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REVIEW

Regulatory mechanisms governing epidermal stem cell function during development and homeostasis

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ABSTRACT

Cell divisions and cell-fate decisions require stringent regulation for proper tissue development and homeostasis. The mammalian epidermis is a highly organized tissue structure that is sustained by epidermal stem cells (ESCs) that balance self-renewal and cell-fate decisions to establish a protective barrier, while replacing dying cells during homeostasis and in response to injury. Extensive work over past decades has provided insights into the regulatory mechanisms that control ESC specification, self-renewal and maintenance during different stages of the lifetime of an organism. In this Review, we discuss recent findings that have furthered our understanding of key regulatory features that allow ESCs to establish a functional barrier during development and to maintain tissue homeostasis in adults.

KEY WORDS: Development, Epidermis, Homeostasis, Skin, Stem cells

Introduction

The epidermis functions as a protective barrier for the body and is crucial in preventing microbial intrusion, external environmental insults and dehydration (reviewed by Gonzales and Fuchs, 2017; Belokhvostova et al., 2018). The epidermis is composed of proliferative and undifferentiated epidermal stem cells (ESCs) located in the basal layer, and several layers of differentiated and non-proliferative suprabasal cells (Blanpain and Fuchs, 2006). In mice, epidermal development begins at around embryonic (E) day 8.5, when the monolayer ectoderm starts expressing the transcription factor p63, which is crucial for the specification of the epidermal lineage (Mills et al., 1999; Yang et al., 1999; Blanpain and Fuchs, 2009). ESCs or basal cells self-renew by symmetric cell division (SCD), which is dictated by the proper apico-basal orientation of the mitotic spindle (Poulson and Lechler, 2010; Xie and Zhou, 2017). At E14.5, epidermal stratification initiates when the orientation of cell division becomes perpendicular to the plane of the epidermis, resulting in asymmetric cell division (ACD) (Lechler and Fuchs, 2005; Ray and Lechler, 2011). The epidermal tissue structure is established before birth and is composed of spinous, granular and stratum corneum layers – each of which contributes to the formation of the functional barrier required for survival (Fig. 1). Cornified cells from the outer layer are continuously shed off the skin surface and renewed by ESC mitotic activity throughout the lifetime of an organism (Gonzales and Fuchs, 2017). In the adult epidermis, a strict balance between ESC self-renewal and differentiation is required to maintain epidermal homeostasis and a functional barrier (Blanpain and Fuchs, 2009; Nassar and Blanpain, 2012). Hence, an enormous effort has been undertaken to understand how ESCs meet the demands of tissue development and homeostasis, and what regulatory mechanisms allow these adaptations. In this Review, we highlight recent studies that have expanded our understanding of ESC regulation during development and that have uncovered the landscape of ESC dynamics during adult homeostasis.

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Epidermal stem cell regulation during development and homeostasis

Regulation of epidermal stem cell division during development

Epidermal development is driven by the precise regulation and coordination of oriented cell division. Planar division or symmetric cell division (SCD) expands the pool of ESCs required for rapid tissue growth during development (Fig. 2A). Perpendicularly oriented division or ACD results in the formation of one basal cell and one suprabasal daughter cell that goes on to differentiate and contribute to epidermal stratification (Fig. 2A) (Lechler and Fuchs, 2005; Ray and Lechler, 2011). Notably, differentiation of basal cells to form the differentiated layers of the epidermis is accompanied by changes in cellular geometry (discussed in this section), and nuclear architecture and microenvironment (Gdula et al., 2013). Tipping the balance between SCD and ACD during epidermal development has severe implications in epidermal differentiation and barrier formation.

Oriented cell division relies upon mitotic spindle orientation that is controlled by intrinsic and external factors (Poulson and Lechler, 2010; Xie and Zhou, 2017). The cell cortex is polarized by the asymmetric distribution of partitioning defective protein 3 (Par3), partitioning defective protein 6 (Par6) and atypical protein kinase C (aPKC) (Fig. 2B) (Knoblich, 2008; Pearce et al., 2010). During division, Par3 recruits inscuteable (Insc), which in turn induces the localization of Leu-Gly-Asn (LGN) protein, which is encoded by G-protein signaling modulator 2 gene (*Gpsm2*) (Fig. 2B) (Schober et al., 1999). LGN belongs to a protein complex that anchors spindle astral microtubules to the cell cortex, orienting the spindle and dictating the direction of division (Siller and Doe, 2009). In ESCs undergoing ACD, Par3 is localized to the apical cortex of the cell, which orients the spindle anchoring proteins perpendicular to the basement membrane (Fig. 2B) (Ray and Lechler, 2011; Williams et al., 2014). Although the importance of SCD and ACD during epidermal development has been well established, several recent studies have identified the novel factors that regulate division orientation during skin development.

Dainichi and colleagues reported that phosphoinositide-dependent kinase 1 (PDK1) is a crucial upstream regulator of ACD. Epidermal loss of PDK1 results in arrested epidermal stratification with PDK1-null basal cells undergoing mostly SCD and not ACD division (Dainichi et al., 2016). Substrates of PDK1, such as AKT and PKC, are involved in regulating several cellular

Development (2020) 147, dev194100. doi:10.1242/dev.194100

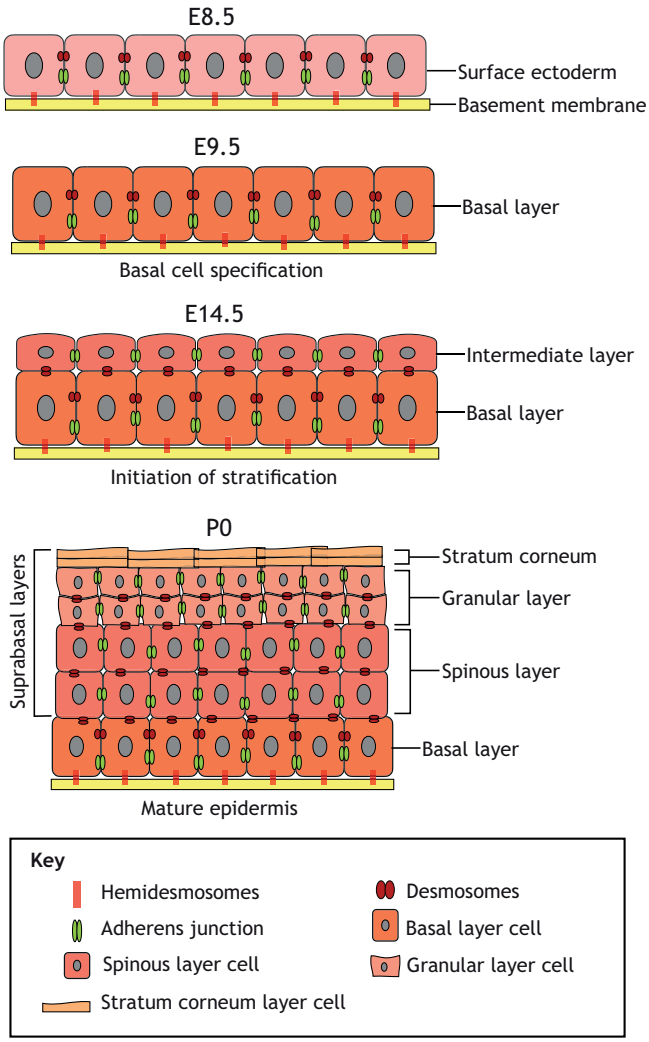

Fig. 1. Epidermal stratification during development. Mammalian epidermal development is a multistage process consisting of cell fate specification, commitment, differentiation and stratification. The epidermis originates from a single embryonic ectoderm layer at embryonic day (E) 8.5. Upon epidermal fate commitment at E9.5, the surface ectoderm becomes the epidermal basal layer. Epidermal stratification begins at E14.5, when the basal layer gives rise to the intermediate layer, which eventually differentiates upward to establish a stratified epidermis. By birth, postnatal day (P) 0, the epidermis is fully formed with a single basal layer and differentiated suprabasal layers that consist of the spinous, granular and stratum corneum layers.

functions, including cell polarity and ACD (Pearce et al., 2010). PDK1 localizes to the apical cortex of the basal cells during ACD and promotes recruitment of protein kinase C (PKC) and Par3. Loss of PDK1 in the epidermis also results in the significant repression of Notch downstream targets, Hes5 and Hey2. Notch3 protein activation restores impaired expression of differentiation markers in PDK1-null basal cells (Dainichi et al., 2016). Therefore, in addition to the essential regulation of ACD for epidermal stratification, PDK1 regulates the Notch-dependent transcriptional network that is required for the switch from a basal to a suprabasal differentiation program.

Like PDK1, Tbx3 (a T-box transcription factor) has recently been shown to regulate oriented cell division and modulate Notch signaling to promote differentiation (Ichijo et al., 2017). Contrary to PDK1 function, depletion of Tbx3 results in diminished SCD, while ACD remains unperturbed (Ichijo et al., 2017). Moreover, the number of *Hes1*-expressing cells is significantly decreased in the suprabasal layer of Tbx3-null epidermis, suggesting that Tbx3 also regulates Notch signaling. Analysis of Tbx3 chromatin association showed enrichment of Tbx3 binding at the genomic region of the transducing-like enhancer of split 3 (*Tle3*), a transcriptional co-repressor that directly interacts with Hes1 (Han et al., 2010). Together, these studies show that PDK1 and Tbx3 play a role in orienting cell division as well as in the regulation of Notch signaling to promote epidermal development, suggesting that the Notch signaling cascade is essential for this process.

A member of a conserved serine-threonine kinase, mTORC2, along with its accessory protein Rictor (Ric), has also been shown to regulate ACD (Hoeffer and Klann, 2010; Ding et al., 2016). Epidermal depletion of Ric results in a stratified epithelium with reduced thickness caused by fewer granular and stratum corneum layers. This phenotype is due to reduced incidences of ACD in basal cells contributing to the defective maturation of epidermal layers. Moreover, the apical localization of Par3 is significantly reduced, and fewer basal cells depict an apical crescent of LGN, suggesting that mTORC2 mediates cell polarization and spindle orientation to promote ACD during epidermal differentiation (Ding et al., 2016).

Although the role of apical-basal polarity cues in directing ACD divisions to drive stratification is clear, how parallel SCD is established is largely unknown (Segalen and Bellaïche, 2009; Goodrich and Strutt, 2011; Devenport, 2016). Recent findings elucidated the role of planar cell polarity (PCP) proteins, which control polarization along an epithelial plane, in the regulation of SCD (Box et al., 2019). The basis of PCP establishment is the localization of a subset of core PCP proteins to opposing domains along the cell cortex and in the epidermis, while conserved PCP proteins cadherin EGF LAG seven-pass G-type receptor 1 (Celsr1) and Van Gogh-like 2 (Vangl2) localize to the basolateral cell junctions (Devenport et al., 2011; Aw et al., 2016). PCP protein asymmetry coincides with the earliest stages of stratification, but whether these proteins control stratification via spindle orientation or by other mechanisms was previously unknown (Devenport and Fuchs, 2008). Box and colleagues reported that the loss of the PCP protein Vangl2 results in a bias toward ACD, driving increased epidermal maturation and producing a significantly thicker epidermis. Surprisingly, this defect is not a result of failed cortical PCP cues or spindle orientation, but the result of compromised cellular geometry. Cells that are flatter and wider tend to divide in parallel when compared with taller and narrower cells, which divide perpendicular to the epithelial plane. Incidentally, loss of PCP proteins results in an increased occurrence of taller and narrower cells, indicating that the height-to-width ratios, i.e. the 3D geometry, of basal cells plays a crucial role in driving planar versus perpendicular cell division (Box et al., 2019). In line with these studies, Luxenberg and colleagues showed that, between E12.5 and E14.5, basal cells decrease their surface area, become rounded and acquire an anterior-posterior orientation from a dorsoventral one, indicating a striking change in their physical properties. Inhibition of cell shape changes is accompanied by the mislocalization of the PCP protein Celsr1 and by defects in PCP establishment. These changes in basal cell geometry are mediated by WD repeat domain 1 (Wrd1), which induces the disassembly of actin filaments by enhancing the activity of cofilin (Chu et al., 2012; Lechuga et al., 2015). Loss of Wrd1 in the epidermis leads to increased F-actin levels that disrupt the actin cytoskeleton of the basal cells (Luxenburg et al., 2015). The actin cytoskeleton network is important for mediating cortical tension that promotes cell shape changes observed during epidermal development (Fernandez-

Development (2020) 147, dev194100. doi:10.1242/dev.194100

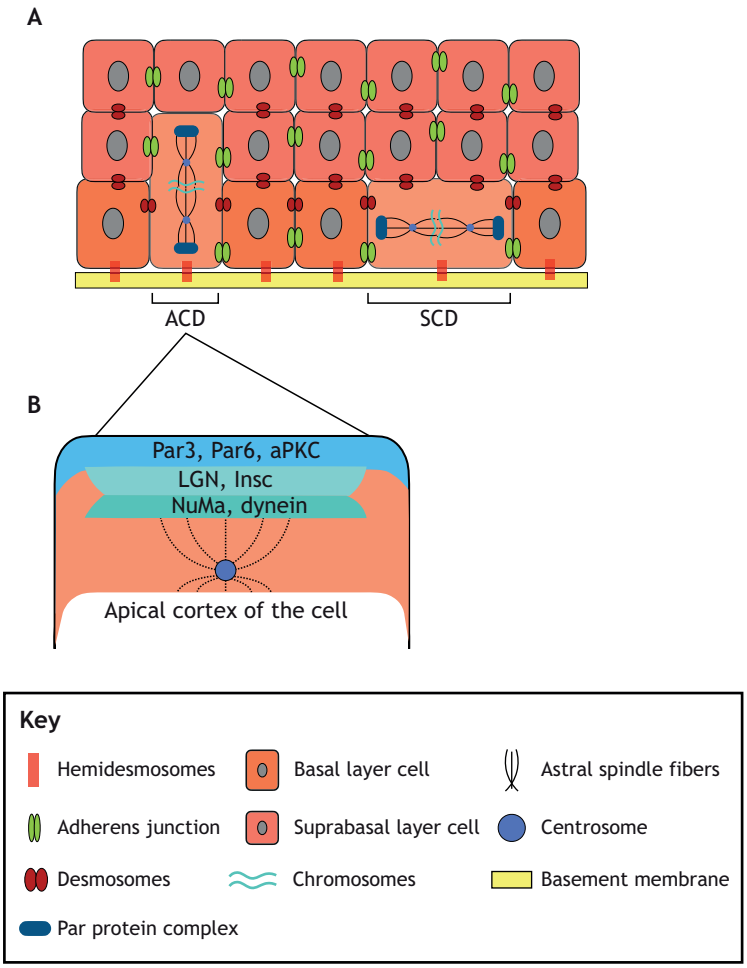

Fig. 2. Oriented cell division during epidermal development. (A) Epidermal development is driven by oriented cell divisions. Divisions parallel to the basement membrane are called symmetric cell divisions (SCDs), which result in the formation of two basal cells. Divisions perpendicular to the basement membrane are called asymmetric cell divisions (ACDs), which give rise to one basal cell and one suprabasal cell that fuel epidermal stratification. (B) Schematic of the molecular machinery located at the apical cortex of a basal cell undergoing ACD. Par complex proteins and atypical protein kinase C (aPKC) localizes to the apical membrane of the cell. Par3 binds to inscuteable (Insc), which in turn recruits G-protein-signaling modulator 2 (LGN) to the apical cortex of the cell. Once anchored, LGN recruits nuclear mitotic apparatus protein 1 (NuMa), a microtubule-binding protein. NuMa directs the mitotic spindle through dynein. Together, this complex orients the mitotic spindle perpendicular to the basement membrane, aiding ACD.

Gonzalez et al., 2009). Wrd1-deficient basal cells have reduced cortical tension, indicating the function of cytoskeletal organization in promoting basal cell architecture for epidermal differentiation (Luxenburg et al., 2015).

Miroshnikova and colleagues proposed a model where the cells of the epidermis are elastic with distinct mechanical properties that influence the balance between cell renewal and differentiation. The proliferation of basal cells induces crowding, which results in certain basal cells becoming elongated with decreased cell surface, and this shape change is directly correlated to changes in cellular mechanics and cell-cell adhesion. Elongated basal cells have low cortical tension, reduced contact with the basement membrane and increased cellular adhesion with the upper differentiated layer. Together, these events promote the cell-fate changes of these basal cells and induce their entry into stratification. Upon loss of contact from the basement membrane, the differentiated progeny in the suprabasal layer switch from P-cadherin- to an E-cadherin-dominated cell adhesion, which results in a high cortical tension state, allowing the cells to stabilize their position, promoting stratification (Miroshnikova et al., 2018). In conclusion, this study not only provides insight into how cortical tension establishes boundaries between epidermal layers to influences differentiation but also highlights how cadherins have specific roles in different layers of the epidermis.

Along with division orientation, recent findings suggest that cell competition plays a functional role during mammalian epidermal development. Ellis and colleagues asked whether unfit cells in the basal epidermis are eliminated by neighboring fit cells via a cell-competition-dependent mechanism. They established an *in vivo* 'winner/loser' model by generating mosaic embryonic skin with $Mycn^{+/-}$ and $Mycn^{+/+}$ cells, where Mycn is a Myc isoform crucial for cell competition (Moreno and Basler, 2004). They found that an 'unfit' $Mycn^{+/-}$ cell has a proliferative disadvantage, undergoes apoptosis and is cleared by its neighboring 'fit' $Mycn^{+/+}$ cell through engulfment machinery. Notably, upon epidermal stratification, engulfment genes are downregulated in basal progenitors and 'loser' cells no longer undergo apoptosis. These observations suggest that, at late embryonic time points, 'loser' cells are outcompeted via a different mechanism. Indeed, the spindle orientation of 'loser' basal cells in E15.5 mosaic skin was skewed toward perpendicular ACD, indicating that ACD-mediated differentiation aids in clearing unfit basal cells after epidermal stratification (Ellis et al., 2019). In conclusion, this study reveals the modes of cell competition in monolayer and stratified developing epidermis, and indicates how the epidermis has evolved to have an alternative mechanism to eliminate unfit cells that escape apoptosis in the single-layered epithelium.

Together, these studies have uncovered the intrinsic regulatory mechanisms that aid in basal cell division orientation and the cellular dynamics that are crucial for dictating ESC cell-fate decisions during development.

Epidermal stem cell dynamics in adult tissue homeostasis and wound healing

It has been long debated whether adult interfollicular epidermis (IFE) is composed of a single stem cell (SC) population or distinct

Development (2020) 147, dev194100. doi:10.1242/dev.194100

populations of SCs that differentially contribute to epidermal homeostasis (Clayton et al., 2007; Doupé et al., 2012; Mascré et al., 2012). Different models of ESC dynamics and differentiation in the adult epidermis have been proposed. The single-progenitor (SP) hypothesis states that each basal cell is equivalent and generates progeny that have equal probability to self-renew or differentiate (Fig. 3A) (Clayton et al., 2007; Doupé et al., 2012). The stem cell-committed progenitor (SC-CP) model states that there is a hierarchy of slow-cycling stem cells that generates actively dividing committed progenitor cells (Fig. 3B) (Mascré et al., 2012). The two SC (2×SC) population model states that the IFE houses two independent populations of stem cells that divide differentially in the epidermis (Fig. 3C) (Sada et al., 2016). Recent studies addressed these models of epidermal differentiation, investigating how ESCs make cell-fate decisions and what mechanisms aid the integration of differentiated cells into the existing tissue structure.

By conducting lineage-tracing experiments, Sada and colleagues found that there are spatially distinct distributions of label-retaining cells (LRCs) and non-LRCs in both the tail and back skin IFE, with distinct gene expression profiles. The LRC-dense regions are predominantly bigger and surround the circular non-LRC regions that are flanked by hair follicles, surprisingly correlating with the previously reported 'interscale' and 'scale' regions of the tail skin (Gomez et al., 2013). On investigating the division and differentiation kinetics of LRCs versus non-LRCs, Sada and colleagues found that both populations proliferate and differentiate, albeit at different rates. The non-LRCs are fast-cycling SCs, and non-LRC skin regions may have a faster overall regeneration speed to maintain homeostatic balance. In response to injury, LRCs and non-LRCs exchange functions by migrating and repopulating their neighboring atypical territories to fuel differentiation in order to establish a functional barrier. However, in the long term after an injury, these cells are lost from their atypical locations while reestablishing their segregated territories, as seen during homeostasis (Sada et al., 2016). In line with this study, single cell transcriptomics performed by Joost and colleagues identified cellular heterogeneity in the IFE of dorsal back skin. Although basal cells seem to have a distinct gene signature, they followed a single terminal differentiation program (Joost et al., 2016). Together, these studies indicate that, although the distinct SC populations give rise to unique lineages, the basal SC heterogeneity in the IFE of the back skin could be the result of the cells being in different cellular states corresponding to their differentiation trajectory.

Contrary to the previous studies, Rompolas and colleagues reported that a single population of cells in the IFE is responsible for maintaining epidermal homeostasis, and no LRCs or slow-cycling cells are observed. They developed an *in vivo* live-imaging system to capture the fate of individual basal cells and track their differentiation potential in the ear IFE. They found that, with each division, a cell undergoes unbiased stochastic fate choice to give rise

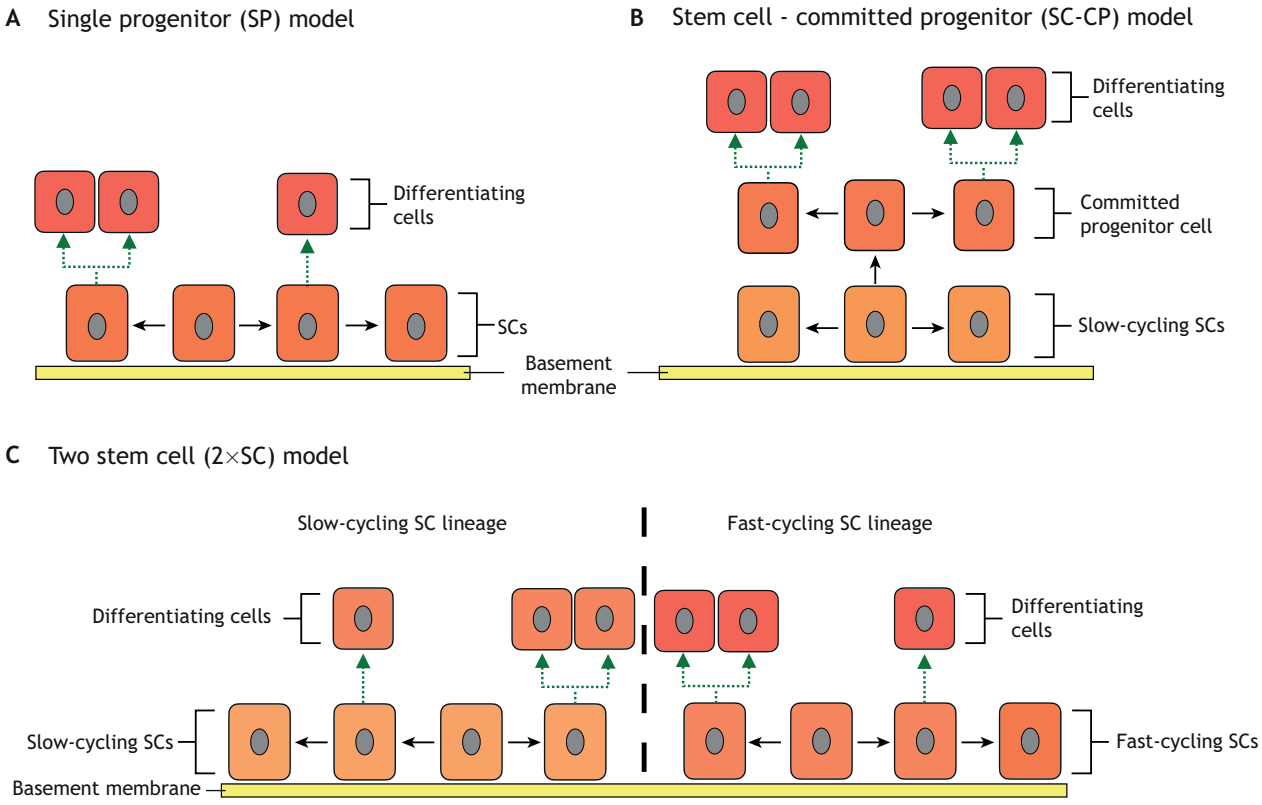

Fig. 3. Cell-proliferation models of adult epidermal stem cells during homeostasis. Several lineage-tracing experiments in the adult interfollicular epidermis (IFE) have led to conflicting models of epidermal stem cell (ESC) proliferation during homeostasis. (A) The single progenitor (SP) model states that each stem cell (SC) has an equal potential of self-renewal or differentiation, and these cell-fate decisions are stochastic. Each basal SC can proliferate to give rise to either two SCs, two differentiated cells or one SC and one differentiated cell. (B) The SC-CP model proposes a hierarchy of rare slow-cycling SCs, which divide to give rise to SCs or committed progenitor (CP) cells. The CP cells are a rapidly dividing pool of cells that are biased towards differentiation, hence establishing a scenario where SCs have to continually undergo proliferation to fuel cells required to maintain tissue homeostasis. (C) The 2×SC model proposes the IFE is composed of two populations of SCs: one fast cycling and one slow cycling. Each population of SCs make stochastic fate choices, as stated in A. The 2×SC model postulates that skin regions with fast-cycling SCs would have faster regeneration rates compared with the regions with slow-cycling SCs.

Development (2020) 147, dev194100. doi:10.1242/dev.194100

to two basal SCs or to a differentiating cell. These findings suggest that division symmetry does not imply fate commitment; rather, sister cells temporally coordinate cell-fate choices. Next, they asked how these random cell-fate decisions contribute to organized suprabasal layers. They found that, rather than a single basal SC contributing to its immediate upper differentiated layers in a perimeter-confined discrete column, newly differentiated cells transit through the layers and align themselves into columns to take up the space occupied by their predecessors (Rompolas et al., 2016). This study proposes that epidermal homeostasis is maintained by spatiotemporal coordination of both basal and differentiated layers. Each basal cell is born as an uncommitted SC with equal chances to proliferate or differentiate that are temporally coordinated by sibling SCs. After fate commitment, the cells funnel themselves into a pre-existing spatial organization.

It has been shown that a density-dependent mechanism drives basal cell-fate choice in embryos (Miroshnikova et al., 2018). However, it is not clear whether basal cell self-renewal is influenced by the differentiation of neighboring cells in the adult epidermis. By developing a spatiotemporal map of all division and differentiation events occurring within a large region of tissue and over a long period of time, Mesa and colleagues shed light on how the differentiation of a single basal SC is influenced by the cell-fate choices of neighboring cells. Contrary to what is observed in the developing embryo, where the proliferation of basal cells drives differentiation, they found that, in the adult tissue, a differentiation event taking place in a neighboring cell results in the self-renewal of an adjacent SC to balance the cell numbers in the basal layer. They further showed that delamination of a cell from the basal layer results in a significant expansion of neighboring basal SCs, which triggers the cell to self-renew (Mesa et al., 2018). This observation posits a model that a differentiation event provides space in the basal layer that allows the neighboring SC to progress through the cell cycle to complete self-renewal.

To address the opposing reports regarding SC populations in IFE, Piedrafita and colleagues conducted extensive genetic lineage-tracing experiments and coupled them with mathematical modeling, employing previously unused parameters to address the issue of whether the SP model represents epidermal differentiation at different body sites. Using genetic tools for lineage tracing, they did not observe the presence of LRCs in various epidermal tissues, with the exception of the interscale epidermis in the tail. Using the same approach, they also eliminated the 2×SC model, because they did not observe multiple populations of cells dividing at different rates. They showed that, with the exception of the tail, the basal layers of the epidermis at multiple body sites divide at a unique average rate with highly homogenous cell cycle periods consistent with the SP model. As the SP, SC-CP and 2×SC models generate development of similar clonal features over time, it is difficult to distinguish them using lineage-tracing data. Therefore, to investigate which model of basal SC is relevant in the epidermis via mathematical modeling, they incorporated the average division rate – a parameter that has been generally overlooked in previously reported stochastic models. Consistent with the SP model, they reported the presence of neutral competition between basal cells in which clonal dynamics resulted from stochastic cell-fate choices, and a cell-division event generated one proliferating and one differentiating cell (Piedrafita et al., 2020). Although their revised computational method could not discard the possibility that the SC-CP model was operative in the interscale of the tail epidermis, basal cell behavior in the scale region aligned with the SP model.

Recent work by Dekoninck and colleagues dove deeper to uncover the clonal dynamics of the basal cells of adult tail IFE using CONFETTI-lineage tracing, proliferation kinetics, single cell–RNA sequencing and mathematical modeling. Notably, they uncovered the clonal dynamics of basal cells during epidermal expansion, which requires an imbalance between basal cell proliferation and differentiation to accommodate the need to produce cells for tissue expansion. They found that, during tissue expansion of the tail IFE, basal cells make stochastic fate choices between division and differentiation at a single-cell level; however, at a population level, the fate choice is tipped toward self-renewal. Together, this ensures a constant density of basal cells in the IFE and a constant ratio of basal to suprabasal cells to maintain proper epidermal thickness. Interestingly, once the animal reaches stable body size during adulthood, the clonal dynamic of the IFE basal cells switches from imbalanced to balanced, supported by the fact that the proliferative rate of basal cells in adults decreases over time, but the ratio between basal to suprabasal cells remains constant (Dekoninck et al., 2020). Interestingly, these studies also identified that postnatal tissue expansion is fueled by a single population of equipotent basal cells in both scale and interscale regions, rejecting the hypothesis of a SC-CP model of clonal dynamic in the postnatal interscale. However, single-cell sequencing identified heterogeneity in the cell types with varying differentiation trajectories in the interscale of adult tail IFE, supporting the SP-CP clonal dynamic of the tail interscale (Piedrafita et al., 2020). Overall, the data from past and recent studies that have used well-established lineage-tracing methodology along with improved mathematical modeling strategies support the hypothesis that basal SCs in the adult mouse IFE largely follow the stochastic SP model of clonal expansion, where fate choices of each basal SC depends on a differentiation event taking place in a neighboring cell. This process allows the basal layer to maintain an optimal balance of cell numbers during homeostasis. Alternatively, basal SCs of the mouse tail interscale region follow the SC-CP model, in which basal cells are composed of both slow cycling basal SCs and quickly dividing committed progenitor population. Interestingly, similar SC-CP model seems to explain the behavior of human epidermal IFE cells (Box 1). Future studies need to be carried out to dissect what molecular events dictate the differences in basal SC clonal dynamics between scale and interscale regions of the mouse IFE, and whether these mechanisms are conserved between human and mouse.

Although the aforementioned studies have shown that basal cell division and differentiation during epidermal homeostasis is a stochastic phenomenon, Liu and colleagues have reported that epidermal homeostasis is coordinated by cell competition mediated by collagen XVII (Col17a1), a component of the hemidesmosomes that connect the basal cells to the basal lamina, which is crucial for maintaining homeostasis (Hatzfeld and Magin, 2019 Liu et al., 2019b). Col17a1 expression is downregulated in aged epidermis, resulting in the destabilization of the hemidesmosomes, leading to basal cell delamination and epidermal thinning, a phenomenon observed in aged skin (Langton et al., 2016; Watanabe et al., 2017). Liu and colleagues showed that basal cell clones in young skin express high levels of Col17a1, contrary to the clones in aged skin, and showed that basal cell clone sizes positively correlate to Col17a1 expression. Moreover, Col17a1^{+} cells undergo SCD, which mechanistically pushes out Col17a1^{-} cells undergoing perpendicular ACD for delamination. Indeed, reduced expression of Col17a1 in basal cells of aged skin results in increased ACD and epidermal thinning. Therefore, Col17a1-dependent SCD generates the mechanical driving force that promotes the horizontal spread of

Development (2020) 147, dev194100. doi:10.1242/dev.194100

Box 1. Human epidermal stem cells

Most of our understanding of human epidermal SC homeostasis and regenerative potential come from *in vitro* culture or epidermal graft studies. Initial clonal analysis of primary human keratinocytes identified three types of clonogenic keratinocytes: the holoclones, with the greatest reproductive capacity; the meroclones, which contain a mixture of cells of different growth potential; and the paraclones, which contain cells with a short lifespan (Barrandon and Green, 1987). Interestingly, this study also identified that aged donor keratinocytes gave rise to lower proportions of holoclones and to a higher proportion of paraclones, indicating that regenerative capacity of human epidermal keratinocytes decreases with age. Intriguingly, holoclones possess all the hallmarks of stem cells and are capable of giving rise to meroclones and paraclones that depict properties of fast cycling progenitors (De Luca et al., 2006). Moreover, past and recent studies have shown that holoclone-forming cells are crucial for successful epidermal grafts in humans and are required for long-term epidermal renewal (Pellegrini et al., 1999; Hirsch et al., 2017). Together, these observations imply that, unlike the mouse IFE basal cells, which follow a SP model of clonal dynamics where each basal cell is equipotent, the human basal layer is heterogenous, and the human IFE basal SCs follow a SC-CP model for clonal dynamics where there is a hierarchy of stem cells that generates actively dividing committed progenitor cells.

Col17a1^{+} 'winner' cells to maintain homeostasis and counteract aging (Liu et al., 2019b). Together, Liu and colleagues show that cell competition along with SCD, rather than stochastic cell-fate division, is crucial for the quality control of basal SCs to maintain epidermal homeostasis.

Signaling and transcriptional axis governing epidermal development and homeostasis

Signaling and transcriptional regulators of epidermal development

Several signaling pathways, such as the Wnt signaling pathway (reviewed by Veltri et al., 2018) and the Notch signaling pathway, serve as a major differentiation cue in the epidermis (Okuyama et al., 2008; Massi and Panelos, 2012; Nowell and Radtke, 2013). Previous studies determined that the loss of *Ift88*, a gene required in cilium biogenesis, results in stunted terminal differentiation and is accompanied by a reduction of canonical Notch signaling (Croyle et al., 2011; Ezratty et al., 2011). However, how ciliogenesis modulates Notch signaling has been largely unexplored. Recent studies determined that the Notch-processing enzyme presinilin 2 (Psen2) localizes to the basal body of cilia, and this localization is mediated by a small GTPase, ADP-ribosylation factor 4 (ARF4). The localization of Psen2 corresponds to the activity of the Notch signaling pathway. Whereas Notch activity is highest in the early differentiating cells, late differentiated layers have diminished Notch activity and lack cilia. In line with these observations, the loss of Psen in the epidermis is coupled with a loss of Notch activity in the early differentiating layers of the epidermis and leads to differentiation defects (Ezratty et al., 2016). This study shows that the basal body of cilia harbors additional proteins that are atypical to the cilia to promote intercellular communication during development.

Receptor-interacting protein kinase 4 (RIPK4), a member of the RIPK/serine threonine kinase family, is a downstream effector of several signaling pathways (Holland et al., 2002). Although RIPK4 has been shown to play an essential role during epidermal development, the requirement of its catalytic activity was recently highlighted (Holland et al., 2002; De Groote et al., 2015; Oberbeck et al., 2019). Epidermis expressing the catalytically inactive form of RIPK4 exhibits epidermal hyperplasia, proliferative suprabasal cells and the absence of stratum corneum, confirming the crucial role of the kinase activity of RIPK4 for epithelial differentiation. Moreover, epidermis expressing catalytically inactive RIPK4 exhibits similar differentiation defects to those seen in epidermis lacking interferon regulatory factor 6 (IRF6), which plays an essential role during epidermal development and acts via a Notch-dependent mechanism (Ingraham et al., 2006; Richardson et al., 2006; Restivo et al., 2011). Biochemical and genetic studies show that RIPK4 directly phosphorylates and activates IRF6, whereas the epidermal expression of IFR6 with mutations at residues Ser413 and Ser424 (targets of RIPK4-mediated phosphorylation) results in epidermal differentiation defects. Moreover, the loss of the kinase activity of RIPK4 and loss of IRF6 in the epidermis result in a change of the gene expression of a highly correlated subset of genes, including those involved in lipid metabolism pathways (Oberbeck et al., 2019). Chromatin immunoprecipitation (ChIP) studies identified direct targets of IRF6, including grainyhead-like protein 3 homolog (Grhl3), patatin-like phospholipase domain-containing protein 1 (Pnpla1) and ceramide synthase 3 (Cers3). Interestingly, mice lacking Grhl3 exhibit altered lipid processing and epidermal barrier defects, and the mutation of *PNPLA1* or *CERS3* in humans results in barrier disruption and skin defects (Ting et al., 2005; de la Garza et al., 2013; Eckl et al., 2013; Grond et al., 2017). Consistent with these findings, Urwyler-Rösselet and colleagues showed that epidermal differentiation defects seen in RIPK4-null epidermis are accompanied by problems in lipid organization and processing (Urwyler-Rösselet et al., 2018). Together, these studies reveal that the RIPK4-IRF6 axis drives the transcriptional program essential for lipid composition of the stratum corneum, which, when perturbed, can lead to disrupted epidermal barrier function.

Several studies established the cross-talk between miRNAs and the Notch pathway during cancer progression, but its interaction in the developing epidermis is yet to be fully uncovered (Majidinia et al., 2018). When miR-184, an evolutionary conserved miRNA, is depleted in the epidermis, it leads to abnormal epidermal differentiation with thick spinous layers indicative of dysregulation of the Notch-p63 signaling axis (Shalom-Feuerstein et al., 2012; Nagosa et al., 2017). The loss of *miRNA-184* leads to increased p63 expression and decreased levels of Notch and its downstream target Hes1, a negative regulator of p63, indicating that *miR-184* tilts the balance between Notch and p63 expression in the epidermis (Nguyen et al., 2006). In line with these observations, *miR-184*-binding sites are identified in the 3′ UTR regions of p63 and F1H1, a negative regulator of Notch activity, and miRNA-184 directly binds to and represses F1H1 to promote Notch activity in the developing epidermis (Nagosa et al., 2017). This in turn decreases p63-mediated regulation and establishes the Notch-p63 signaling balance that promotes a proper differentiation program.

Transcription factor p63 is expressed in basal cells and is essential for maintaining ESC stemness, proliferation and ACD, whereas the p63-null epithelium remains as a monolayer of nonproliferating cells (Yang et al., 1999; Soares and Zhou, 2018). Although molecular cues controlled by p63 in the epidermis continue to be thoroughly investigated, recent studies have identified key regulators of p63. Inhibitors of histone acetyltransferases (INHATs) are part of a multiprotein complex capable of inhibiting the acetyltransferase activity of p300/CBP and PCAF (Seo et al., 2001). Nir (Noc21), an INHAT, associates with hypoacetylated histones (Hublitz et al., 2005). Ablation of Nir in mouse epidermis results in severe barrier formation defects, which include nonstratification of the epidermis and partial detachment or complete loss of epidermis at birth. RNA-seq analysis of Nir-null

Development (2020) 147, dev194100. doi:10.1242/dev.194100

epidermis revealed that while epidermal genes are downregulated, ectodermal genes are strongly upregulated, suggesting that Nir is required for shifting the ectodermal transcriptional repertoire to that of a stratified epidermis. ChIP studies showed Nir is recruited to the p63 gene promoter and inhibits the acetylation of H3K18, which when hyper-acetylated leads to misregulated p63 expression. Moreover, p63 regulated proteins that are essential for ACD are also downregulated in Nir-null epidermis, leading to nonstratification of the epidermis (Duteil et al., 2018). In conclusion, Nir plays a key role in epithelial stratification and barrier development by directly regulating p63 expression.

In line with this work, several chromatin regulators have been shown to control epidermal development (reviewed by Miroshnikova et al., 2019). For example, the Polycomb group (PcG) of proteins, which are crucial epigenetic regulators, have emerged as key regulators of epidermal differentiation during development (Dauber et al., 2016). PcG proteins are classified into two multi-subunit complexes: Polycomb repressive complex 1 and 2 (PRC1 and PRC2; Simon and Kingston, 2013). The mammalian PRC2 complex consists of Eed, Suz12 and Ezh1/2 core subunits that tri-methylate H3 on lysine residue 27 (H3K27me3). Even though there are several mammalian PRC1 complexes, each complex contains an E3 ubiquitin ligase, RING1A or RING1B, which catalyzes the monoubiquitylation of H2A on lysine residue 119 (H2AK119ub; Cohen et al., 2020). Previous reports have shown that depletion of any of the PRC2 core subunits results in the accelerated formation of differentiated granular and cornified suprabasal layers (Ezhkova et al., 2009). Although PRC1 and PRC2 are known to co-regulate gene transcription, recent studies show epidermal loss of PRC1 alone results in epidermal phenotypes different from that of PRC2. In contrast to PRC2, the loss of PRC1 results in the loss of epidermal integrity and results in epidermal fragility and skin blistering (Cohen et al., 2019). RNA-seq analysis of PRC1-null epidermis identified genes such as Lama3, Dst and Col17a1, which are known to be mutated in skin blistering disorders and are also upregulated in PRC1-null epidermis. ChIP-seq analysis of Ring1B confirmed that genes downregulated in PRC1-null epidermis were directly bound by Ring1B, whereas no PRC2 binding at these genes was observed. These data show that PRC1 functions independently of PRC2 to regulate cell adhesion and cytoskeleton genes to maintain epidermal integrity. In addition to regulating adhesion genes, PRC1 also activates epidermal fate-promoting lineage genes, independent of PRC2, during development (Cohen et al., 2018). Although the most popular dogma of Polycomb canonical function postulates that both PRC1 and PRC2 facilitate their mutual recruitment to target genes to ensure transcriptional repression, these studies have identified that PRC1 has PRC2-independent noncanonical functions that activate expression of genes essential for epidermal lineage specification and the maintenance of epidermal integrity.

Although our understanding of the transcriptional and epigenetic control of ESCs during development has broadened over the years, the post-transcriptional regulation of ESCs mediated by RNA-binding proteins (RBPs) remains elusive. An RBP, Y-box-binding protein 1 (YBX1), has been shown to modulate protein synthesis and enhance translation of cancer stem cell factors (Di Costanzo et al., 2012; El-Naggar et al., 2015). Using a human organotypic culture system it has been shown that YBX1 partners with DDX6, an RNA helicase, to control ESCs by binding to the 3′ UTR of the self-renewal regulators Cdk1 and Ezh2 to facilitate their translation (Wang et al., 2015). Kwon and colleagues showed that in the developing mouse epidermis, YBX1 maintains epidermal basal cells by inhibiting the translation of senescence-promoting cytokines. The loss of YBX1 results in the decreased proliferation of basal cells and consequently in reduced epidermal thickening. The loss of YBX1 results in the increased translation of cytokine proteins that directly bind to the 3′ UTR of cytokines IL8 and CXCl1, which, when upregulated, lead to senescence-related phenotypes (Kwon et al., 2018). In conclusion, this study provides insight into how RBPs may play a crucial role in basal cells to regulate pathways that govern epidermal stem cell proliferation and maintenance.

Transcriptional regulators of epidermal homeostasis

Similar to the observations made during epidermal development, recent studies have also highlighted the importance of several transcriptional regulatory mechanisms in maintaining homeostasis in the adult epidermis. Hippo signaling is an evolutionarily conserved signaling network that plays a crucial role in regulating cell-fate decisions, proliferation, tissue growth and regeneration. The core Hippo pathway consists of a kinase cascade that leads to the phosphorylation and inactivation of the transcriptional co-activators YAP and TAZ (Zheng and Pan, 2019). When Hippo signaling is inactive, unphosphorylated YAP and TAZ translocate to the nucleus, where they associate with TEADs to induce the transcription of target genes that promote cell proliferation, survival and migration (Wu et al., 2008; Koontz et al., 2013). The epidermal deletion of YAP during embryogenesis results in thin epidermis and a reduction in basal cell proliferation and differentiation (Schlegelmilch et al., 2011). Recent work has also shown that the Hippo pathway and its downstream effector, YAP, are not only involved in maintaining epidermal homeostasis (reviewed by Rognoni and Walko, 2019) but also cause diseased skin when de-regulated (De Rosa et al., 2019). Elbediwy and colleagues showed that although YAP and TAZ localize in the nucleus of IFE basal cells, they become cytoplasmic in the stratified suprabasal layers, indicating that subcellular localization of YAP and TAZ is dependent on the apical-basal mechanistic cues of epidermal cells. Additionally, upon differentiation, basal cell progenitors lose contact with the basement membrane, which induces the cytoplasmic localization of YAP and the downregulation of YAP-mediated gene expression. The loss of YAP and TAZ in basal cells results in a reduction of basal SC proliferation, in a significant downregulation of YAP target genes (which include cell cycle regulators, cell growth factors and components of the EGFR signaling pathway) and in delayed wound repair (Elbediwy et al., 2016). This study has identified YAP/TAZ as important factors that regulate the signaling axis required for maintaining ESC identity in adult skin. When progenitor cells lose contact with the basal layer, it interrupts this axis, which in turn allows for the establishment of differentiation-promoting signaling pathways.

Skin atrophy is a general phenomenon associated with aging (Hsu et al., 2014). Concomitantly, telomere shortening is also tied to premature aging, and patients with mutations of telomerase components exhibit telomere shortening and skin atrophy phenotypes (Flores et al., 2005; Buckingham and Klingelhutz, 2011). Liu and colleagues showed that epidermal telomeric shortening induced by the loss of the telomerase RNA component, TERC, results in epidermal thinning, skin atrophy and reduced expression of p63 and Krt14 in mice. Transcriptome analysis of cells lacking Terc revealed upregulation of follistatin (Fst), a negative regulator of the BMP/Smad pathway (Fainsod et al., 1997). Prevention of telomere shortening results in downregulation of Fst, and rescues p63 and Krt14 expression levels, indicating that elevated levels of Fst upon telomeric shortening downregulate the BMP pathway (Liu et al.,

2019a). The shortening of telomeres has been implicated in the altering of heterochromatic architecture at subtelomeric regions where the *Fst* gene is located (Benetti et al., 2007). Interestingly, the loss of telomeric repeats leads to decreased levels of H3K9me3 and H3K27me3 abundance in ES cells, and ChIP-qPCR analysis revealed that H3K27me3 was markedly reduced at the *Fst* promoter in cells lacking Terc, when compared with control cells (Liu et al., 2019a). Notably, the expression of the PRC2 components Ezh1 and Ezh2, which are responsible for catalyzing the deposition of H3K27me3, is reduced upon Terc loss. This study has revealed how the functional telomere regulates the epigenetic-signaling axis, which when dysregulated leads to impaired epidermal homeostasis and the onset of premature aging phenotypes.

Epidermal homeostasis has been shown to be cooperatively regulated via various epigenetic mechanisms. Although the PcG and trithorax groups of proteins (TrxG) have been shown to be crucial for epidermal development, their function in maintaining epidermal homeostasis remains largely unexplored (Mulder et al., 2012). Ash1l, a SET-domain histone lysine methyltransferase, is a component of the trithorax group of proteins that methylates H3K36, which antagonizes H3K27me3, a PRC2-dependent mark, in embryonic SCs (Tanaka et al., 2007; Yuan et al., 2011). The loss of Ashl1 results in epidermal hyperplasia, skin lesions, ectopic Krt14 expression in suprabasal layers and the increased proliferation of basal cells in adult skin (Li et al., 2017). Upon wounding, even though Ash1l-null cells proliferate, they fail to spread into the wounded region required for initiating re-epithelization. Moreover, the loss of Ash1l results in upregulation of *Myc* expression in hyperplasia epidermis, but Ash1l does not directly regulate *Myc* promoter activity (Li et al., 2017). Notably, Myc is known to be activated by PcG-dependent mechanisms in tumors (Shi et al., 2007). The loss of Ash1l results in the upregulation of the PcG-dependent H3K27me3 mark in 293-T cells, indicating that Ash1l modulates *Myc* expression in the epidermis via histone modification–dependent mechanisms (Li et al., 2017). In line with the above study, Kang and colleagues reported that H3 K4/9/27 hypomethylation is crucial for proper re-epithelization during wound repair (Kang et al., 2020). Together, these studies broaden our understanding of how histone modifiers and the modulation of histone modifications regulate the basal SC dynamics in adult skin to maintain homeostasis and promote mechanisms that aid in wound healing.

Conclusions and future perspectives

The development of mammalian epidermis from a monolayer in the embryo to a multilayered organized structure is orchestrated by highly regulated processes, with the oriented cell division of ESCs playing a central role. Although previous work has paved the way to understanding how SCD and ACD contribute to ESC self-renewal, differentiation and epidermal stratification, studies highlighted in this Review have broadened our understanding of intrinsic factors that lie upstream of regulatory cues that influence oriented cell division, such as spindle orientation proteins. Particularly, these factors regulate the Notch signaling pathway, expanding our understanding of how the Notch signaling axis, in addition to controlling the differentiation-promoting transcriptional network, is also coupled to oriented cell division in ESCs. Moreover, modulation of cortical tension as a result of cell shape changes that are aided by PCP proteins, PCP regulators and the cytoskeletal network, has also emerged as a vital player in epidermal differentiation during development. Intriguingly, not much is known about the role of PCP proteins in maintaining homeostasis in the adult epidermis. Moreover, the regulators of the PCP pathway and cytoskeletal network in ESCs remain to be identified.

In the adult epidermis, several different models of stem cell-based epidermal maintenance have been proposed, but the development of superior experimental and mathematical modeling techniques has given way to the unified idea that epidermal homeostasis is maintained by a single equipotent progenitor population, with the exception of the adult tail interscale region. Why and how the tail interscale region follows the SC-CP model during homeostasis is not yet understood, and little is known about the contribution of the microenvironment of the SC niches between scale and interscale regions. Recent work has also shed light on ESC competition mechanisms in both developing and adult epidermis to eliminate unfit cells for proper epidermal establishment and maintenance. Interestingly, the mechanisms of cell competition in monolayer epidermis during epidermal stratification and homeostasis are very different. Further work needs to be carried out to understand what intrinsic factors mediate this switch and whether changes in microenvironments in different epidermal stages aid ESCs to adapt a differential cell competition mechanism.

Although significant contributions over the past few years have been made in understanding how histone modifications and chromatin regulators, especially the PcG and TrxG proteins, play a role during epidermal development, their role in regulating ESC fate in adulthood is largely unexplored. Epigenetic modifications have been shown to play a role in modulating SC function and influencing the clonogenicity potential of cultured human keratinocytes, indicating that epigenetic modifiers might be controlling key transcriptional networks in adult ESCs to maintain tissue homeostasis. Key questions to answer would be whether ESCs alter their chromatin landscape with age and how this dynamic landscape affects cell differentiation potential. These efforts will help us understand how and whether a dynamic epigenetic landscape in adult epidermis contributes to aging and development of diseases such as psoriasis, atrophy and cancer. Concomitantly, our knowledge of post-transcriptional gene regulatory mechanisms regulating ESCs during development and homeostasis remains limited. Recent studies that shed light on the role of post-transcriptional regulatory mechanisms controlling ESC function via miRNAs and RBPs pave the way for future investigations to identify other post-transcriptional regulators during development and homeostasis.

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Competing interests

The authors declare no competing or financial interests.

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