Transcriptional Control of Epidermal Stem Cells

9

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Abstract

Transcriptional regulation is fundamentally important for the progression of tissue stem cells through different stages of development and differentiation. Mammalian skin epidermis is an excellent model system to study such regulatory mechanisms due to its easy accessibility, stereotypic spatial arrangement, and availability of well-established cell type/lineage differentiation markers. Moreover, epidermis is one of the few mammalian tissues the stem cells of which can be maintained and propagated in culture to generate mature cell types and a functional tissue (reviewed in [1]), offering in vitro and ex vivo platforms to probe deep into the underlying cell and molecular mechanisms of biological functions.

Keywords

Epidermis • Hair follicle • Stem cell • Transcription factor • Chromatin regulation

Abbreviations

BrdU	Bromodeoxyuridine
DNMT	DNA Methyltransferase
EGF	Epidermal Growth Factor
EMT	Epithelial-To-Mesenchymal Transition
FACS	Fluorescence Activated Cell Sorting
GFP	Green Fluorescent Protein
H3K27	Histone H3 Lysine 27
HDAC	Histone Deacetylase
IFE	Interfollicular Epidermis
K	Keratin
LRC	Label Retaining Cell
NICD	Notch Intracellular Domain
ORS	Outer Root Sheath
PcG	Polycomb Group Proteins
SC	Stem Cell
Shh	Sonic hedgehog
TA	Transit Amplifying
HG	Secondary Hair Germ

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9.1 Introduction

Mammalian skin is a complex organ with a multitude of epithelial and stromal cell types, and harbors various appendages such as hair follicles which themselves are "miniorgans". Skin epidermis and its associated appendages are established during embryogenesis. In postnatal life these structures are regenerated by several distinct pools of stem cells which have the ability to self-renew as well as to give rise to the different lineages that form the mature tissues of the skin [2, 3] (Fig. 9.1).

At least some of the cellular and molecular blueprint for homeostasis in adult skin is specified during mid-late embryogenesis (e.g., [4–6]; reviewed in [7, 8]). Thus, the study of embryonic epidermal stem/progenitor cells will likely shed light on how the behaviors of adult skin epithelial stem cells, such as their proliferative potential and lineage differentiation, are regulated. Experimental analysis of epidermal morphogenesis enjoys the additional benefit of having relatively synchronous development, and that stem/progenitor/differentiating cells are not only spatially but also temporally laid out.

In this chapter, we review recent literature on the understanding of skin epithelial stem cells, and knowledge of transcriptional and chromatin regulation of the development and differentiation of these cells. There have been a number of excellent recent reviews that discuss adult stem cells in the mammalian skin, particularly those that reside in the hair follicle as well as on developing follicular stem/progenitor cells [7–12]. We therefore focus our discussion primarily on stem cells that produce and replenish the interfollicular epidermis (IFE) and provide an update on transcription and chromatin factors that regulate the activity of these cells during development.

9.2 Overview of Adult Skin Epithelial Stem Cells

Adult skin stem cells have been identified based on their slow cycling nature or unique surface marker expression. The well-known DNA label-retention assay is based on the assumption that stem cells are generally quiescent and retain tritiated thymidine or bromodeoxyuridine (BrdU) label of genomic DNA much longer than their rapidly cycling progenies [13, 14]. An elegant variant of this strategy is the use of histone H2B-Green Fluorescent Protein (GFP) to label the chromatin [15]. Approximately 95 % of the

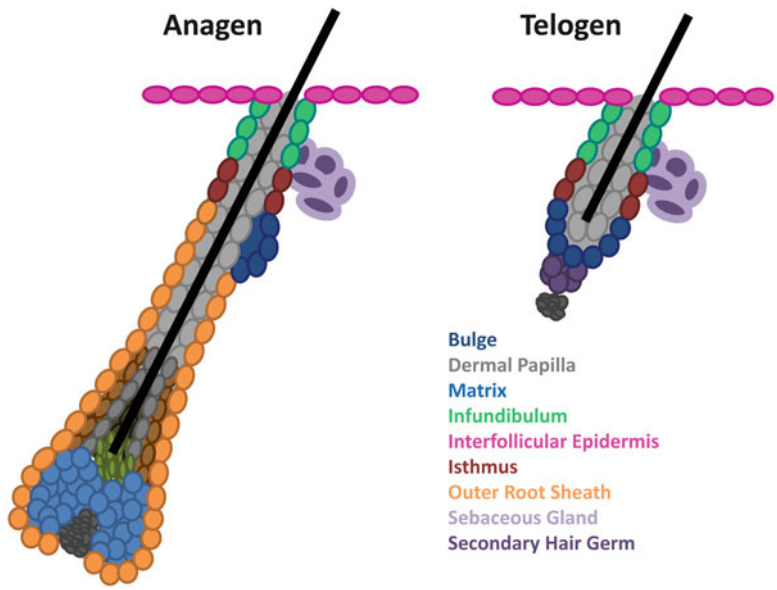

Fig. 9.1 Schematic diagram of anagen and telogen hair follicles and their cellular compositions. Each cellular compartment is color coded and those relevant in this review are labeled accordingly

Table 9.1 Summary of markers of adult mouse skin epithelial stem/progenitor cells

Marker	Location	References
α6 Integrin	ORS, bulge, IFE, SG	[27, 28]
Sca1	Infundibulum, IFE	[29]
K15[a]	Bulge, ORS	[27, 30, 31]
ΔNp63	ORS, bulge, HG, matrix, IFE	Reviewed in [28, 32]
CD34	Bulge	[31]
Lgr5	ORS, bulge, HG	[33]
Lgr6	Isthmus	[34]
Lrig1	Isthmus, ORS	[35]
MTS24	Isthmus, infundibulum occasionally	[36]
Blimp1	Isthmus, SG opening	[37]
Gli1	Bulge, HG	[38]
Lhx2	ORS, bulge, HG	[39]
Sox9	Bulge, ORS	[6, 40]
Nfatc1	Bulge	[41]
Tcf3	Bulge, ORS	[5, 42]
Tcf4	Bulge, ORS	[5]
Runx1	ORS, bulge, HG	[43, 44]

[a]While K15 protein is detected in basal layer of IFE and ORS/bulge of hair follicle, a fragment of the K15 promoter has been found to be selectively active in the bulge

slow-cycling, label-retaining cells (LRCs) in skin reside within the bulge, the lower permanent part of the hair follicle close to the site of attachment of the arrector pili muscle [16, 17] (Fig. 9.1). Using a double label technique to monitor the fate of the LRCs, Taylor et al. demonstrated that they are multipotent and can give rise to both the upper and lower portions of the follicle; however, their repopulation of the upper follicle only occurs during times of need such as wounding or during neonatal expansion of the skin [18]. Further supporting the presence of multipotent stem cells in the bulge, dissected human hair follicle bulge regions possess the ability to generate all skin epithelial lineages upon transplantation onto immunodeficient mice [19, 20].

LRCs can also be detected within the mouse IFE and previously have been shown to comprise about 0.2–10 % of the basal population, depending on the duration of the chase period in nucleotide pulse-chase experiments [21–23]. Using organotypic culture, LRCs have also been observed in the basal layer of human epidermis [24]. Whether or not the basal LRCs are true stem cells is debatable. Transplantation studies using limiting dilution of GFP-positive neonatal murine keratinocytes to recreate an epidermis in vivo suggest that the basal layer contains only a few functional long-term repopulating cells (~0.01 %) [25]. In addition, stem cell frequency in the IFE may vary depending on the tissue area (e.g. back, tail, ear) and age of the mouse (neonatal versus adult) [16].

In recent years, Fluorescence Activated Cell Sorting FACS based on stem cell-enriched surface and/or fluorescent markers has emerged as a powerful strategy to identify and isolate several distinct populations of skin epithelial stem cells. This advancement has led to the accumulation of tremendous amount of knowledge about adult skin stem cells, which reside within discrete physical locations called niches, where their proliferation and differentiation can be regulated by myriad intracellular and extracellular signals from the surrounding microenvironment (reviewed in [3, 7, 26]) (Fig. 9.1, Table 9.1). For example, the bulge contains CD34/alpha6-integrin/K15-positive cells that are relatively quiescent and contribute to hair follicle regeneration under physiological conditions but give rise to all skin epithelial lineages upon transplantation [20, 27, 30, 31]. The secondary hair germ (HG), a transient follicular structure that is responsible for the formation of the new hair follicle during postnatal cycling, also contains multipotent stem cells, which are Lgr5-positive and more proliferative than the bulge stem cells [33]. Recently, two different populations of cells that express Gli1, a target of the Shh pathway, have been reported: one resides in the HG/lower bulge and the other in the upper bulge of the telogen follicle [38]. This finding further illustrates the cellular/molecular heterogeneity within the stem cell-rich bulge/HG region. Another stem cell-rich zone in the hair follicle is the isthmus and infundibular region in the upper permanent part of the follicle. Residing in this region are MTS24-positive, Lrig1-positive, or Lgr6-positive cells that have the capability to reconstitute all skin epithelial components in transplantation assays, as well as Blimp1-positive cells that seed the sebaceous gland (SG), and Sca-1-positive cells that can regenerate IFE but not hair follicle upon transplantation [29, 34–37].

9.3 Stem/Progenitor Cells That Feul IFE Homeostasis and Repair

9.3.1 Stem Cells Within the IFE

In early studies, the observation of epidermal proliferative unit within the IFE led to the suggestion that there is one stem cell that supplies basal and suprabasal progeny in a hexagonal column of differentiated cells [23]. A popular hypothesis had been that one single self-renewing stem cell exists within each unit, whereas the other basal cells are the so-called transit-amplifying (TA) progeny that only divide a given number of times before withdrawing from the cell cycle and undergoing terminal differentiation [45–47]. While this exact unit of organization does not seem to hold true in subsequent analysis, support for heterogeneity in proliferative potential within the epidermis came from in vitro/ex vivo cell culture studies, where primary human keratinocytes can be distinguished in clonogenecity assays with regard to the size and lifespan of the colonies they produce [48, 49].

Although the lineage relationship between basal and suprabasal cells has been confirmed using in vivo experiments to clonally mark IFE cells (e.g., [50]), the notion of the existence of a TA cell compartment has been challenged by Clayton et al., in their lineage tracing experiments [51–53]. A combination of lineage tracing experiments and mathematic modeling have led the Jones's group to suggest a simple model where a single population of progenitor cells, which make stochastic choices between proliferation and differentiation is sufficient to maintain homeostasis of adult mouse tail epidermis. Whether this model is generally applicable to all regions of the epidermis remains to be tested.

9.3.2 Stem Cells in Non-IFE Locations

There is strong evidence supporting the contribution of hair follicle stem cells to IFE homeostasis and repair (reviewed in [26]). Earlier transplantation studies suggest that bulge cells give rise to the IFE [15, 18]. However, later lineage tracing experiments indicate that these bulge cells do not contribute to the IFE under physiological conditions, but do so upon injury [4, 13, 54, 55]. Lrig1, an EGF receptor antagonist that was first shown to mark human IFE stem cells [56], was identified as a marker of a population of stem cells, located in the junctional zone between the IFE and bulge in mouse skin, with potential to generate all epithelial lineages of the adult skin in transplantation assays [35]. During normal homeostasis, however, Lrig1-expressing cells only support the renewal of the IFE and SG [35]. More recently, it has been shown via lineage tracing that the non-label-retaining, Lgr6-positive cells located in the central isthmus of the hair follicle directly above the bulge are able to contribute to the IFE and SG under normal homeotic conditions at all ages, whereas their contribution to the hair follicle decreases with age [34]. In vivo lineage analysis of Shh-expressing cells originating from within the hair follicle has suggested that cells from the upper isthmus or infundibulum contribute to epidermal wound repair [55].

9.4 Lineage Progression of Epidermal Stem/Progenitor Cells During Morphogenesis

The epidermis originates from the surface ectoderm during embryonic development. In the mouse, commitment of the single-layered surface ectoderm to becoming epidermal precursor cells occurs at around embryonic (E) day 9.5 (reviewed in [57]) (Fig. 9.2). The biochemical hallmark of this is the switching-off of keratin (K) 8/18 (K8/K18) expression, and turning-on of K5 and K14, markers of the future basal layer of mature epidermis. Around E10.5, cells of the embryonic basal layer give rise to a transient layer called periderm, which covers the developing epidermis until stratification is complete. The early-stage K5/K14-positive cells are presumably multipotent, being capable of contributing to multiple subsequent lineages, including the IFE, hair follicle, and SG. Starting at E14.5, lineage distinction is evident as a subset of cells in the hair

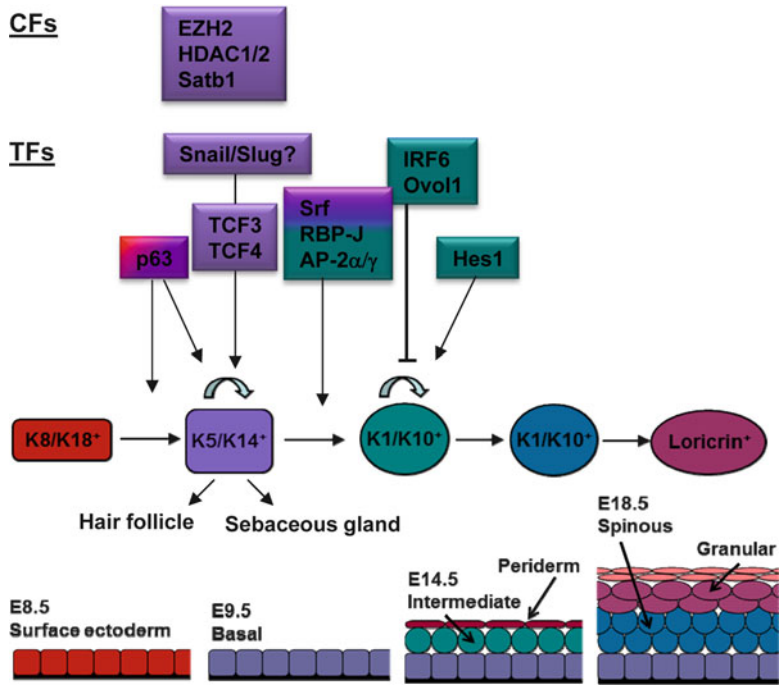

Fig. 9.2 Critical morphological/molecular events and transcriptional/chromatin regulators of epidermal development. Each lineage stage is color coded, and the roles of key transcription factors (TFs) and chromatin factors (CFs) are *highlighted* to indicate positive or negative influence on their cognate (matching color) lineage stage(s)

follicle placode that arises around this time retain multipotency, whereas the surrounding K5/K14-positive basal cells symmetrically or asymmetrically divide to expand the basal layer or to leave the underlying basement membrane and migrate upward to produce suprabasal cells of the IFE [4, 6, 26, 55]. The latter results in the formation of a transient suprabasal cell layer, namely the intermediate cell layer, between the basal layer and the periderm. These intermediate cells express K1 and K10, terminal differentiation markers of the mature epidermis, but are still proliferative [58]. Once these cells withdraw from the cell cycle (E15.5), they mature into spinous cells, which undergo further differentiation to produce granular keratinocytes that express yet another set of terminal differentiation markers such as loricrin. The epidermal maturation program is finalized by the formation of the cornified layers, which provide an outer front that acts as a permeability barrier essential for the organism's ex utero survival. How embryonic epidermal morphogenesis is orchestrated at a molecular level has been an active area of research. Below we review recent literature on the involvement of transcription and chromatin regulators that control the self-renewal, proliferation, and initiation of differentiation during epidermal development (Table 9.2).

9.5 Transcription Factors That Regulate Developing Epidermal Stem/Progenitor Cells

9.5.1 p63

p63 is a transcription factor homologous to the p53 tumor suppressor. p63 encodes two classes of protein isoforms, TAp63 and ΔNp63, with the latter being the predominant isoform expressed in epidermis (reviewed in [28, 82]). $p63^{-/-}$ mice cannot form a stratified epidermis or epidermal

Table 9.2 Summary of selected publications on the involvement of transcription and chromatin factors in regulating mouse epidermal stem/progenitor cells

Mutation	Targeted tissue	Phenotype	References
p63			
p63 knockout	Germline	Single-layered epidermis at birth, lack of limbs and epidermal appendages	[59, 60]
ΔNp63 knock-in	Germline	Single-layered epidermis in some and patches of prematurely differentiating keratinocytes in other areas, lack of epidermal appendages, no or defective limbs	[61]
ΔNp63 transgenic	Basal (K5)	Expansion of basal and spinous layers	[32]
TAp63 knockout	Germline	Develop blisters, ulcerated wounds and exhibit premature aging	[62]
TAp63 knockout	Basal (K14-Cre)	No defects	[62]
Notch			
RBP/J knockout	Basal (K14-Cre)	Thinner epidermis, reduced keratin network in suprabasal cells and decreased granular layer	[63]
NICD constitutive activation	Basal (K14)	Repressed basal gene expression, blistering between epidermis and dermis, expanded spinous layers, reduced granular layer and defective barrier	[63]
Hes1 transgenic	Basal (K14)	No defects	[64]
NICD1 transgenic	Spinous (K1)	Expanded granular layer	[64]
Hes1 transgenic	Spinous (K1)	Expanded spinous layer	[64]
Hes1 knockout	Germline	Premature differentiation of spinous cells into granular cells	[64]
Ascl2 transgenic	Spinous (K1)	Thinner epidermis, similar to Hes1 KO	[64]
AP-2			
AP-2α knockout	Basal (K14-Cre)	Epidermal hyperproliferation	[65]
AP-2γ knockout	Epiblast (Sox2-Cre)	Delayed basal gene expression, differentiation and barrier formation	[66]
AP-2α/γ double knockout	Basal (K14-Cre)	Thinner epidermis, defective differentiation and delayed barrier formation	[67]
Ovol			
Ovol1 knockout	Germline	Expanded spinous layer, failure of cell cycle exit, defective granular differentiation	[68]
Ovol2 knockout	Germline	Embryonic lethality, increased surface ectoderm	[69]
Ovol2 knockout	Basal (K14-Cre)	No defects, Ovol1 expression upregulated	Unpublished
TCF			
Tcf3 transgenic	Basal (K14)	Repression of terminal differentiation	[42]
Tcf4 transgenic	Basal (K14)	Repression of terminal differentiation	[5]
Tcf3/4 double knockout	3-Basal (K14), 4 -germline	Thinner epidermis, flattened basal cells	[5]
Srf			
Srf knockout	Basal (K5-Cre)	Edema and embryonic skin blistering	[70]
Srf knockout	Basal (K14-Cre)	Disorganized skin epithelium, loss of proper cell adhesion, increased proliferation in suprabasal cells, defective differentiation, random spindle orientation, increased apoptosis	[71, 72]
IRF6			
IRF6 mutant/ knockout	Missense mutation; germline	Hyperproliferation and failure to undergo terminal differentiation	[73, 74]
Satb1			
Satb1 knockout	Germline	Thinner epidermis, decreased granular layer, and decreased proliferation	[75]

(continued)

Table 9.2 (continued)

Mutation	Targeted tissue	Phenotype	References
EMT			
Slug knockout	Germline	Reduced epidermal thickness, delay in hair follicle morphogenesis	[76, 77]
Snail transgenic	Basal (K14)	Hyperproliferation and expansion of basal compartment	[78]
Snail knockout	Germline	No defects	[79]
Chromatin factors			
EZH2 knockout	Basal (K14-Cre)	Hyperthickened stratum corneum, reduced basal proliferation, pronounced granular layer, accelerated epidermal maturation	[80]
HDAC1/2 double knockout	Basal (K14-Cre)	Single-layered epidermis throughout embryogenesis, failure of eyelid fusion, and failure of limb-digit separation	[81]

appendages [59, 60]. Instead, a single-layered epithelium that expresses K8 but not K5 and K14 persists at birth. ΔNp63 knock-in mice, in which the ΔNp63-specific exon is replaced by GFP, phenocopies $p63^{-/-}$ mice by exhibiting similar developmental abnormalities including a poorly stratified epidermis [61]. Unlike $p63^{-/-}$ mice, however, patches of keratinocytes that are able to stratify exhibit signs of premature terminal differentiation, possibly due to alterations in the Notch signaling pathway. On the other hand, TAp63 knockout mice develop blisters as young adults and ulcerated wounds and premature aging later in life, but display no apparent defect in epidermal morphogenesis [62, 83]. Ectopic expression of either TAp63 or ΔNp63 in simple lung epithelium converts it into a K5/K14-expressing stratified epithelium, whereas overexpression of ΔNp63 in epidermal basal layer causes hyperproliferation and partially rescues the skin phenotypes of $p63^{-/-}$ mice [32, 84, 85]. Together, these studies highlight p63, particularly ΔNp63, as a master regulator of epidermal morphogenesis.

The cellular mechanism of p63 function has been an issue of controversy, with evidence supporting its roles in initiating epidermal stratification, maintaining stem/progenitor cell proliferation potential, as well as tuning the process of terminal differentiation (reviewed in [26, 28, 57, 61, 86, 87]) (Fig. 9.2). Consistent with such diverse functions, p63 regulates a wide array of genes involved in cell cycle, cell motility and adhesion, chromatin regulation, as well as skin tissue-specific markers such as K14, involucrin and loricrin [75, 88, 89].

Of note, p63/ΔNp63 is expressed in the nuclei of proliferating cells in the IFE basal layer and hair follicle, but shows reduced expression in the suprabasal layers [82, 84, 85]. This expression pattern is compatible with a key role for p63 in maintaining the proliferation of epidermal stem/progenitor cells. Additional support for this notion came from studies of human epidermal keratinocytes, where p63 functions to antagonize p53 in proliferation control [90, 91]. Interestingly, depletion of p53 rescues the p63 knockdown phenotype in cell growth but not terminal differentiation, suggesting that p63 plays a p53-independent role in controlling differentiation potential of epidermal stem/progenitor cells [90].

9.5.2 TCF3 and TCF4: Transcription Factors of the Wnt/β-Catenin Signaling Pathway

Wnt/β-catenin signaling plays important roles in the hair follicle lineage by promoting placode formation during embryogenesis, maintaining adult follicle bulge stem cell identity, activating quiescent stem cells during transition of postnatal follicle from a resting to a growing phase, and promoting terminal differentiation within the

follicle (reviewed in [6]). Members of the LEF/TCF family of transcription factors are downstream effectors of the Wnt/β-catenin pathway, by forming bipartite transcriptional complexes with β-catenin to regulate gene expression [92]. Recent studies reveal novel involvement of TCF3 and TCF4 in the developing epidermis, where both are expressed in embryonic basal cells [5, 42, 93]. K14 promoter-mediated overexpression of TCF3 or TCF4 in transgenic mice leads to repression of terminal differentiation in the IFE, which is likely due to the Wnt-independent transcriptional repressor function of these TCF factors [5, 42]. Neither TCF3-deficient skin nor TCF4-deficient skin grafts show any overt phenotype [5]. Loss of both TCF3 and TCF4 results in a thinner epidermis at birth with flattened basal cells and increased cell death, defective hair follicle downgrowth, and failure of epidermal cells to populate skin grafts [5]. Interestingly, the IFE defects displayed by TCF3/TCF4 double knockout mice are not shared by β-catenin loss-of-function mutant, suggesting that this aspect of the TCF3/4 function may be independent of Wnt/β-catenin signaling [5]. Together, these findings implicate TCF3 and TCF4 as gatekeepers of an epidermal stem/progenitor cell state (Fig. 9.2).

9.5.3 RBP-J and Hes1: Transcription Factors of the Notch Pathway

Notch signaling plays complex, context-dependent roles in skin epithelial differentiation and has recently been implicated as an effector linking asymmetric division to differentiation of embryonic epidermal stem cells (reviewed in [64, 94, 95]). Signaling is initiated by ligand binding to the Notch receptor followed by cleavage and nuclear translocation of Notch intracellular domain (NICD) that in turn binds to transcription factor RBP-J and regulates gene expression [96]. In the developing epidermis, Notch signaling activation occurs at the basal-suprabasal juncture [63, 97]. Consistently, K14-Cre-mediated deletion of RBP-J results in a thinner epidermis with reduced keratin network in suprabasal cells and fewer granular layers [63]. Conversely, precocious activation of Notch signaling by way of overexpressing NICD in basal cells leads to repressed basal gene expression and expanded spinous layers [63]. Together, these studies are consistent with an in vivo role for RBP-J in mediating canonical Notch signaling to promote a basal to spinous switch of epidermal stem cells (Fig. 9.2).

Hes1 transcriptional repressor is a downstream target of Notch signaling. Loss of Hes1 causes premature differentiation of suprabasal keratinocytes and is important for maintaining proliferation in both basal and spinous compartments [64]. Interestingly, overexpression of Hes1 in basal cells does not suppress basal fate and induce spinous fate as NICD does, suggesting that the spinous fate-promoting function of Notch signaling may be Hes1-independent; instead Hes1 is required for maintenance of the immature state of spinous cells [64] (Fig. 9.2). Hes1 directly represses the expression of transcription activator Ascl2, the overexpression of which in epidermal basal layer causes a similar skin phenotype as Hes1 knockout mice including reduced basal and spinous cell proliferation [64]. How suprabasally expressed Hes1 affects the proliferation potential of basal cells remains unclear.

9.5.4 AP-2α and AP-2γ

AP-2 transcription factors have long been implicated in regulating epidermal gene expression [98, 99], but their functional importance has only been recently demonstrated. Loss of AP-2α in the epidermis results in persistent EGFR activity in differentiating cells and localized epidermal hyperproliferation [65]. AP-2γ is induced by p63 to activate K14 expression [100], and its deficiency results in a transient developmental delay in epidermal stratification [66]. K14-Cre-mediated deletion of both AP-2α and AP-2γ leads to suppression of terminal differentiation in vivo and in vitro [67], uncovering redundant roles for these AP-2 proteins in skin.

Given that the AP-2α/γ mutant skin phenotype is reminiscent of that of RBP-J mice, Wang et al. examined in detail the relationship between the

AP-2 and Notch regulatory pathways [67]. This led to the discovery that AP-2 factors and RBP-J-mediated Notch signaling act in concert to regulate the expression of C/EBP transcription factors, which may in turn contribute to the basal-spinous transition [67] (Fig. 9.2). Cross-talk between Notch signaling and p63 has also be reported: Notch activation suppresses p63, and the two regulate common direct transcriptional targets such as Hes1 [101]. Moreover, sustained p63 function inhibits Notch-induced epidermal cell differentiation. Collectively, these findings highlight the importance for epidermal stem/progenitor cells to integrate multiple transcriptional inputs in order to intricately regulate the balance between self-renewal/proliferation and differentiation at the basal-suprabasal juncture.

9.5.5 Serum Response Factor (Srf)

Recent studies on the involvement of transcription factor Srf in epidermal development solidify the interesting in vivo link between the actin cytoskeleton and the control of epidermal stem/progenitor cell proliferation and differentiation. K5-Cre-mediated ablation of Srf results in embryonic skin blistering, whereas K14-Cre-mediated Srf loss leads to persistent suprabasal proliferation and a disorganized skin epithelium at birth suggestive of defective differentiation [70, 71]. A finer developmental analysis of the K14-Cre/Srf-deficient skin reveals faulty cellular organization at the basal-spinous juncture, which seems to be the root cause of later defects in proliferation and differentiation [72]. The earliest molecular alterations reside in the expression of genes encoding actins and their regulators, and genes involved in intercellular adhesion/signaling and cell–substratum adhesion. Probing further with elegant cell biological experiments, Luxenburg et al. suggest an intriguing model where the reduced expression of actin/actin regulators are responsible for changes in cortical framework and cell shape, which may in turn cause mitotic defects in spindle orientation, ultimately leading to skewed asymmetric cell division and defective stratification in Srf-deficient epidermis [72].

9.5.6 Ovol Transcription Factors

The *Ovo* gene family encodes evolutionarily conserved zinc-finger transcription factors with its prototype in *Drosophila* being critical for epidermal denticle formation [102]. Three mammalian Ovol homologs (Ovol1, Ovol2, and Ovol3) exist [103, 104]. Both fly Ovo and mammalian Ovol1 reside downstream of key developmental signaling pathways such as Wg/Wnt, BMP/TGF-β and FOXO [68, 102, 105, 106], constituting a central hub of signaling cross-talk. In the developing epidermis, Ovol1 expression coincides with the appearance of intermediate cells and persists in the more mature suprabasal layers [103], whereas Ovol2 is expressed predominantly in the basal layer [107]. Interestingly, Ovol1 and Ovol2 seem to repress the expression of each other, and Ovol1 auto-represses [107, 108]. Collectively, these data suggest the likely importance to intricately control Ovol expression levels, and are compatible with both distinct and redundant/compensating functions of Ovol1 and Ovol2 in epidermal morphogenesis.

Ovol involvement in epidermal development has been studied using knockout approaches. Germline ablation of Ovol1 results in a thickened epidermis at birth with expanded spinous layers [68]. The spinous cells in Ovol1-deficient embryos fail to down-regulate c-Myc expression and undergo proliferation arrest, and Ovol1-deficient keratinocytes do not exit cell cycle in response to calcium or TGF-β signaling [68]. Overall these studies underscore a function for Ovol1 in the growth arrest of late epidermal progenitor cells at least in part via direct repression of c-Myc transcription. Germline ablation of Ovol2 results in mid-gestation lethality, and mutant embryos display an overemphasized surface ectoderm [69]. siRNA-mediated knockdown to deplete Ovol2 in HaCaT cells, a human keratinocyte line, results in populational expansion but a loss of colony forming-cells upon clonal passaging [109]. Results of mathematical modeling suggest that both faster cycling and precocious withdrawal from the cell cycle may underlie this phenotype. Moreover, Ovol2 depletion accelerates extracellular signal-induced K1 expression in

2-D and 3-D culture models. Ovol2 directly represses the expression of c-Myc and Notch1 by binding to their promoters. Inhibiting c-Myc function rescues the transient increase in proliferation, whereas inhibiting Notch signaling rescues the precocious K1 expression of Ovol2-deficient cells. Thus, in vitro, Ovol2 functions to suppress HaCaT cell proliferation and K1 expression, but seems to promote long-term colony formation. The in vivo function of Ovol2 as well as the full scope of Ovol function in developing epidermis is under active investigation in the Dai laboratory.

9.5.7 IRF6

IRF6, a member of the interferon regulatory factor (IRF) family of transcription factors, has been shown to be involved in controlling the balance between epidermal stem/progenitor cell proliferation and differentiation. IRF6 null embryos display a hyperproliferative epidermis with expanded spinous layers that fail to silence p63 expression, exit the cell cycle, and undergo terminal differentiation [73]. Embryos carrying homozygous missense mutations in IRF6 show a similar skin phenotype [74]. Additionally, IRF6$^{-/-}$ primary mouse keratinocytes and IRF6-overexpressing primary human keratinocytes display increased and decreased, respectively, colony-formation in culture, suggesting a cell-autonomous role for IRF6 in repressing long-term proliferation of epidermal keratinocytes [110, 111]. IRF6 is expressed at low levels in proliferating keratinocytes but becomes significantly up-regulated upon calcium-induced differentiation [110], leading one to speculate that it may primarily function by causing growth arrest of late epidermal progenitor cells (Fig. 9.2), a role reminiscent of that of Ovol1. Interestingly, Ovol1 has been identified as a direct transcriptional target of IRF6 in squamous carcinoma cells [112]. Moreover, IRF6 is a direct transcriptional target of ΔNp63, and induces degradation of ΔNp63, presenting a negative feedback mechanism that regulates the switch between keratinocyte proliferation and differentiation [111].

9.5.8 Transcription Factors That Regulate Epithelial-Mesenchymal Transition (EMT)

An underexplored area in skin epithelial biology is how epithelial remodeling contributes to stem cell biology. This is an intriguing issue especially given the recent discovery of the association between passing through EMT and acquisition of self-renewal capability, and that normal multipotent mammary epithelial stem cells express EMT markers [113]. In light of this, it is interesting to note that ΔNp63α overexpression-induced EMT endows human keratinocytes with stem cell traits, namely multipotency to differentiate into non-keratinocyte cell types [114].

Limited evidence implicates the importance of known transcriptional regulators of EMT, Snail and Slug, in embryonic skin. Snail is expressed, in a transient manner, in hair placodal cells but not detectable in the IFE [79]. Slug (Snai2 or Snail2) is expressed in all epidermal layers at mid-gestation but becomes gradually restricted to the basal layer that harbors epidermal precursor cells and hair placode that harbors hair follicle precursor cells, and progressively disappears after birth [76, 78, 115]. These expression patterns correlate temporally with the increasingly restricted lineage and morphogenic potential of embryonic epidermal progenitor cells. Interestingly, overexpression of Snail in skin basal cells leads to loss of E-cadherin, epidermal hyperproliferation and expansion of the basal compartment [78]. Furthermore, Slug knockout mice show a thinner epidermis and delayed hair follicle development [76, 77]. Future work is needed to explore the potential functional importance of these EMT transcription factors in controlling the "stemness" of epidermal stem cells (Fig. 9.2).

9.6 Chromatin Factors That Regulate Epidermal Stem/Progenitor Cells

Chromatin regulation is intimately related to transcriptional control. Fiona Watt's group examined the global patterns of histone modifications

in mammalian skin using immunostaining, providing a first glimpse at the "histone code" that associates with quiescent cells present in human IFE as well as mouse hair follicle bulge [116]. This "histone code" appears to be characterized by high levels of histone H3 lysine 9 and histone H4 lysine 20 (H4K20) trimethylation and low levels of histone H4 acetylation and H4K20 mono-methylation. Interestingly, tampering with the "code" by application of inhibitors of histone deacetylases (HDAC) or overexpression of c-Myc, a proto-oncogene that has been suggested to regulate the conversion of epidermal stem cells to committed TA cells (reviewed in [87, 117]), results in altered proliferation/differentiation characteristics of epidermal stem cells. Investigation of stem cell epigenetics promises to be an exciting direction in epidermal biology.

9.6.1 Enhancer of Zeste Homolog 2 (EZH2)

Polycomb group (PcG) proteins are evolutionally conserved chromatin remodeling proteins involved in gene silencing [118]. EZH2 is a PcG member, and a methyltransferase component of the Polycomb repressive complex 2 (PRC2) that trimethylates primarily histone H3 at lysine 27 (H3K27) to initiate gene repression. EZH2 is expressed in embryonic stem/progenitor cells of the epidermis, and its ablation leads to reduced basal cell proliferation, premature induction of late-differentiation genes, and accelerated epidermal maturation [80]. EZH2 has also been shown to control the proliferative potential of basal stem/progenitors by repressing the Ink4B-Arf-Ink4A tumor suppressor locus and preventing premature recruitment of the AP1 transcriptional activator to genes involved in differentiation of the epidermis [80]. This differentiation-preventing function is opposite to that of H3K27me3 demethylase JMJD3 in human epidermal keratinocytes [119]. These studies collectively underscore the importance of epigenetic repression vs. derepression in controlling the balance between epidermal stem/progenitor cell proliferation and differentiation.

The PRC2 complex has been shown to recruit DNA methyltransferases (DNMTs) to cognate target genes, providing a direct link between H3K27 trimethylation and DNA methylation [120]. Consistent with this, DNMT1 is enriched in undifferentiated human keratinocytes, and is required cell-intrinsically for maintaining epidermal stem/progenitor cell proliferation and for preventing premature terminal differentiation [121]. Whether DNA methylation plays a similar role in mouse epidermal stem/progenitor cells has not yet been reported.

9.6.2 HDAC1 and HDAC2

HDAC1 and HDAC2, two histone deacetylases that remove histone acetylation marks to cause chromatin compaction and gene repression, are dynamically expressed in the developing epidermis [81]. While K14-Cre-mediated deletion of either one produces no overt skin defects, deletion of both results in the generation of a single-layered epidermis and lack of hair follicles at birth, phenotypes reminiscent of those in p63 knockout mice [81]. Moreover, the double mutant embryos display reduced basal cell proliferation and increased cell apoptosis that become increasingly severe with age, suggestive of failure in maintaining embryonic epidermal progenitor cells. At least one mechanism of HDAC1/2 action in these cells seems to be directly mediating the repressive aspect of p63 function on downstream targets such as Ink4A. A budding scenario from the HDAC/EZH2/JMJD3/DNMT1 studies is that all three modes of chromatin/transcriptional repression (histone deacetylation, H3K27me3, and DNA methylation) operate in epidermal progenitor cells to maintain a self-renewing and/or undifferentiating state, albeit with distinct underlying molecular mechanisms. The involvement of the epigenetic activating machinery in epidermal development and differentiation awaits future investigation.

9.6.3 Satb1

Satb1, a genome organizer that regulates high-order chromatin structure, is expressed in basal progenitor cells as a direct target of p63 [75].

Newborn skin deficient in Satb1 show reduced thickness and epidermal proliferation, as well as altered chromatin configuration at, and gene expression from, the epidermal differentiation complex (EDC) locus [75]. The similarity in Satb1 and p63's effect on epidermal development, chromatin architecture and gene expression has prompted further experiments by Fessing et al., which demonstrate that restoration of Satb1 expression in p63-deficient embryonic skin explants partially rescues the epidermal phenotypes of the latter. This study opens the door to future exploration of how high-order chromatin organization contributes to the regulation of epidermal gene expression and lineage development.

9.7 Transcriptional and Chromatin Regulation of Adult Hair Follicle Stem Cells

An understanding of the transcriptional control of adult skin stem cells is also emerging. Transcription factors expressed in hair follicle bulge stem cells include Nfatc1, Lhx2, Sox9, Runx1, Tcf3, Tcf4, and Gli1, which themselves are functionally important players in stem cell biology [5, 6, 9, 38, 39, 41, 43]. For example, loss of NFATc1 causes loss of stem cell quiescence [41], whereas ablation of Lhx2 results in increased proliferation of CD34-positive stem cells but reduced CD34 expression within the follicle [39]. Sox9, Runx1, c-Myc, and Blimp1 have also been reported to regulate the emergence, maintenance, and/or proliferation of adult skin epithelial stem and progenitor cells [6, 37, 40, 43, 44, 122, 123].

Particularly worth noting are TCF3 and TCF4 that, as discussed above, play a role in epidermal morphogenesis. In adult skin, TCF3 and TCF4 become restricted to the bulge and outer root sheath (ORS), and are barely detected in the IFE [5, 42, 93]. Although a role for TCF3 and TCF4 in bulge stem cells has not yet been directly assessed, the finding that TCF3/4-deficient epidermal cells fail to populate skin grafts is suggestive of a TCF3/4 function in maintaining long-term epidermal homeostasis [5]. The similarity in the TCF3-responsive gene signature and the bulge/ORS gene signature [42] further supports this notion. As such, molecular parallels exist between the transcriptional regulation of embryonic epidermal stem/progenitor cells (including but not exclusive to those in the developing hair follicle) and that of adult bulge stem cells. Along the same vein, double ablation of EZH2, a regulator of epidermal maturation, and its homolog EZH1 adversely affects hair follicle homeostasis and wound repair [124].

9.8 Summary and Perspectives

This chapter reviews the recent progress on the transcriptional and chromatin control of epidermal stem cells. The self-renewal/proliferation/survival of embryonic epidermal stem/progenitor cells, their decision to initiate the terminal differentiation program and become spinous cells, and their lineage stay as committed progenitor cells are all under regulation by multiple transcription factors (Fig. 9.2). Interfacing with this layer of regulation is the active remodeling of the local as well as high-order configuration of chromatin by histone/DNA modifying enzymes and genome organizer. At least some components of the transcriptional/chromatin control strategies are reused to govern the behaviors of adult hair follicle stem cells.

Looking forward, we anticipate future research to address the following questions. First, what additional transcription factors are important in epidermal stem cells and how do they interact with each other to constitute regulatory networks that produce a normal epidermis with intricately balanced proliferation and differentiation? Second, exactly how do transcription factors communicate with chromatin factors and what additional epigenetic factors are functionally required for epidermal morphogenesis? While existing studies on the identification of downstream targets of, and functional interactions between, various transcription/chromatin factors have already offered tantalizing clues (e.g., [125]; also see above), a systems biology approach may be necessary to provide an integrated, comprehensive picture.

Third, what are the roles of non-coding RNAs and how do they interface with transcriptional/chromatin regulation? Leading this direction are recent studies on the identification of microRNAs in skin and the demonstration of functional requirements for the microRNA biogenic machinery as well as for specific microRNAs ([126]; reviewed in [127]). Continued identification of critical targets of important miRNAs, such as ΔNp63 for microRNA-203 [128, 129] will add a new dimension to the regulatory networks controlling gene expression in epidermal stem/progenitor cells. Finally, how do transcription and chromatin factors regulate the epigenomic landscape of epidermal stem/progenitor cells? Studies to address such issues rely on the ability to isolate sufficient quantities of relatively homogeneous stem/progenitor cell populations for genome-wide interrogations, as recently accomplished by the Fuchs group [130].

The ability of epidermal stem cells to be cultured over long periods of time without losing their stemness has been vastly beneficial in treating burn victims [131]. Multipotent skin stem cells hold the promise to treat human disorders such as alopecia, and their alterations are implicated in the ageing process [132]. Therefore, understanding the transcriptional/chromatin mechanisms that regulate epidermal stem cell lineage progression and homeostasis may facilitate the development of stem cell-based regenerative medicine and other therapeutic agents.

Authors' Notes

A number of research and review articles have been published since the submission of this review on the topic of epidermal stem cells and their molecular control. Most notably, Mascré et al. performed elegant lineage tracing studies to track YFP expression at clonal density in mouse tail epidermis [133]. Their results suggest that two distinct pools of progenitors with a hierarchical relationship, namely slow-cycling stem cells and committed progenitor cells, are present in the IFE. Furthermore, several novel players, including iASSP, Setd8, and Jarid2, have been identified that participate in epidermal stem cell-regulatory pathways and/or regulate epidermal morphogenesis and homeostasis [134, 135, 136]. Readers are referred to a recent review for additional update and details [137].

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References

1. Green H (2008) The birth of therapy with cultured cells. Bioessays 30(9):897–903
2. Fuchs E (2008) Skin stem cells: rising to the surface. J Cell Biol 180(2):273–284
3. Woo WM, Oro AE (2011) SnapShot: hair follicle stem cells. Cell 146(2):334–334, e332
4. Levy V, Lindon C, Harfe BD, Morgan BA (2005) Distinct stem cell populations regenerate the follicle and interfollicular epidermis. Dev Cell 9(6): 855–861
5. Nguyen H, Merrill BJ, Polak L, Nikolova M et al (2009) Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. Nat Genet 41(10): 1068–1075
6. Nowak JA, Polak L, Pasolli HA, Fuchs E (2008) Hair follicle stem cells are specified and function in early skin morphogenesis. Cell Stem Cell 3(1):33–43
7. Barker N, Bartfeld S, Clevers H (2010) Tissue-resident adult stem cell populations of rapidly self-renewing organs. Cell Stem Cell 7(6):656–670
8. Watt FM, Jensen KB (2009) Epidermal stem cell diversity and quiescence. EMBO Mol Med 1(5):260–267
9. Blanpain C, Fuchs E (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol 10(3):207–217
10. Jaks V, Kasper M, Toftgard R (2010) The hair follicle-a stem cell zoo. Exp Cell Res 316(8):1422–1428
11. Schneider MR, Schmidt-Ullrich R, Paus R (2009) The hair follicle as a dynamic miniorgan. Curr Biol 19(3):R132–R142
12. Yang L, Peng R (2010) Unveiling hair follicle stem cells. Stem Cell Rev 6(4):658–664
13. Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous

unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61(7):1329–1337
14. Morris RJ, Potten CS (1999) Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. J Invest Dermatol 112(4):470–475
15. Tumbar T, Guasch G, Greco V, Blanpain C et al (2004) Defining the epithelial stem cell niche in skin. Science 303(5656):359–363
16. Ambler CA, Maatta A (2009) Epidermal stem cells: location, potential and contribution to cancer. J Pathol 217(2):206–216
17. Kobayashi K, Rochat A, Barrandon Y (1993) Segregation of keratinocyte colony-forming cells in the bulge of the rat vibrissa. Proc Natl Acad Sci U S A 90(15):7391–7395
18. Taylor G, Lehrer MS, Jensen PJ, Sun TT et al (2000) Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102(4): 451–461
19. Oshima H, Rochat A, Kedzia C, Kobayashi K et al (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells. Cell 104(2): 233–245
20. Rochat A, Kobayashi K, Barrandon Y (1994) Location of stem cells of human hair follicles by clonal analysis. Cell 76(6):1063–1073
21. Bickenbach JR (1981) Identification and behavior of label-retaining cells in oral mucosa and skin. J Dent Res 60(Spec No C):1611–1620
22. Fuchs E, Horsley V (2008) More than one way to skin. Genes Dev 22(8):976–985
23. Potten CS (1974) The epidermal proliferative unit: the possible role of the central basal cell. Cell Tissue Kinet 7(1):77–88
24. Muffler S, Stark HJ, Amoros M, Falkowska-Hansen B et al (2008) A stable niche supports long-term maintenance of human epidermal stem cells in organotypic cultures. Stem Cells 26(10):2506–2515
25. Schneider TE, Barland C, Alex AM, Mancianti ML et al (2003) Measuring stem cell frequency in epidermis: a quantitative in vivo functional assay for long-term repopulating cells. Proc Natl Acad Sci USA 100(20):11412–11417
26. Fuchs E (2009) The tortoise and the hair: slow-cycling cells in the stem cell race. Cell 137(5):811–819
27. Braun KM, Niemann C, Jensen UB, Sundberg JP et al (2003) Manipulation of stem cell proliferation and lineage commitment: visualisation of label-retaining cells in wholemounts of mouse epidermis. Development 130(21):5241–5255
28. Blanpain C, Fuchs E (2007) p63: revving up epithelial stem-cell potential. Nat Cell Biol 9(7):731–733
29. Jensen UB, Yan X, Triel C, Woo SH et al (2008) A distinct population of clonogenic and multipotent murine follicular keratinocytes residing in the upper isthmus. J Cell Sci 121(Pt 5):609–617
30. Morris RJ, Liu Y, Marles L, Yang Z et al (2004) Capturing and profiling adult hair follicle stem cells. Nat Biotechnol 22(4):411–417
31. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G et al (2003) Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. J Invest Dermatol 120(4):501–511
32. Romano RA, Smalley K, Liu S, Sinha S (2010) Abnormal hair follicle development and altered cell fate of follicular keratinocytes in transgenic mice expressing DeltaNp63alpha. Development 137(9):1431–1439
33. Jaks V, Barker N, Kasper M, van Es JH et al (2008) Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet 40(11):1291–1299
34. Snippert HJ, Haegebarth A, Kasper M, Jaks V et al (2010) Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. Science 327(5971):1385–1389
35. Jensen KB, Collins CA, Nascimento E, Tan DW et al (2009) Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. Cell Stem Cell 4(5):427–439
36. Nijhof JG, Braun KM, Giangreco A, van Pelt C et al (2006) The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristics of progenitor cells. Development 133(15):3027–3037
37. Horsley V, O'Carroll D, Tooze R, Ohinata Y et al (2006) Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. Cell 126(3):597–609
38. Brownell I, Guevara E, Bai CB, Loomis CA et al (2011) Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells. Cell Stem Cell 8(5):552–565
39. Rhee H, Polak L, Fuchs E (2006) Lhx2 maintains stem cell character in hair follicles. Science 312(5782):1946–1949
40. Vidal VP, Chaboissier MC, Lutzkendorf S, Cotsarelis G et al (2005) Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. Curr Biol 15(15):1340–1351
41. Horsley V, Aliprantis AO, Polak L, Glimcher LH et al (2008) NFATc1 balances quiescence and proliferation of skin stem cells. Cell 132(2):299–310
42. Nguyen H, Rendl M, Fuchs E (2006) Tcf3 governs stem cell features and represses cell fate determination in skin. Cell 127(1):171–183
43. Osorio KM, Lee SE, McDermitt DJ, Waghmare SK et al (2008) Runx1 modulates developmental, but not injury-driven, hair follicle stem cell activation. Development 135(6):1059–1068
44. Raveh E, Cohen S, Levanon D, Negreanu V et al (2006) Dynamic expression of Runx1 in skin affects hair structure. Mech Dev 123(11):842–850
45. Potten CS (1981) Cell replacement in epidermis (keratopoiesis) via discrete units of proliferation. Int Rev Cytol 69:271–318
46. Potten CS, Wichmann HE, Loeffler M, Dobek K, Major D (1982) Evidence for discrete cell kinetic subpopulations in mouse epidermis based on mathematical analysis. Cell Tissue Kinet 15:305–329

47. Potten CS, Loeffler M (1987) Epidermal cell proliferation. I. Changes with time in the proportion of isolated, paired and clustered labelled cells in sheets of murine epidermis. Virchows Arch B Cell Pathol Incl Mol Pathol 53:279–285
48. Barrandon Y, Green H (1987) Three clonal types of keratinocyte with different capacities for multiplication. Proc Natl Acad Sci U S A 84(8):2302–2306
49. Jones PH, Watt FM (1993) Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. Cell 73(4):713–724
50. Ghazizadeh S, Taichman LB (2001) Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. EMBO J 20(6):1215–1222
51. Clayton E, Doupe DP, Klein AM, Winton DJ et al (2007) A single type of progenitor cell maintains normal epidermis. Nature 446(7132):185–189
52. Jones PH, Simons BD, Watt FM (2007) Sic transit gloria: farewell to the epidermal transit amplifying cell? Cell Stem Cell 1(4):371–381
53. Doupè DP, Klein AM, Simons BD, Jones PH (2010) The ordered architecture of murine ear epidermis is maintained by progenitor cells with randomfate. Dev Cell 18:317–323
54. Ito M, Liu Y, Yang Z, Nguyen J et al (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. Nat Med 11(12):1351–1354
55. Levy V, Lindon C, Zheng Y, Harfe BD, Morgan BA (2007) Epidermal stem cells arise from the hair follicle after wounding. FASEB J 21(7):1358–1366. Epub Jan 25 2007
56. Jensen KB, Watt FM (2006) Single-cell expression profiling of human epidermal stem and transit-amplifying cells: Lrig1 is a regulator of stem cell quiescence. Proc Natl Acad Sci U S A 103(32): 11958–11963
57. Koster MI, Roop DR (2007) Mechanisms regulating epithelial stratification. Annu Rev Cell Dev Biol 23:93–113
58. Koster MI, Dai D, Roop DR (2007) Conflicting roles for p63 in skin development and carcinogenesis. Cell Cycle 6(3):269–273
59. Mills AA, Zheng B, Wang XJ, Vogel H et al (1999) p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 398(6729): 708–713
60. Yang A, Schweitzer R, Sun D, Kaghad M et al (1999) p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 398(6729):714–718
61. Romano RA, Smalley K, Magraw C, Serna VA et al (2012) {Delta}Np63 knockout mice reveal its indispensable role as a master regulator of epithelial development and differentiation. Development 139(4):772–782
62. Su X, Paris M, Gi YJ, Tsai KY et al (2009) TAp63 prevents premature aging by promoting adult stem cell maintenance. Cell Stem Cell 5(1):64–75
63. Blanpain C, Lowry WE, Pasolli HA, Fuchs E (2006) Canonical notch signaling functions as a commitment switch in the epidermal lineage. Genes Dev 20(21):3022–3035
64. Moriyama M, Durham AD, Moriyama H, Hasegawa K et al (2008) Multiple roles of Notch signaling in the regulation of epidermal development. Dev Cell 14(4):594–604
65. Wang X, Bolotin D, Chu DH, Polak L et al (2006) AP-2alpha: a regulator of EGF receptor signaling and proliferation in skin epidermis. J Cell Biol 172(3):409–421
66. Guttormsen J, Koster MI, Stevens JR, Roop DR et al (2008) Disruption of epidermal specific gene expression and delayed skin development in AP-2 gamma mutant mice. Dev Biol 317(1): 187–195
67. Wang X, Pasolli HA, Williams T, Fuchs E (2008) AP-2 factors act in concert with Notch to orchestrate terminal differentiation in skin epidermis. J Cell Biol 183(1):37–48
68. Nair M, Teng A, Bilanchone V, Agrawal A et al (2006) Ovol1 regulates the growth arrest of embryonic epidermal progenitor cells and represses c-myc transcription. J Cell Biol 173(2):253–264
69. Mackay DR, Hu M, Li B, Rheaume C et al (2006) The mouse Ovol2 gene is required for cranial neural tube development. Dev Biol 291(1):38–52
70. Koegel H, von Tobel L, Schafer M, Alberti S et al (2009) Loss of serum response factor in keratinocytes results in hyperproliferative skin disease in mice. J Clin Invest 119(4):899–910
71. Verdoni AM, Ikeda S, Ikeda A (2010) Serum response factor is essential for the proper development of skin epithelium. Mamm Genome 21(1–2):64–76
72. Luxenburg C, Pasolli HA, Williams SE, Fuchs E (2011) Developmental roles for Srf, cortical cytoskeleton and cell shape in epidermal spindle orientation. Nat Cell Biol 13(3):203–214
73. Ingraham CR, Kinoshita A, Kondo S, Yang B et al (2006) Abnormal skin, limb and craniofacial morphogenesis in mice deficient for interferon regulatory factor 6 (Irf6). Nat Genet 38(11):1335–1340
74. Richardson RJ, Dixon J, Malhotra S, Hardman MJ et al (2006) Irf6 is a key determinant of the keratinocyte proliferation-differentiation switch. Nat Genet 38(11):1329–1334
75. Fessing MY, Mardaryev AN, Gdula MR, Sharov AA et al (2011) p63 regulates Satb1 to control tissue-specific chromatin remodeling during development of the epidermis. J Cell Biol 194(6):825–839
76. Parent AE, Newkirk KM, Kusewitt DF (2010) Slug (Snai2) expression during skin and hair follicle development. J Invest Dermatol 130(6): 1737–1739
77. Newkirk KM, Parent AE, Fossey SL, Choi C et al (2007) Snai2 expression enhances ultraviolet radiation-induced skin carcinogenesis. Am J Pathol 171(5):1629–1639

78. Jamora C, Lee P, Kocieniewski P, Azhar M et al (2005) A signaling pathway involving TGF-beta2 and snail in hair follicle morphogenesis. PLoS Biol 3(1):e11
79. Murray SA, Gridley T (2006) Snail family genes are required for left-right asymmetry determination, but not neural crest formation, in mice. Proc Natl Acad Sci U S A 103(27):10300–10304
80. Ezhkova E, Pasolli HA, Parker JS, Stokes N et al (2009) Ezh2 orchestrates gene expression for the stepwise differentiation of tissue-specific stem cells. Cell 136(6):1122–1135
81. LeBoeuf M, Terrell A, Trivedi S, Sinha S et al (2010) Hdac1 and Hdac2 act redundantly to control p63 and p53 functions in epidermal progenitor cells. Dev Cell 19(6):807–818
82. Candi E, Cipollone R, Rivetti di Val Cervo P, Gonfloni S et al (2008) p63 in epithelial development. Cell Mol Life Sci 65(20):3126–3133
83. Guo X, Keyes WM, Papazoglu C, Zuber J et al (2009) TAp63 induces senescence and suppresses tumorigenesis in vivo. Nat Cell Biol 11(12): 1451–1457
84. Koster MI, Kim S, Mills AA, DeMayo FJ et al (2004) p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev 18(2):126–131
85. Romano RA, Ortt K, Birkaya B, Smalley K et al (2009) An active role of the DeltaN isoform of p63 in regulating basal keratin genes K5 and K14 and directing epidermal cell fate. PLoS One 4(5):e5623
86. Candi E, Dinsdale D, Rufini A, Salomoni P et al (2007) TAp63 and DeltaNp63 in cancer and epidermal development. Cell Cycle 6(3):274–285
87. Dai X, Segre JA (2004) Transcriptional control of epidermal specification and differentiation. Curr Opin Genet Dev 14(5):485–491
88. Su X, Cho MS, Gi YJ, Ayanga BA et al (2009) Rescue of key features of the p63-null epithelial phenotype by inactivation of Ink4a and Arf. EMBO J 28(13):1904–1915
89. Vigano MA, Mantovani R (2007) Hitting the numbers: the emerging network of p63 targets. Cell Cycle 6(3):233–239
90. Truong AB, Khavari PA (2007) Control of keratinocyte proliferation and differentiation by p63. Cell Cycle 6(3):295–299
91. Truong AB, Kretz M, Ridky TW, Kimmel R et al (2006) p63 regulates proliferation and differentiation of developmentally mature keratinocytes. Genes Dev 20(22):3185–3197
92. Clevers H, van de Wetering M (1997) TCF/LEF factor earn their wings. Trends Genet 13(12):485–489
93. DasGupta R, Fuchs E (1999) Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development 126(20):4557–4568
94. Ambler CA, Watt FM (2007) Expression of Notch pathway genes in mammalian epidermis and modulation by beta-catenin. Dev Dyn 236(6):1595–1601
95. Williams SE, Beronja S, Pasolli HA, Fuchs E (2011) Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 470(7334):353–358
96. Tanigaki K, Honjo T (2010) Two opposing roles of RBP-J in Notch signaling. Curr Top Dev Biol 92: 231–252
97. Okuyama R, Nguyen BC, Talora C, Ogawa E et al (2004) High commitment of embryonic keratinocytes to terminal differentiation through a Notch1-caspase 3 regulatory mechanism. Dev Cell 6(4):551–562
98. Byrne C, Tainsky M, Fuchs E (1994) Programming gene expression in developing epidermis. Development 120(9):2369–2383
99. Leask A, Byrne C, Fuchs E (1991) Transcription factor AP2 and its role in epidermal-specific gene expression. Proc Natl Acad Sci U S A 88(18):7948–7952
100. Koster MI, Kim S, Huang J, Williams T et al (2006) TAp63alpha induces AP-2gamma as an early event in epidermal morphogenesis. Dev Biol 289(1):253–261
101. Nguyen BC, Lefort K, Mandinova A, Antonini D et al (2006) Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. Genes Dev 20(8):1028–1042
102. Payre F, Vincent A, Carreno S (1999) ovo/svb integrates wingless and DER pathways to control epidermis differentiation. Nature 400(6741):271–275
103. Dai X, Schonbaum C, Degenstein L, Bai W et al (1998) The ovo gene required for cuticle formation and oogenesis in flies is involved in hair formation and spermatogenesis in mice. Genes Dev 12(21): 3452–3463
104. Li B, Dai Q, Li L, Nair M et al (2002) Ovol2, a mammalian homolog of Drosophila ovo: gene structure, chromosomal mapping, and aberrant expression in blind-sterile mice. Genomics 80(3):319–325
105. Descargues P, Sil AK, Sano Y, Korchynskyi O et al (2008) IKKalpha is a critical coregulator of a Smad4-independent TGFbeta-Smad2/3 signaling pathway that controls keratinocyte differentiation. Proc Natl Acad Sci U S A 105(7):2487–2492
106. Gomis RR, Alarcon C, He W, Wang Q et al (2006) A FoxO-Smad synexpression group in human keratinocytes. Proc Natl Acad Sci U S A 103(34): 12747–12752
107. Teng A, Nair M, Wells J, Segre JA et al (2007) Strain-dependent perinatal lethality of Ovol1-deficient mice and identification of Ovol2 as a downstream target of Ovol1 in skin epidermis. Biochim Biophys Acta 1772(1):89–95
108. Nair M, Bilanchone V, Ortt K, Sinha S et al (2007) Ovol1 represses its own transcription by competing with transcription activator c-Myb and by recruiting histone deacetylase activity. Nucleic Acids Res 35(5):1687–1697
109. Wells J, Lee B, Cai AQ, Karapetyan A et al (2009) Ovol2 suppresses cell cycling and terminal differentiation of keratinocytes by directly repressing c-Myc and Notch1. J Biol Chem 284(42):29125–29135
110. Biggs LC, Rhea L, Schutte BC, Dunnwald M (2012) Interferon regulatory factor 6 is necessary, but not

sufficient, for keratinocyte differentiation. J Invest Dermatol 132(1):50–58
111. Moretti F, Marinari B, Lo Iacono N, Botti E et al (2010) A regulatory feedback loop involving p63 and IRF6 links the pathogenesis of 2 genetically different human ectodermal dysplasias. J Clin Invest 120(5):1570–1577
112. Botti E, Spallone G, Moretti F, Marinari B et al (2011) Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas. Proc Natl Acad Sci U S A 108(33):13710–13715
113. Mani SA, Guo W, Liao MJ, Eaton EN et al (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133(4):704–715
114. Oh JE, Kim RH, Shin KH, Park NH et al (2011) DeltaNp63alpha protein triggers epithelial-mesenchymal transition and confers stem cell properties in normal human keratinocytes. J Biol Chem 286(44):38757–38767
115. Hudson LG, Newkirk KM, Chandler HL, Choi C et al (2009) Cutaneous wound reepithelialization is compromised in mice lacking functional Slug (Snai2). J Dermatol Sci 56(1):19–26
116. Frye M, Fisher AG, Watt FM (2007) Epidermal stem cells are defined by global histone modifications that are altered by Myc-induced differentiation. PLoS One 2(8):e763
117. Watt FM, Frye M, Benitah SA (2008) MYC in mammalian epidermis: how can an oncogene stimulate differentiation? Nat Rev Cancer 8(3):234–242
118. Sparmann A, van Lohuizen M (2006) Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 6(11):846–856
119. Sen GL, Webster DE, Barragan DI, Chang HY et al (2008) Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. Genes Dev 22(14):1865–1870
120. Vire E, Brenner C, Deplus R, Blanchon L et al (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439(7078):871–874
121. Sen GL, Reuter JA, Webster DE, Zhu L et al (2010) DNMT1 maintains progenitor function in self-renewing somatic tissue. Nature 463(7280):563–567
122. Osorio KM, Lilja KC, Tumbar T (2011) Runx1 modulates adult hair follicle stem cell emergence and maintenance from distinct embryonic skin compartments. J Cell Biol 193(1):235–250
123. Waikel RL, Kawachi Y, Waikel PA, Wang XJ et al (2001) Deregulated expression of c-Myc depletes epidermal stem cells. Nat Genet 28(2):165–168
124. Ezhkova E, Lien WH, Stokes N, Pasolli HA et al (2011) EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. Genes Dev 25(5): 485–498
125. Nascimento EM, Cox CL, Macarthur S, Hussain S et al (2011) The opposing transcriptional functions of Sin3a and c-Myc are required to maintain tissue homeostasis. Nat Cell Biol 13(12):1395–1405
126. Zhang L, Stokes N, Polak L, Fuchs E (2011) Specific microRNAs are preferentially expressed by skin stem cells to balance self-renewal and early lineage commitment. Cell Stem Cell 8(3):294–308
127. Yi R, Fuchs E (2010) MicroRNA-mediated control in the skin. Cell Death Differ 17(2):229–235
128. Lena AM, Shalom-Feuerstein R, Rivetti di Val Cervo P, Aberdam D et al (2008) miR-203 represses 'stemness' by repressing DeltaNp63. Cell Death Differ 15(7):1187–1195
129. Yi R, Poy MN, Stoffel M, Fuchs E (2008) A skin microRNA promotes differentiation by repressing 'stemness'. Nature 452(7184):225–229
130. Lien WH, Guo X, Polak L, Lawton LN et al (2011) Genome-wide maps of histone modifications unwind in vivo chromatin states of the hair follicle lineage. Cell Stem Cell 9(3):219–232
131. Gallico GG III, O'Connor NE, Compton CC, Kehinde O et al (1984) Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med 311(7):448–451
132. Zouboulis CC, Adjaye J, Akamatsu H, Moe-Behrens G et al (2008) Human skin stem cells and the ageing process. Exp Gerontol 43(11):986–997
133. Mascré G, Dekoninck S, Drogat B, Youssef KK, Broheé S, Sotiropoulou PA, Simons BD, Blanpain C (2012) Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature 489(7415):257–262
134. Chikh A, Matin RN, Senatore V, Hufbauer M, Lavery D, Raimondi C, Ostano P, Mello-Grand M, Ghimenti C, Bahta A, Khalaf S, Akgül B, Braun KM, Chiorino G, Philpott MP, Harwood CA, Bergamaschi D (2011) iASPP/p63 autoregulatory feedback loop is required for the homeostasis of stratified epithelia. EMBO J 30(20):4261–4273
135. Driskell I, Oda H, Blanco S, Nascimento E, Humphreys P, Frye M (2011) The histone methyltransferase Setd8 acts in concert with c-Myc and is required to maintain skin. EMBO J 31(3):616–629
136. Mejetta S, Morey L, Pascual G, Kuebler B, Mysliwiec MR, Lee Y, Shiekhattar R, Di Croce L, Benitah SA (2011) Jarid2 regulates mouse epidermal stem cell activation and differentiation. EMBO J 30(17):3635–3646
137. Beck B, Blanpain C (2012) Mechanisms regulating epidermal stem cells. EMBO J 31(9):2067–2075