaging studies in zebrafish revealed that those smaller protrusions are dynamic, densely filled with actin, and coalesce to form and elongate microridges (Figure 1c–e, Figure 2) [9]. These precursors have been named "pegs" [9] to reflect their distinctive shapes and properties. Notably, pegs never aggregate into clumps, suggesting that mechanical or biochemical constraints ensure that they assemble into linear structures. One ultrastructural study noted that tight actin bundles, like those in microvilli, were embedded within microridges [17], raising the intriguing possibility that the internal peg scaffold is retained when pegs join microridges, perhaps serving as "posts" for a microridge "fence." Indeed, visualizing microridges with fluorescent actin reporters reveals bright spots within microridges that resemble pegs [3,23] (Figure 1d, arrowheads). The formation of microridges from peg precursors distinguishes them from other elongated protrusions, like lamellipodia, which form and grow as unitary structures. Virtually nothing is known about how pegs form, but studying their composition and morphogenesis will be critical for understanding how microridges attain their modularity, stability, and uniform dimensions.

Actin-binding proteins in microridges

The properties of all actin-based protrusions are determined by the identity and regulation of the actin-binding proteins that build them—including nucleators, bundlers, crosslinkers, motors, capping proteins, and severing proteins. Several actin-binding proteins have been identified as microridge components, including proteins associated with branched actin networks (the Arp2/3 complex, cortactin, and WASL) [3,4],

Figure 2



Model of microridge morphogenesis. Molecules involved in distinct morphogenetic steps are indicated.

as well as proteins associated with bundled actin (VASP and α-actinin) [3,17]. Other actin-binding proteins in microridges include ezrin [6], a membrane-actin linking protein, paxillin [24], a component of focal adhesion complexes, and cofilin [4], an F-actin-severing protein. This diverse array of actin-regulatory proteins may indicate that microridges are built from a combination of cytoskeletal architectures—perhaps both a branched network that extends them laterally and bundled components derived from pegs.

Although the roles of most actin-binding microridge proteins have yet to be investigated, inhibiting the Arp2/3 complex with the drug CK666 revealed a requirement for the branch-nucleating complex in microridge morphogenesis (Figure 2) [3,4,9]. Arp2/3 inhibition not only prevented microridge coalescence from pegs, but also caused the actin in mature microridges to disassemble back into peg-like structures in less than an hour [3]. This observation indicates that pegs themselves likely do not contain branched actin, supports the speculation that peg-like structures remain embedded within microridges, and reveals that the actin network within microridges is constantly renewed, similar to actively tread-milling actin within microvilli [25]. Since microridge formation relies on the activity of the membrane-associated actomyosin cortex (see below) [9], and Arp2/3 is required to build the cortex [26], it is not clear if Arp2/3's contribution to microridge formation and maintenance reflects a role in the cortex, within microridges themselves, or in both structures.

A recent study identified two cytolinker proteins of the plakin family [27], envoplakin and periplakin, as key organizers of the microridge cytoskeleton [8]. In zebrafish, envoplakin and periplakin are expressed almost exclusively in microridge-containing periderm cells, and localize to microridges [8,28,29]. These proteins have a coiled-coil-containing "rod" domain that allows them to heterodimerize or oligomerize [30]. Their N-terminal regions bind F-actin and contain plakin domains with the potential to coordinate multiple actin-regulatory proteins [31,32]; their C-terminal regions bind keratin filaments [33,34]. Thus, plakin proteins are well positioned to integrate the two cytoskeletal components of microridges. Indeed, plakin mutants lack mature microridges but retain pegs [8], indicating that plakins are required for peg coalescence (Figure 2). The plakin N-terminus is required to rescue this defect [8], suggesting that their actin-associated activities help integrate pegs into microridges. The keratin-binding plakin C-terminus is not required for peg coalescence, but rather for microridges to stabilize and fully elongate [8], perhaps suggesting that plakins recruit keratin into microridges to provide mechanical strength and stability. Indeed, when fish were treated with an Arp2/3 inhibitor, microridge-associated keratin filaments retained their structure even though actin was severely disrupted [8]. This finding suggests that keratin may help maintain microridge stability, despite frequent actin turn-over within them. Given the requirement for plakins in multiple steps of microridge morphogenesis and their potential to coordinate several activities, better understanding plakin functions will help explain how microridges attain the unique properties that distinguish them from other protrusions.

Apicobasal polarity, membrane trafficking, and cortical contraction regulate microridge formation

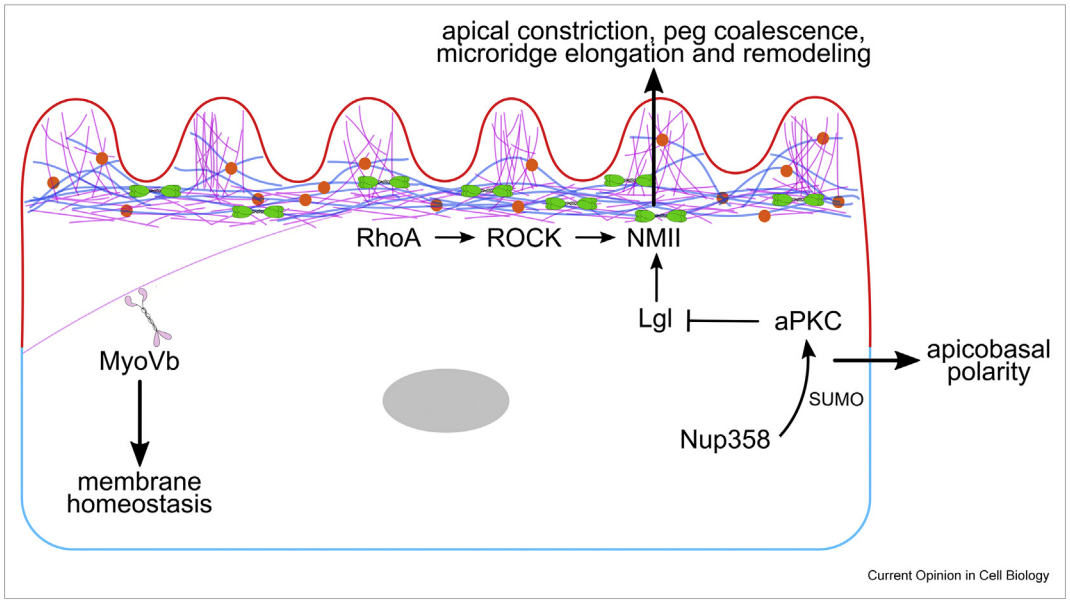
Studying microridges can be valuable not only for revealing how the cytoskeleton builds these unique protrusions, but also for investigating the regulation of universal epithelial properties, including apicobasal polarity, membrane homeostasis, and cortical contractility. For example, specification of apicobasal polarity and

membrane transport are essential for proper microridge formation (Figure 3). Knocking down the myosin Myo5b dramatically increased endocytosis from the apical membrane of periderm cells, resulting in smooth membranes lacking microridges [5]. Myo5b also regulates apicobasal polarity in drosophila follicle cells [35], so it is possible that Myo5b-deficient periderm cells have impaired polarity, which is essential for proper microridge formation [6]. Atypical protein kinase C (aPKC), an apical domain protein regulated by SUMOylation by the nucleoporin Nup358 [7], controls microridge length by removing the basolateral domain protein Lgl from the apical membrane, preventing precocious microridge elongation [6].

Non-muscle myosin II (NMII)-based contraction of the apical cortex, another common epithelial process, was recently found to regulate membrane tension to permit the formation and elongation of microridges from pegs (Figure 2) [6,9]. Zebrafish periderm cells experience pulsatile NMII contractions that drive apical constriction during the first two days of development [9], similar to other systems [36]. Apical area is inversely correlated with microridge length, and computational modeling suggested that cortical contraction lowers membrane tension to allow the coalescence of pegs into elongating microridges [9]. Indeed, treating zebrafish cells with hyperosmolar media promoted rapid apical cell shrinkage and microridge formation [9], demonstrating that reducing membrane tension alone is sufficient to induce microridge elongation from pegs. These results show that cortical contraction not only drives the constriction of epithelial cell surface areas, but can also regulate their three-dimensional topology.

The regulation of microridge morphogenesis by cortical NMII contraction provides an opportunity to investigate how cortical contractility is regulated in space and time to enable a specific, quantitatively sensitive morphogenetic outcome. Microridge formation begins at a stereotyped developmental stage and progresses inwards from cell borders, correlating closely with pulsatile NMII contractions, as predicted by computational modeling [9], thus suggesting that upstream regulators of cortical contraction determine the spatial and temporal progression of microridge morphogenesis. In other systems, cortical NMII activity is regulated by the small GTPase RhoA and its effector the Rho GTPase effector kinase (ROCK) to drive apical constriction [37,38]. Similarly, inhibiting ROCK [9], or expressing constitutively active or dominant negative versions of RhoA [3], in zebrafish periderm cells disrupted apical constriction, microridge formation, and microridge patterning. Another mechanism of NMII regulation is through aPKC, which suppresses NMII activity at the apical membrane by removing the basolateral NMII-interacting protein Lgl, preventing microridge fusion and restricting microridge length [6]. aPKC localization and activity are dependent on another small GTPase, Cdc42 [39] and, interestingly, Cdc42 antagonizes RhoA-mediated NMII activity in cells with microvilli [40]. Better understanding the

Figure 3



Myosin regulation of micoridge morphogenesis. Non-muscle myosin II (NMII) is regulated by the GTPase RhoA and apicobasal polarity pathway to control microridge morphogenesis spatially and temporally. Myosin Vb regulates membrane homeostasis to permit microridge formation.

temporal and spatial regulation of cortical contractility and microridge formation will require determining how these upstream regulatory pathways are activated and coordinated.

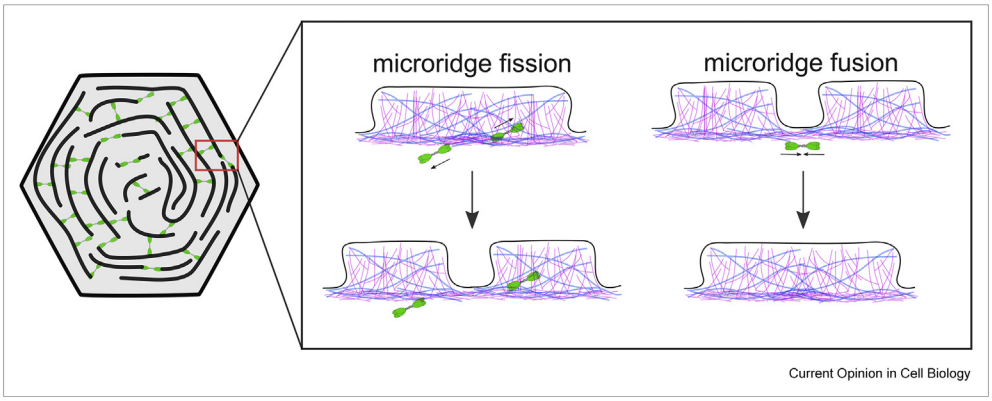
Creating a cellular maze: Microridge patterning across cell surfaces

Perhaps the most striking feature of microridges is their periodic spacing and maze-like arrangement on cell surfaces. Microridges thus offer a rare opportunity to study the formation of subcellular patterns. These highly ordered arrangements develop through a progressive maturation process: following their initial elongation, microridge density increases, their spacing becomes more regular, and they increasingly align with one another [10]. During this process, microridge patterns continuously rearrange through fission and fusion—a microridge can break along its length into two small microridges, and two microridges can fuse end-to-end to form one longer microridge (Figure 4) [3,10]. These unusual fission and fusion events occur with roughly equal frequency, allowing cells to sample different arrangement patterns. The increasing order of microridge patterns as they evolve suggests that they mature towards an energetically favorable state, perhaps optimizing membrane bending energy. Indeed, mature microridge patterns resemble the nematic organization of molecules in liquid crystals [41], in which boundaries between locally aligned regions are associated with an energy penalty.

Like peg fusion and elongation, microridge fission and fusion is driven by cortical NMII contraction [10]. Inhibiting NMII blocks these rearrangements and disrupts microridge density, alignment, and spacing, supporting the hypothesis that rearrangements help microridge patterns mature (Figure 2). High-resolution imaging of the periderm cortex revealed that cortical NMII minifilaments connect adjacent microridges and coordinate rearrangement events by pulling directly on microridges [10]. This arrangement of NMII minifilaments in the cortex is unusual—since minifilaments span the cortex between two microridges, they are oriented radially in the cell, rather than isotropically, as is typical of interphase cells (Figure 4) [42]. This cortical organization and the impact it has on microridge patterning highlights the close relationship between the cortex and apical protrusions. Understanding how this minifilament organization emerges and how it affects contractile activity will provide insight into how cortical architecture affects protrusion formation and patterning.

It is often noted that microridge arrangements resemble complex larger-scale biological patterns, ranging from fish stripes [43] to desert vegetation patterns [44]. Such patterns can arise from signaling interactions obeying Alan Turing's reaction–diffusion model [45,46]. Although Turing model variables are most commonly thought of as concentrations of biochemical signals, integration of mechanical and biochemical factors could extend the original Turing model to explain morphogenesis on many scales [47]. The opposing roles of contraction and tension in microridge formation and elongation [9] may thus fit within this paradigm to explain microridge subcellular patterns. The ability to quantitatively document the development of microridge patterns over time [10,23], the tissue and organism diversity in microridge pattern features [2], and the ability to molecularly alter microridge patterns (for example, by manipulating RhoA activity [3]), provides an exciting opportunity to uncover mathematical principles, physical factors, and molecular processes that create these amazing biological patterns.

Figure 4



Microridge fission and fusion. Schematics to the right show how NMII minifilaments in the cortex pull on microridges to execute rearrangement events. The schematic on the left shows that by attaching to microridges, NMII minifilaments orient radially in the periderm cell cortex.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Acknowledgments

We thank Andrew Goryachev, Evan Takayoshi, Khaled Nassman and Kadidia Adula for insightful comments on the manuscript.

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** of outstanding interest

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