interesting to determine whether such a role for IFNγ is seen in vivo, for example by studying branching in IFNγ or IFNγ receptor knockout mice or by using blocking antibodies.

In summary, this new function for the immune system in altering pubertal mammary gland development occurs via APC stimulation of CD4+Th1 interactions. This results in localized secretion of IFNγ, which inhibits luminal differentiation and thereby suppresses ductal development and branching.

Although lots of details about the cells and cytokines involved still need to be uncovered, this new role of the adaptive immune system may also be important for disease development. For example, in the current model there is a feedback loop between APCs and Th1 cells that suppresses epithelial development and could have a role in protecting against cancer. However, if in cancer the T cells switch more to a Th2 type, this may have an opposite effect, contributing to breast epithelial invasion and metastases. Additionally, infection with pathogens that induce certain CD4+ T cell responses, and thereby alter the Th1 bias, may have previously unappreciated roles in regulating breast development and differentiation.

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Secrets of the Hair Follicle: Now on Your iPhone

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Skin development requires communication between epithelial and mesenchymal cells, melanocytes, and neurons. In this issue of *Developmental Cell*, Sennett et al. (2015) shed new light on these mechanisms by simultaneously profiling multiple different cell types in embryonic mouse skin at the onset of hair follicle formation.

Ever since the publication of Margaret Hardy's classic review on hair follicle morphogenesis (Hardy, 1992), biologists have turned to this mini organ as an accessible and intricately beautiful model system for unraveling general principles of development, regenerative growth, and adult stem cell behavior. Delineating the mechanisms by which hair follicles develop in the embryo remains an area of great interest. Although we understand the basic mechanisms by which epithelial and mesenchymal cells communicate during hair follicle morphogenesis, precisely how this crosstalk results in a functioning, multi-layered hair follicle is still unclear. Little is known of the signals by which emerging hair follicles communicate and coordinate with other cell types in the skin, such as nerve fibers and melanocytes. Regional differences in hair follicles, for instance in the size and androgen responsiveness of human scalp versus body hair, are thought to be dictated by signals from the dermis (Hardy, 1992); however, the signaling interactions involved in establishing this variation are virtually unknown. Furthermore, the regulatory factors that control formation of hair follicles versus sweat glands, and how these have evolved to match the needs of different mammalian species, remain obscure. Solving these puzzles will be important in the quest to regenerate normally functioning skin for therapeutic purposes. In an elegant Resource study in this issue of *Developmental Cell*, Sennett et al. (2015) provide fresh insight into some of these questions by simultaneously profiling multiple different cell types in embryonic mouse skin at a single, critical time point, when hair follicles first start to develop.

The earliest morphological sign of hair follicle formation is the appearance of a thickening, or placode, in the surface ectoderm. Almost simultaneously, dermal cells under the placode coalesce to form

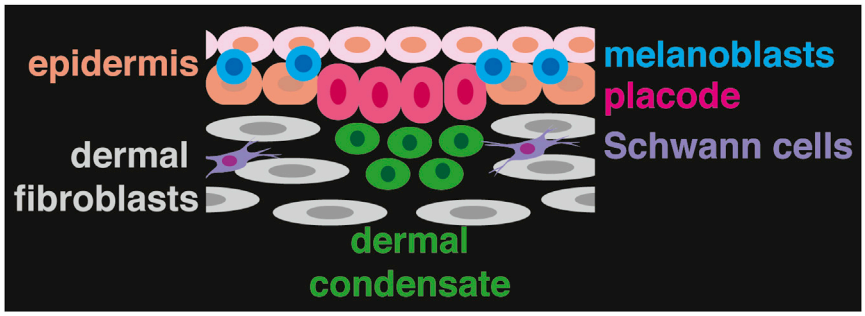

Figure 1. Cell Types in Embryonic Mouse Skin at E14.5

a dermal condensate (DC), the precursor of the hair follicle dermal papilla (DP) (Figure 1). Subsequent signaling from the DC is required for proliferation and downgrowth of the follicle. Secreted signaling molecules mediate interactions of developing hair follicles with their environment, as well as the communications required for morphogenesis.

Gene expression analyses form an important foundation for delineating these signaling mechanisms. The first studies in this area relied on labor-intensive in situ hybridization and immunostaining techniques. While such approaches could identify only limited numbers of candidate genes, they were informative, leading to functional analyses that defined major signaling pathways required for hair follicle development. These include Wnt signaling through the canonical β-catenin pathway, which is essential in both epithelial and dermal cells for hair follicle formation (Tsai et al., 2014; Zhang et al., 2009). Activation of the Eda/Edar pathway, mutated in the majority of human ectodermal dysplasias, operates immediately downstream of Wnt/β-catenin signaling and maintains primary hair placodes in embryonic mouse skin. Eda/Edar signaling is required for expression of Sonic hedgehog (*Shh*), which in turn promotes development of the DC into a DP and hair follicle epithelial proliferation and downgrowth (Zhang et al., 2009). Subsequent microarray-based transcriptional profiling studies of EDA-A1-treated skin explants lead to identification of FGF20 as a key epithelial signal that induces DC formation (Huh et al., 2013). Rendl et al. developed sophisticated profiling approaches using transgenic technology and immunolabeling to purify dermal fibroblasts, melanocytes and hair follicle matrix cells, outer root sheath cells, and DP from mouse skin at postnatal day 4. By examining multiple cell types at the same time point, this study revealed potential interactions of mesenchymal and epithelial cells at early stages of postnatal hair growth (Rendl et al., 2005).

In the current paper, Sennett et al. (2015) have undertaken the ambitious goal of simultaneously profiling all of the cells in the skin at a single embryonic time point when hair follicles first start to develop. This global approach allows inference of signaling interactions between multiple different cell types and provides an advance over earlier studies of embryonic hair follicles that focused on either the epithelial or mesenchymal compartment or utilized mixed-cell populations. Sennett et al. (2015) also used next-generation RNA sequencing for transcriptional profiling, which provides a less-biased and more-quantitative analysis than the microarray-based approaches employed in most of the prior studies. For FACS isolation of specific cell types, the authors utilized a novel combination of immunolabeling with anti-E-cadherin and P-cadherin together with transgenic expression of *Sox2*^{GFP} and *Lef1-RFP* that allowed them to separate placode cells, DCs, melanocytes, interfollicular keratinocytes, non-DC fibroblasts, Schwann cells, and all remaining cells, which were a mixture of endothelial cells, smooth muscle cells, and unidentified dermal cells. Importantly, the authors were able to define new molecular signatures for each of the specifically isolated populations. Placode-specific genes included *Crim1* and *Kremen2*, which modulate signaling, gap junction and calcium-sensing genes, and the transcription factor gene *Sox21*. DC-expressed genes included many associated with Shh and Fgf signaling, *Dclk1*, encoding a kinase implicated in cell migration, and transcription factors of the SOX and FOX families. Mining of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database revealed distributions of ligand, receptor, and inhibitor expression for pathways of known importance in morphogenesis. The authors also identified DC-specific expression of potentially significant novel factors, including axon guidance regulators involved in chemokine, Semaphorin, Neuropilin, Netrin, Ephrin, and Slit/Robo signaling, suggesting involvement of these pathways in sorting and clustering of cells to form the DC and/or in guiding Schwann cells to developing hair follicles.

The hair follicle placode gives rise to all of the epithelial lineages of the hair follicle, including the adult hair follicle bulge epithelial stem cell compartment (Levy et al., 2005). The new dataset provided by Sennett et al. (2015) allowed these authors to explore whether hair follicle embryonic precursors have similar transcriptional characteristics to previously characterized adult hair stem cells and their DP niche. Interestingly, the authors found little overlap in gene expression between placodes and bulge stem cells (13 genes). This finding is consistent with the observation that bulge formation is dependent on *Sox9*, which is expressed in hair follicle epithelial cells after the placode stage (Nowak et al., 2008). Comparison of the DC and adult DP identified only 31 overlapping genes, suggesting that DC cells undergo major changes as they develop into the DP, consistent with observations of heterogeneous cell types within the adult DP. By contrast, embryonic and postnatal melanocytes had relatively similar gene expression patterns.

A major strength of this study is the development and ready availability of a companion website, Hair-gel (http://hair-gel.net/) that has been optimized for use on mobile as well as standard devices and provides an easily searchable gene expression database and a link to the raw data. Such a resource has been lacking in the hair biology field, and its usefulness may be inferred from the popularity of a similar site called Bite-it (http://bite-it.helsinki.fi/) that provides expression data on developing teeth (hosted by the Tooth and Craniofacial Development Group at the University of Helsinki). Access to such data, together with the

advent of CRISPR/Cas9 technology, will significantly accelerate the pace of functional analyses of skin morphogenesis and the development of new tools for visualizing, and tracing the lineages of, specific cell populations. In the future it will be of interest to employ similar approaches to explore the molecular basis of regional differences, for instance in dorsal versus ventral and in hairy versus non-hairy skin. Extending these analyses to include microRNAs and large noncoding RNAs, which are increasingly recognized as playing key roles in stem cell function and differentiation, will also be valuable.

Skin development is a highly dynamic process. The availability of fluorescent reporters of gene expression, cell-cycle stage, and signaling pathway activity, as well as exciting recent advances in live imaging of skin tissues (Ahtiainen et al., 2014; Rompolas et al., 2012), are now making it possible to monitor changes in expression patterns and visualize their effects on signaling, cell division, and cell movements, in real time. Sennett et al.'s fascinating "snapshot" of gene activity will facilitate such studies and provides us with an exciting preview of Technicolor movies yet to come.

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Adhesive Enrichment and Membrane Turnover at the Heart of Cardiopharyngeal Induction

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Differential inductive signaling during asymmetric division of progenitor cells specifies the heart lineage in *Ciona intestinalis*. In this issue of *Developmental Cell*, Cota and Davidson (2015) show that differential induction is mediated by FGF receptor regionalization, resulting from asymmetric cell-matrix adhesion and reduced mitotic turnover of polarized Caveolin-rich membrane domains.

Inductive signaling plays a central role in cell fate acquisition, for example in driving mesodermal progenitor cells toward a cardiac fate. The vertebrate heart and pharyngeal muscles are derived from evolutionarily conserved cardiopharyngeal mesoderm that shares developmental regulators and sequential fate decisions with tunicates, our closest invertebrate chordate relative and a powerful model system for investigating the earliest steps of heart development (Diogo et al., 2015). Cota and Davidson now demonstrate in this issue of *Developmental Cell* that polarized induction of cardiopharyngeal progenitor cells in the ascidian tunicate *Ciona intestinalis* is driven by spatially restricted receptor localization (Cota and Davidson, 2015). Furthermore, they identify the intersection between asymmetric cell-matrix adhesion and mitotic membrane turnover as the mechanism controlling receptor regionalization (Figure 1). This elegant study of the cell biology underlying cardiopharyngeal specification has broad implications for our understanding of how basic cellular mechanisms—such as adhesion, membrane turnover, and mitosis—impact embryonic cell fate decisions.

The cardiac lineage in *Ciona* is derived from bilateral B7.5 blastomeres in the 110-cell embryo that express the transcription factor Mesp (reviewed in Kaplan et al., 2015). Activation of the cardiac genetic program requires Mesp together with MAPK-Ets activity downstream of fibroblast growth factor (FGF) signaling and is restricted to two anterior/ventral granddaughter cells of B7.5 that arise by asymmetric cell division at the late neurula stage (Kaplan et al., 2015). These cells, termed trunk ventral cells (TVCs), migrate into the trunk region to give rise to the heart and pharyngeal muscles at metamorphosis (Kaplan et al., 2015). In contrast, the posterior/dorsal granddaughter cells, expressing Mesp but not active MAPK-Ets, form anterior tail muscle.

FGF signaling plays a conserved role in cardiogenesis, being required for