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Leah C. Biggs, Christine S. Kim, Yekaterina A. Miroshnikova, Sara A. Wickström

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Mechanical forces in the skin: roles in tissue architecture, stability, and function

Leah C. Biggs[1-3] 0000-0002-4990-8664, Christine S. Kim[4,5] 0000-0001-7512-7960, Yekaterina A. Miroshnikova[1-5] 0000-0001-9771-4963, Sara A. Wickström[1-5*] 0000-0001-6383-6292

[1]Helsinki Institute of Life Science, Biomedicum Helsinki, University of Helsinki, Finland

[2]Wihuri Research Institute, Biomedicum Helsinki, University of Helsinki, Finland

[3] Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Finland

[4]Max Planck Institute for Biology of Ageing, Cologne, Germany

[5] Cologne Excellence Cluster for Stress Responses in Ageing-associated diseases (CECAD), University of Cologne, Germany

CORRESPONDING AUTHOR
Sara A. Wickström sara.wickstrom@helsinki.fi
TWITTER **@WickstromLab**

SHORT TITLE
Mechanical properties and regulation in the skin

ABBREVIATIONS

AJ	Adherens junction
BM	Basement Membrane
DEJ	Dermal-Epidermal Junction
ECM	Extracellular Matrix
K	Keratin
TJ	Tight junction
SRF	Serum response factor
YAP	Yes-associated protein-1

ABSTRACT

Tissue shape emerges from the collective mechanical properties and behavior of individual cells and the ways by which they integrate into the surrounding tissue. Tissue architecture and its dynamic changes subsequently feed back to guide cell behavior. The skin is a dynamic, self-renewing barrier that is subjected to large-scale extrinsic mechanical forces throughout its lifetime. The ability to withstand this constant mechanical stress without compromising its integrity as a barrier requires compartment-specific structural specialization, and capability to sense and adapt to mechanical cues. This review discusses the unique mechanical properties of the skin and the importance of signals that arise from mechanical communication between cells and their environment.

INTRODUCTION

The skin is a multilayered organ positioned between the organism and the outer world, thus serving a critical barrier function. This barrier function is mediated by the epidermis, a stratified epithelium consisting of an outermost cornified layer, a number of differentiated layers, and the basal stem cell layer anchored to the basement membrane (BM).

Below the BM lies the dermis, composed primarily of extracellular matrix (ECM) which is produced to a large extent by dermal fibroblasts (Jiang and Rinkevich 2018; Nyström and Bruckner-Tuderman 2019), and the dermal adipose tissue, which participates in cutaneous homeostasis through mediating immune responses, hair follicle cycling, and wound healing (Guerrero-Juarez and Plikus 2018; Shook et al. 2016).

The skin is subject to constant mechanical stresses, including stretch and compression, due to body movement, touch, and growth of underlying tissues. Here, we discuss the structural and mechanical compartmentalization that allows skin to withstand external forces without compromising its integrity and function. Further, we address how these mechanical signals are converted to biochemical cues to alter cell behavior.

Epidermis, a dynamic cellular composite organized by adhesions and the cytoskeleton

Epidermal keratinocytes preserve tensile strength of the tissue and bear variable loads, while at the same time executing dynamic, homeostatic turnover and maintaining a tight, bidirectional barrier. Keratinocytes are tethered to each other via cell-cell adhesions and, in the basal layer, also to the BM via cell-matrix adhesions. The adhesions are linked to dense cytoskeletal networks of actin, microtubules, and keratins, which collectively determine the mechanical properties of the cell (Pegoraro et al. 2017; Rübsam et al. 2018; Simpson et al. 2011). Thus, forces within the epidermis are transmitted both within and across layers (Fig. 1). Of the cytoskeletal networks, keratin intermediate filaments (IF) exhibit the greatest ability to withstand mechanical load and strain and are thus critical for tissue integrity (Janmey et al. 1991; Ramms et al. 2013; Seltmann et al. 2013; Wagner et al. 2007). In contrast, the viscoelastic actin network is critical for force generation and propagation (Xu et al. 1998). Microtubules are organized in a centrosomal array in the basal layer, but become cortically localized upon differentiation. They modulate epidermal integrity via fortifying the strength of cell-cell adhesions by recruiting myosin to apply tension and mechanically link them to the actin cytoskeleton (Hatzfeld et al. 2017; Sumigray et al. 2012).

A similar division of labor exists among adhesions. Classical cadherins form actin-linked adherens junctions (AJs) that are critical for cell-cell attachment, force generation, and mechanotransduction (Simpson et al. 2011). AJs also coordinate the assembly of the other intercellular junctions, namely desmosomes that associate with keratin IFs and provide tight adhesion and mechanical resistance, as well as tight junctions (TJs) that prevent water loss from the organism and provide additional intercellular cohesion (Furuse et al. 2002; Niessen 2007). Importantly, both the cytoskeleton and cell-cell junctions exhibit layer-specific organization and composition, reflecting layer-specific functional and mechanical requirements (Jacob et al. 2018; Rübsam et al. 2017).

Basal stem/progenitor layer

Stem/progenitor cells within the basal layer must balance self-renewing cell divisions and BM adhesion with differentiation and concomitant departure to the suprabasal layers. Two mechanisms have been proposed: perpendicular spindle orientations that position one daughter directly to the suprabasal layer, and delamination where cells detach from the BM and move upwards (Lechler and Fuchs 2007; Miroshnikova et al. 2018) (Gonzales and Fuchs 2017; Mesa et al. 2018; Watt and Green 1982) (Fig. 1). The current consensus is that both modes likely operate in parallel, exploiting mechanisms of stem cell competition as an additional homeostatic control (Ellis et al. 2019; Liu et al. 2019). Delamination requires combined loss of cell-matrix contacts and dynamic junctional rearrangements to reduce cortical stiffness of the delaminating cell (Miroshnikova et al. 2018; Nekrasova et al. 2018; Rübsam et al. 2018). In contrast, perpendicular spindle orientation is coordinated by cell-matrix adhesions and polarity regulators, a highly conserved process that depends on microtubule interactions with membrane-bound polarity complexes (Muroyama and Lechler 2012; Seldin et al. 2016; Williams et al. 2011). Regardless of the model, coordination of cell adhesion and mechanics appears critical for proper departure of differentiating cells from the basal layer.

In addition to dynamic regulation of adhesion, mechanical integrity and resilience must be maintained in all epidermal layers, and this is mediated by the keratin IFs. The basal layer expresses K5/K14 that are responsible for the stiffness of undifferentiated keratinocytes (Ramms et al., 2013, Seltmann et al., 2013). Defects in the K5–K14 network cause basal keratinocyte fragility in and account for their rupture upon mechanical stress. Similarly, dominant negative mutations in K10/K1 cause suprabasal keratinocyte fragility (Coulombe and Lee 2012), highlighting the role of keratins in the mechanical stability of the epidermis and the basal layer as a hotspot of mechanical stresses between the stiff BM and the suprabasal layers.

Suprabasal layers

The spinous layers exhibit more complex cell-cell adhesions and cytoskeletal networks to allow continuous cell renewal as well as rigid and mechanically strong desmosome-intermediate filament complexes (Broussard et al. 2017). High mechanical resistance and absorption barrier function is maintained in the adjacent granular layers (Furuse et al. 2002; Rübsam et al. 2017) which display the most intense network of F-actin in the epidermis (Fig. 1). While not as strong as the desmosome-keratin network, the actin network facilitates TJ assembly by recruiting actin nucleator Arp2/3 (Zhou et al. 2013). Notably, TJs are formed only in the third granular layer despite expression of necessary components in all layers. TJ formation requires mechanotransduction through cadherins and formation of tension-high lateral AJs that occurs specifically in this layer. These features highlight the unique, layer-specific distribution of tension within the epidermis and the role of this tension in barrier maintenance (Rübsam et al. 2017; Tunggal et al. 2005) (Fig. 1).

Positioned at the interface with the external environment, the upper layers of the epidermis withstand large amounts of load and are thus of high mechanical strength. The dry, dead outermost cornified layer is highly keratinized and stiff to prevent dehydration, abrasion, and microbial insults to the underlying layers (Candi et al. 2005). Together, these data outline the mechanical interconnectivity of the epidermis, which maintains its mechanical integrity, and define critical roles for adhesion and the cytoskeleton in regulation of tissue.

The dermal-epidermal junction, guardian of skin mechanical stability

Critical for mechanical stability of the skin is the BM, a thin but rigid structure of ECM that physically separates the epidermis and dermis (Humphrey et al. 2014). This zone, the dermal-epidermal junction (DEJ), is composed of cellular and extracellular components produced by both dermal and epidermal cells. BM evolved to ensure skin integrity upon mechanical challenge, as highlighted by human skin blistering diseases that result from mutations in genes associated with the DEJ (Has and Nyström 2015; Nagy and McGrath 2010; Nyström and Bruckner-Tuderman 2019).

Precise mechanical characterization of skin BMs *in vivo* is challenging due to technical limitations of measuring intact tissue. Elasticity of mouse or human skin BM has not been reported, but atomic force microscopy measurements of chicken and mouse retinal BMs indicate stiffness ranging from 1-1000 kPa (Candiello et al. 2007; Henrich et al. 2012), whereas the dermis is in the KPa range (Kao et al. 2016; Saavedra et al. 2018). This indicates that the BM is the stiffest structure encountered by epidermal cells (Fig. 1).

The skin BM is a composite of four main components: laminins (-332 and -511), collagen IV, perlecan, and nidogen, but its precise composition differs between hair follicles and interfollicular skin (Sugawara et al. 2008; Yurchenco 2011). Of these, laminins are considered to account for the functional differences between BMs through their integrin-binding roles. Both laminin-332 and -511 interact with integrins $\alpha3\beta1$ and $\alpha6\beta4$ thereby providing the essential adhesive connection to both actin-linked focal adhesions and intermediate filament-linked hemidesmosomes, respectively (Carter et al. 1991; Delwel et al. 1994; Niessen et al. 1994; Stepp et al. 1990). Laminin-332 is the most abundant skin BM protein, and its lack in humans leads to generalized severe junctional epidermolysis bullosa with a lethal course due to extreme skin fragility and blistering (Meng et al. 2003; Nakano et al. 2002). Collagen IV forms a flexible, extensively cross-linked oligomeric network within the BM and is thought to be primarily responsible for providing stiffness to the BM (Khoshnoodi et al. 2008) and its depletion in the embryo results in reduced mechanical stability of the BM (Poschl et al. 2004). Compound mutation of nidogen 1 and 2 results in perinatal lethality due to heart defects arising from disrupted BM morphogenesis (Bader et al. 2005). However, in contrast to the glomerular BM, where patients with COL4A1 and COL4A2 gene mutations suffer from mechanical instability of the BM, these patients with mutations in Collagen IV-encoding genes do not display obvious skin phenotypes (Has and Nyström 2015). Thus, the precise role of Collagen IV in the mechanical stability of postnatal skin remains unclear.

Dermis, the force-bearing structure of the skin

In contrast to the epidermis, the dermis consists largely of ECM and is sparsely populated with fibroblasts. Although dermal architecture differs in mouse compared to human, there are key common features. The collagen-dominated dermal ECM is arranged in a basket weave-like structure that provides mechanical strength (Ferguson and O’Kane 2004). The tensile strength and compressibility of fiber-forming collagens make them key proteins of the dermal ECM. Although 28 types of collagen have been identified, collagens I and III comprise the bulk of human skin collagen (Smith et al. 1986). Whereas the fiber-forming collagens and most likely their crosslinks define the rigid mechanical structure of skin, elastic fibers confer extensibility and recoil of collagen, thus enabling stretching of the skin (Gosline et al. 2002). Additional components, such as proteoglycans and glycoproteins, create an osmotically active, hydrated interstitial space, while matricellular proteins, such as tenascins, organize paracrine signaling without contributing to the bulk mechanical properties (Mecham 2012). Thus, within the dermis, mechanical stress is primarily dissipated across collagen and elastin fibrils. Long-range elastin fibers dictate mechanical behavior at small stresses and strains and during recoiling, while large stresses are dissipated by linearizing wavy collagen networks, effectively stiffening the skin (Sherman et al. 2015).

The dermis can be divided into the upper, papillary dermis, which is more densely populated with fibroblasts and contains thin collagen fibrils and elastin, and the underlying reticular dermis characterized by thick collagen bundles and fewer cells. Fibroblasts are responsible for depositing and remodeling connective tissue matrix during morphogenesis, injury, fibrosis, and scarring (Jiang and Rinkevich 2018). They have long been recognized as a heterogeneous pool of cells with specialized functions, gene expression profiles, and contractile properties critical for ECM deposition (Driskell and Watt 2015; Geiger and Bershadsky 2001; Jiang and Rinkevich 2018; Korosec et al. 2019; Philippeos et al. 2018; Rinn et al. 2008; Sorrell and Caplan 2004; Tabib et al. 2018). Recently, markers to distinguish various fibroblasts were identified, allowing lineage tracing and deciphering functional significance of distinct cell lineages/states (Driskell et al. 2013). Current evidence indicates that the subsets of fibroblasts that deposit ECM can be distinguished based on transient, early embryonic expression of Engrailed-1 (Rinkevich et al. 2015). While the

Engrailed-negative cells produce a provisional ECM made up of fibronectin, Engrailed-positive cells deposit collagens to form mature ECM with high tensile strength (Jiang et al. 2018). Another model suggests that collagen deposition late during embryonic development is, in fact, guiding the fibroblast cell fate switch (Rognoni et al. 2018). Additional work is required to uncover the exact mechanisms by which the differential ECM architecture is produced and maintained.

Biophysical forces modulate cell fate decisions in the epidermis

In addition to serving as force-bearing and –transducing entities, skin cells also actively sense the physical properties of their environment, and respond by activating signaling cascades to control their fate and function. An elegant example of skin mechanoresponsiveness is the establishment of global tissue polarity, mediated by asymmetric localization of planar cell polarity components, where mechanical forces within the skin are sufficient to direct this asymmetric localization (Aw and Devenport 2017).

Matrix rigidity and topology

Beyond providing structural support to the basal keratinocytes, BM directs cell fate and function by signaling via adhesion receptors, modulating the bioavailability of growth factors and morphogens, and providing biomechanical cues through dynamic changes in rigidity. BM adhesion has long been known to regulate epidermal stemness, as keratinocytes deprived of adhesion differentiate unless their β1 integrins are ligated (Adams and Watt 1990; Adams and Watt 1989). This effect depends on the ability of the integrins to gauge matrix rigidity. Interestingly, instead of reading bulk stiffness of the matrix, keratinocytes respond to the mechanical feedback of the collagen anchored to the substrate by modulating their focal adhesion size (Trappmann et al, 2012) and increasing proliferation through integrin-mediated mechanosensing pathways (Samuel et al. 2011), indicating that rigidity sensing can operate on multiple scales to impact keratinocyte behavior, including migration (Wickert et al. 2016) and proliferation (Kenny et al. 2018).

Additional topographical features of the ECM, including pore size, fiber diameter, and feature elevation, are sensed by cells (Young et al. 2016). Due to the heterogeneous nature of BMs, it is not surprising that each microenvironment presents a unique topographical fingerprint that can locally influence cell behavior. Human BM exhibits undulations and epidermal stem cell patterning is dependent on mechanical forces exerted at intercellular junctions. Cell density is highest, and thus cell-matrix adhesion area the lowest, at the bases of undulations, whereas stem cells accumulate at tips (Helling et al. 2019; Mobasseri et al. 2019). Consistently, topographies that prevent cell spreading or restrict surface coverage promote differentiation through a contractility-dependent mechanism (Zijl et al. 2019). Although the precise mechanisms are unclear, these findings suggest that the 3-dimensional organization and topological features of the ECM play instructional roles in mediating epidermal stem cell fate decisions. Coupling cell fate decisions to dynamic changes in ECM properties likely allows cells to adjust their behavior to the changing needs of the tissue.

Cell shape, size, and contractility

Cells generate and sense forces to control their fate. This is of particular importance for the ability to adjust skin surface area to changing body size. The switch between lateral expansion of the monolayer and delamination events to trigger stratification coincides with a mechanical transition of the monolayer from a fluid-like to a solid state (Miroshnikova et al. 2018). *In vivo* work further indicates that the basal layer of the actively stratifying embryonic epidermis exists in such a solid-like state. Proliferation in this jammed cell layer causes crowding and lateral compression of cells, which is sufficient to trigger differentiation (Miroshnikova et al. 2018). This is consistent with studies showing that individual human epidermal stem cells cultured on micropatterned surfaces undergo differentiation when cell spreading is restricted (Connelly et al. 2010). Restriction of cell spreading triggers reduction in cellular cortical tension, and increased cell-cell adhesion subsequently triggers delamination of cells from the basal layer, allowing epidermal cells to couple cell fate, mechanics, and position (Miroshnikova et al. 2018) (Fig. 2). Consistent with the idea of cell size dictating cell fate, in the adult epidermis where cell divisions are rare, space liberated by delamination triggers the division of a neighboring cell (Mesa et al. 2018). Thus cell shape and size balance division and differentiation of stem cells in both embryonic and adult epidermis. Stem cells in the embryo constantly cycle to provide sufficient material for the lateral expansion of the epidermis, whereas during adult homeostasis stem cells divide only upon demand to replace delaminating cells.

Mechanosensitive signaling networks

Mechanical cues from a variety of sources ultimately alter gene expression programs to regulate cell fate. Transcriptional regulation has been shown to be mediated via mechanosensitive transcription factors, notably actin-regulated transcription co-regulators MAL-SRF and YAP/TAZ. YAP/TAZ transcriptional activity is dependent on dephosphorylation and subsequent nuclear translocation. YAP phosphorylation is regulated, among others, by Src and LATS kinases in response to changes in monolayer density sensed by AJs and ECM stiffness through integrins to specify stem cell fate under differential tension (Totaro et al. 2017). Activation of nuclear YAP to coordinate transcription with its partner TEAD promotes cell growth and inhibits terminal differentiation in the epidermis (Elbediwy et al. 2016; Lee et al. 2008; Schlegelmilch et al. 2011; Zhang et al. 2011) (Fig. 2). Which specific transcriptional targets of YAP are critical for these effects remain unclear.

Serum response factor (SRF) is another mechanosensitive transcription factor that works in concert with its coactivator MAL to control cell fate (Miralles et al. 2003). SRF is maintained in the cytoplasm by binding to G-actin; when F-actin is increased via Rho activity or other signals, SRF translocates into the nucleus to activate transcription (Miralles et al. 2003; Sotiropoulos et al. 1999). MAL-SRF transcription factors regulate expression of cytoskeleton and contractility-related genes required for proper cell division and induction of differentiation in response to restricted cell-matrix area (Connelly et al. 2010; Luxenburg et al. 2011) (Fig. 2).

In addition to transcription factor-mediated signals, direct propagation of mechanical force into the nucleus to modify chromatin state and transcriptional activity has been described. Rapid, direct mechanical loading of the nucleus induces chromatin stretching and transcription (Tajik et al. 2016). If mechanical force persists, chromatin becomes condensed, leading to suppression of gene expression (Heo et al. 2016; Le et al. 2016) (Fig. 2). In epidermal stem cells, this type of long-term stress results in reduced nuclear actin content, which attenuates global transcription. This allows the polycomb repressive complex 2 through tri-methylation of histone 3 at lysine 27 to condense chromatin at differentiation gene promoters, preventing keratinocyte differentiation (Le et al. 2016) (Fig 2). Interestingly, keratins have also been observed in the nucleus, with roles in cell cycle regulation and transcription (Escobar-Hoyos et al. 2015; Hobbs et al. 2015). However, these observations have been made in states of inflammation and cancer, and the role of nuclear keratins in homeostasis remains unclear.

Taken together, epidermal cells integrate changes in cell density and thereby cell shape and actin dynamics to both activate specific signaling pathways and tune overall transcriptional activity and chromatin state to regulate differentiation. This allows the tissue to balance lateral expansion and stratification to adjust skin surface area to the changing needs of the organism (Fig. 2).

CONCLUSIONS

Development and homeostatic self-renewal are highly organized and efficient processes, achieved partly by tight genetic control. However, ability to integrate signaling noise and fluctuations to establish robustness is paramount. Recent interdisciplinary research has enabled rapid progress in understanding how cells utilize changes in their mechanical landscape as effective and self-adjusting means of communication. Further, the tight mechanical coupling of cell density, mechanics, fate, and position has emerged as a key mechanism of morphogenesis and homeostasis. Critical next steps are constructing tools to dynamically quantitate cell-ECM, cell-cell, and tissue mechanics *in vivo* during cell fate changes and couple them to uncovering the precise molecular mechanisms by which cell shape and tension changes connect tissue architecture and cell fate.

CONFLICT OF INTEREST

The authors state no conflict of interest.

DATA AVAILABILITY STATEMENT

No datasets are related to this article

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AUTHOR CONTRIBUTIONS (CRediT statement)

Writing - Original Draft Preparation: LCB and SAW; Writing - Review and Editing: CSK and YAM; Visualization: SAW, CSK and YAM: Supervision: SAW

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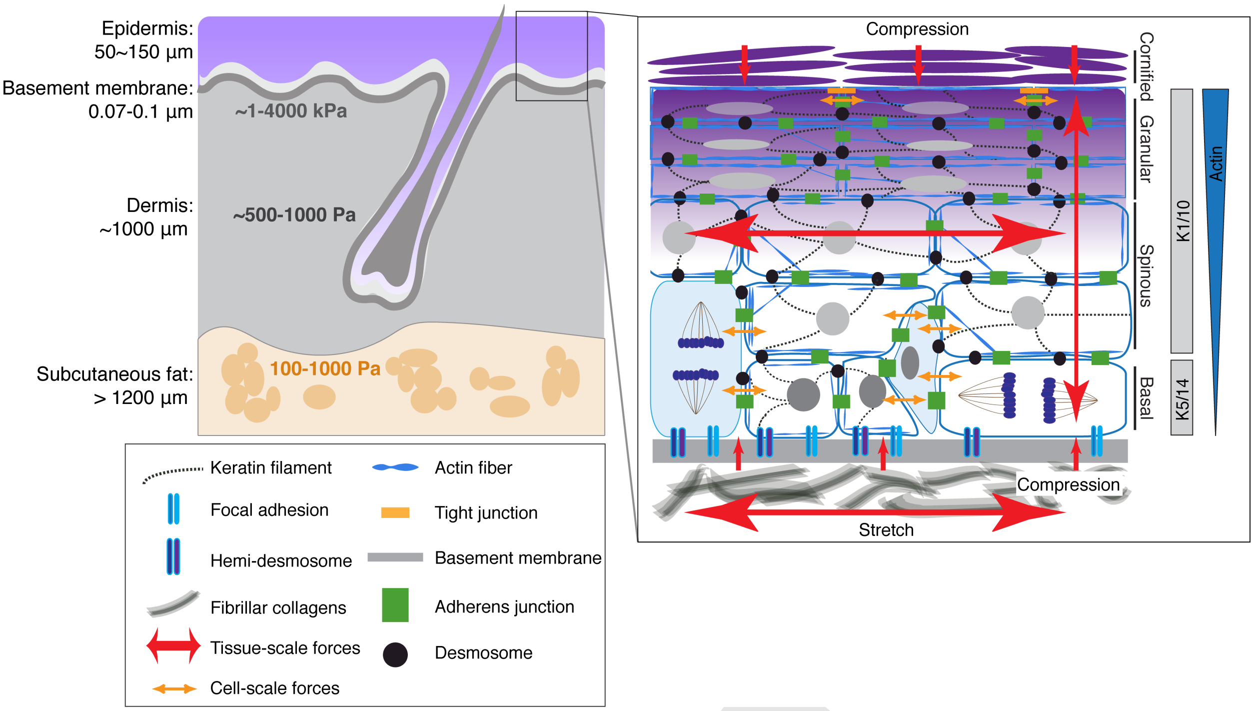
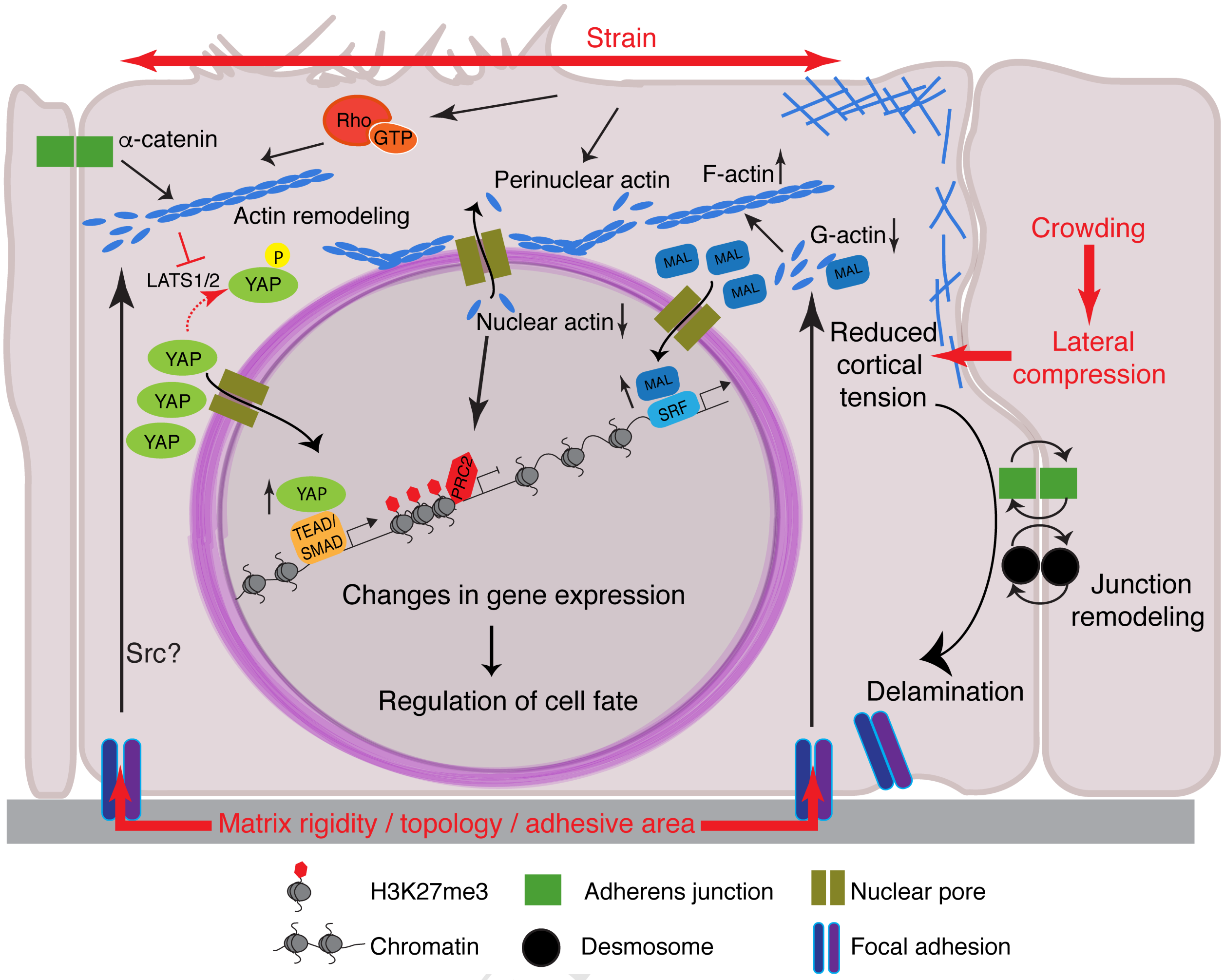
FIGURE LEGENDS

Figure 1. Mechanical properties and force coupling in the skin. The skin consists of the epidermis, dermis, and subcutaneous fat, with compartment-specific mechanical properties and the basement membrane representing the stiffest structure (Pascals (Pa); left panel). The epidermis (right panel) is a load bearing element subjected to large-scale forces including compression and stretch (red arrows), which are transmitted within and across layers (red arrows). The epidermis consists mainly of keratinocytes connected via actin-linked adherens junctions and keratin-linked desmosomes. The basal layer adheres to the basement membrane via integrins that form actin-linked focal adhesions and keratin-linked hemidesmosomes. Tight junctions are formed only in the last viable layer through adherens junctions through mechanotrandsuction by high-tension adherens junctions. Differentiating cells depart from the basal layer through perpendicular cell divisions and delamination. Cell shape changes, divisions and movement within the layers generate forces between cells (orange arrows), modulating cell fate and position.

Figure 2. Mechanical forces alter actin organization and gene expression to control keratinocyte fate and position. Keratinocytes sense basement membrane rigidity/topology, strain and cell layer density (red arrows), that trigger intracellular signaling, altering the polymerization state of the actin cytoskeleton to induce changes in chromatin organization and gene expression. YAP can be activated (de-phosphorylated) by increased matrix stiffness through integrins and Src, by decreased cell density through alpha-catenin, or by strain through RhoA, resulting in YAP nuclear entry to collaborate with TEAD to induce pro-proliferative gene expression.

Restricted cell adhesion area promotes MAL/SRF by limiting availability of G-actin that sequesters MAL, promoting MAL nuclear entry and SRF-dependent differentiation gene expression. Perinuclear actin polymerization by strain depletes nuclear G-actin to repress transcription, allowing polycomb repressive complex 2 (PRC2) to silence differentiation genes. Basal layer crowding also decreases cortical tension and induces junction remodeling to promote delamination, thus coupling cell fate with position.


Epidermis:
50~150 µm
Basement membrane:
0.07-0.1 µm
Dermis:
~1000 µm
Subcutaneous fat:
> 1200 µm
~1-4000 kPa
~500-1000 Pa
100-1000 Pa
Compression
Cornified
Granular
Spinous
Basal
K1/10
K5/14
Actin
Compression
Stretch
Keratin filament
Actin fiber
Focal adhesion
Tight junction
Hemi-desmosome
Basement membrane
Fibrillar collagens
Adherens junction
Tissue-scale forces
Desmosome
Cell-scale forces


Strain
Rho
GTP
α-catenin
Actin remodeling
Perinuclear actin
F-actin↑
G-actin↓
LATS1/2
P
YAP
YAP
YAP
YAP
MAL
MAL
MAL
MAL
Nuclear actin↓
MAL
SRF
↑ YAP
TEAD/ SMAD
PRC2
Crowding
Lateral compression
Reduced cortical tension
Junction remodeling
Delamination
Changes in gene expression
Regulation of cell fate
Src?
Matrix rigidity / topology / adhesive area
H3K27me3
Chromatin
Adherens junction
Desmosome
Nuclear pore
Focal adhesion