Cell biology: More than skin deep

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In studying how stem cells make and maintain tissues, nearly every chapter of a cell biology textbook is of interest. The field even allows us to venture where no chapters have yet been written. In studying this basic problem, we are continually bombarded by nature's surprises and challenges.

As a student in physical chemistry, my initial view of cell biology was that it was a science with too many variables to design a well-controlled experiment. After learning that there are cells in our body with the amazing ability to replenish and repair our tissues all through our lives, I began to venture into the study of living cells, learning to be comfortable with being uncomfortable. I began to realize that in cell biology, even though experiments rarely if ever deliver unequivocal answers, they almost always lead to new exciting questions.

My big leap was to carry out my postdoctoral studies with a quintessential cell biologist, Howard Green. I emerged from his laboratory as a molecular biologist but fascinated by a plethora of cell biological questions about the fundamental properties of adult tissue stem cells. How do polarized stratified epithelial tissues such as the epidermis of the skin form from a single layer of unspecified progenitors? How is the epidermis able to replace dying cells and maintain the tissue barrier that keeps harmful microbes out and essential body fluids in? And upon injury, how does the epidermis repair itself? The answers to these fundamental questions about how tissues form and maintain themselves are at the crux of cell biology. Yet the knowledge also shapes the foundation for understanding the basis of human diseases and for advancing regenerative medicine.

While I got hooked on cell biology from the start of my independent career, I didn't think I'd stay with skin as a model system for so long. However, I began to realize that the skin epithelium is the perfect system for studying tissue biology and stem cells, and it can be tackled from a myriad of different angles. At the surface of our body, it is readily accessible and plentiful, and its cells can be cultured in 3D to recreate a skin epidermis. The skin is subjected to daily assault and its epithelium has a vast reserve of stem cells to rejuvenate the body surface and repair wounds. During development, the epidermis goes from a monolayer to a stratified tissue, and creates appendages—hair follicles, sweat, and sebaceous glands. The epithelium receives many of its signaling cues from other cells within the skin—a plethora of communication signals whose language begs to be deciphered.

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Indeed, understanding how skin epithelial tissues form draws upon nearly every facet of cell biology. Maintaining a monolayer of polarized cells requires knowledge of cell–cell adhesion, cell–substratum adhesion, and cytoskeletal dynamics. Generating a self-sustaining, stratifying, and differentiating tissue requires an additional understanding of spindle orientation, asymmetric cell divisions, and balancing of proliferation with differentiation. And to understand how normal homeostasis is achieved, one needs to identify which signaling pathways are involved, which cells transmit them, and how the epidermal stem cell perceives one or more heterologous and cell autonomous signals to adjust its program of gene expression and strike the right balance between growth and differentiation. Too much growth can lead to hyperproliferative disorders of the skin, including cancers; too little can contribute to skin aging.

As my group and I began to navigate the many facets of tissue biology and work to identify the long-lived stem cells of the skin, we learned that these cells reside in specific locations or "niches" within the skin epithelia. Moreover, as epidermis stratifies, only its innermost layer harbors proliferative self-renewing capacity (Fuchs and Green, 1980; Jones et al., 1995, 2007; Mascré et al., 2012; Lim et al., 2013). Hair follicles and glands have their own separate of stem cell niches (Morris et al., 2004; Tumbar et al., 2004; Snippert et al., 2010; Lu et al., 2012). By having different stem cell niches, each tissue can replenish itself as necessary, and during injury, there is nearly always a nearby stem cell niche to receive and respond to the 911 call (Ito et al., 2007; Page et al., 2013).

We've spent much of the past decade dissecting the complex crosstalk between the hair follicle stem cells and their niche. Unexpectedly, we learned that signaling comes not only from mesenchymal cells but also from stem cell progeny within the niche (Fig. 1; Hsu et al., 2014, and references therein). Given the impact of the microenvironment to the stem cells, it perhaps not too surprising to find that when the surrounding niche components are removed, e.g., by skin burns, laser ablation, or genetic manipulation, tissue regeneration grinds to a halt (Green, 1991; Rompolas et al., 2013; Hsu et al., 2014). Quite remarkably, however, when stem cells are ablated, their early progeny can fill niche vacancies and resume tissue activity (Ito et al., 2004; Buczacki et al., 2013; Rompolas et al., 2013). This plasticity provides compelling evidence that the stem cell niche is as critical to tissue homeostasis and maintenance as its popular residents (Blanpain and Fuchs, 2014).

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The Rockefeller University Press $30.00
J. Cell Biol. Vol. 209 No. 5 629–631
www.jcb.org/cgi/doi/10.1083/jcb.201503129

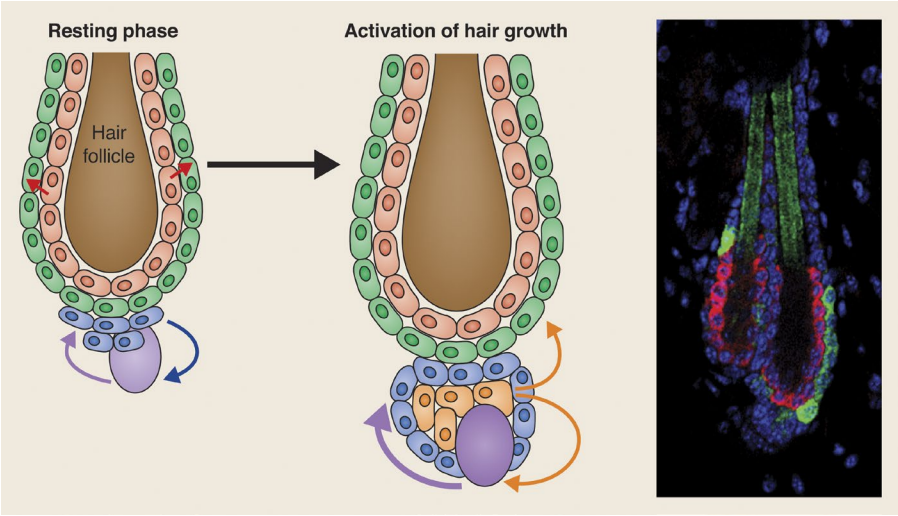

Figure 1. **Stem cells and their niche.** The hair follicle is a prominent example of how cell biology can help understand tissue homeostasis. Each hair follicle requires a niche of stem cells to undergo cyclical bouts of hair growth. The schematic (left) depicts the niche in its resting (nontissue generating) and activated (tissue generating) stages. The arrows indicate communication signals within the niche (color-coded according to the cells that are transmitting the signal and pointing to the recipient cells). Quiescent stem cells (green) receive inhibitory signals from their differentiated progeny (red). During the resting phase, crosstalk between the mesenchymal cells (purple) and "primed" stem cells (blue) builds up the threshold of activating signals that overpower the inhibitory signals to launch the tissue-generating phase. The primed stem cells begin to make short-lived progeny (orange). In the early stages of tissue growth, these stem cell progeny act as a transient signaling center of the niche to fuel tissue growth. For more details, see Greco et al. (2009) and Hsu et al. (2014). The immunofluorescence image (right) marks the nuclei of the skin in blue (DAPI), the inner niche layer in red (keratin 6), and a subset of stem cells that received a transgene expressing green fluorescent protein under the control of an enhancer that is active only in quiescent hair follicle stem cells (Adam et al., 2015).

If the stem cell niche is so overbearing, does it also define the characteristics of stem cells? At least with regard to some stem cell niches, this appears to be the case. Thus, when hair follicle stem cells are taken out of their niche and placed in culture, many of their features change. In fact, with the advent of genome-wide RNA sequencing, chromatin immunoprecipitation, and high-throughput sequencing (ChIP-seq), we've learned that there are literally hundreds of changes in chromatin remodeling and gene expression that occur in vitro that are restored when the stem cells are engrafted and return to their normal niche microenvironment (Adam et al., 2015). For most cell biologists who, like me, studied their favorite cells in culture for many years, where the cells were submerged in serum-containing media supplemented with growth factors, such news is a bit unsettling! Intriguingly, however, we've gone on to show that many of the changes we see are also induced after injury. Thus, the changes that arise when stem cells are placed in vitro mimic a wound or stress environment. As long as we cell biologists walk the line between in vivo and in vitro, we can appreciate these nuances and add physiological relevance to them.

One of my favorite systems now for probing cell biology is the surface of the embryo in utero. Here, the cells receive their normal systemic and environmental cues at all the right times in development and under conditions where they are not subjected to the stress of an in vitro situation. Under these conditions, the growth of epidermal progenitors is remarkably uniform (Beronja et al., 2010). With our ability to use lentiviruses to selectively transduce the skin epithelium at a stage when it exists as one single layer of unspecified progenitors, we can very rapidly induce expression of a desired fluorescently tagged protein or signaling reporter, or knock down expression of a particular gene in a matter of days. With CRISPR/CAS now on the horizon, the prospect of switching genes on or off will revolutionize the pace at which we are able to unravel the mysteries of tissue biology and stem cells.

Indeed, a plethora of variations on the theme of stem cell biology underlie the epidermis and its appendages, and await our investigation. The diversity of techniques and approaches that must go into developing a molecular framework for adult tissue homeostasis still has no blueprint. This is what excites me most about the scientific problem. By going beyond the biology of single cells, and understanding how tissue homeostasis works in vivo, we can begin to apply our knowledge to wound repair, aging, regenerative medicine, and cancer.

In closing, I was fortunate as a graduate student at Princeton University to have professors of cell biology who have had a lasting impact on my own career: Marc Kirschner was focusing on the cytoskeleton and cell cycle; Hal Weintraub and Bruce Alberts were studying chromatin, transcriptional regulation, and gene expression. They were all fabulous teachers and I was fascinated by the questions they were asking. I was also taught by Art Pardee and Arnie Levine—wow—cancer and human biology too! And even though my graduate mentor, Charles Gilvarg, was not a cell biologist, he taught me the importance of rigorous science and of taking a multidisciplinary and molecular approach to tackling scientific questions. Looking back, I see the threads of influence from all my professors woven into my career. In many respects, I was destined to investigate all of cell biology wrapped into the complex problem of tissue biology. Hopefully, this lesson will not fall on deaf ears, as it tells us as a cell biology community that education and mentorship are as important to our profession as being passionate about the science we do.

In the now over three decades of my career, I cannot imagine focusing on any other science. I have become a card-carrying cell biologist with a cell biology–centric view of life. This is the field at the interface with physics, engineering, chemistry, and medicine. The challenge we face for the future is to make sure we convey this message to our government and private philanthropic organizations. It is not so obvious to the society that cell biology should be at the center of attention—that we cell biologists, who are so focused on the basic science of living cells, are in fact forging the paths to new and improved therapeutics for human disease. We are well aware of the importance of what we do. We must pass on our passion not only to those who we mentor, but also to those who fund us. So long as we cell biologists

place some of our collective energy and creativity into dazzling the public with what we can do, the future of cell biology will continue to be rightfully "where it's at" in the world of science.

Acknowledgments

Illustrations were provided by Neil Smith (www.neilsmithillustration.co.uk).

Submitted: 27 March 2015
Accepted: 15 May 2015

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