

# Studying cell biology in the skin

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**ABSTRACT** Advances in cell biology have often been driven by studies in diverse organisms and cell types. Although there are technical reasons for why different cell types are used, there are also important physiological reasons. For example, ultrastructural studies of vesicle transport were aided by the use of professional secretory cell types. The use of tissues/primary cells has the advantage not only of using cells that are adapted to the use of certain cell biological machinery, but also of highlighting the physiological roles of this machinery. Here we discuss advantages of the skin as a model system. We discuss both advances in cell biology that used the skin as a driving force and future prospects for use of the skin to understand basic cell biology. A unique combination of characteristics and tools makes the skin a useful *in vivo* model system for many cell biologists.

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## INTRODUCTION

A primary reason for this perspective is to highlight the utility of *in vivo* model systems, and specifically the skin, in studies of basic cell biological regulators. For basic cell biologists looking for *in vivo* systems to expand into, the skin offers a combination of advantages that other tissues do not possess. We discuss these, along with examples of success stories in which research in skin has uncovered mechanisms of basic cell biology, most prominently in the understanding of the cell adhesion/intermediate filament systems. In addition, we discuss how genetic studies often reveal unexpected phenotypes and roles for cell biological regulators. These are especially enlightening and often yield insights into novel functions for core machinery and additional levels of regulation. Although we focus on the skin as a useful model, it is important to emphasize that cell biological machinery is adapted for diverse functions in different tissues. Therefore lessons learned in cultured cells and in studies of the skin may not necessarily explain the complexity of how these proteins function and interact within a different tissue context. In addition, this is clearly a two-way street, as more cell biological studies on the skin will increase our understanding of the physiology and pathology of this essential organ.

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Abbreviations used: UV, ultraviolet; XP, xeroderma pigmentosum.

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## ADVANTAGES OF THE SKIN AS A CELL BIOLOGY MODEL SYSTEM

Advantages of using of the skin as a model system include the following:

1. Accessibility for microscopy. The medical study of the skin and its diseases has a long history because the skin, being on the body surface, could be described and catalogued before present imaging technologies and surgical procedures were commonplace. This is also a boon to biologists, as it allows live microscopic analysis in living embryos or adults. Imaging has allowed visualization of diverse phenomena, including immune cell recruitment to invading pathogens, stages of hair follicle morphogenesis, migration of melanoblasts, and the dynamics of cell adhesion turnover, to name just a few (Peters *et al.*, 2008; Mort *et al.*, 2010; Rompolas *et al.*, 2012; Foote *et al.*, 2013). Techniques such as spinning disk microscopy are sufficient for some studies in the interfollicular epidermis, as the cells being imaged are very near the surface. In other cases, two-photon microscopy has been used to peer further into the skin. Lightsheet microscopy promises to further propel these studies and our abilities to observe subcellular phenomena in tissues. This relies on the existence of transgenic mouse lines with fluorescent reporters—an emerging field in which the skin is well positioned to play a vital role.
2. Genetic tools and fluorescent transgenic lines. There are many genetic lines that allow the marking and lineage tracing of specific cell populations, as well as genetic fluorescent markers of signaling pathway activation. However, a toolkit of transgenic lines for the visualization of subcellular phenomena has also been gradually developing in the mouse (Table 1). Although we

Structure/protein	Genetic model	Expression	Reference
Chromatin	K14-H2B-GFP	Epidermis	Tumbar <i>et al.</i> (2004)
Cytoskeleton			
Actin	K14-GFP-actin	Epidermis	Vaezi <i>et al.</i> (2002)
Microtubules	CMV-LifeAct-GFP	Ubiquitous	Riedl <i>et al.</i> (2010)
Myosin	K14-EMTB-3GFP	Epidermis	Lechler and Fuchs (2007)
	NMIIC-GFP	Endogenous pr.	Ebrahim <i>et al.</i> (2013)
Cell adhesion			
Adherens junctions	E-cadherin-CFP	Endogenous pr.	Snippert <i>et al.</i> (2010)
Tight junctions	ZO-1-GFP	Endogenous pr.	Zhou <i>et al.</i> (2013)
Desmosomes	DPI-GFP	Endogenous pr.	Footo <i>et al.</i> (2013)
Centrosomes/centrioles	K14-centrin-GFP	Epidermis	Lechler and Fuchs (2005)
	CAG-centrin2-GFP	Ubiquitous	Higginbotham <i>et al.</i> (2004)
Spindle poles	NuMA-GFP	Epidermis	Poulson and Lechler (2010)
Cell cycle status	Fucci reporter	Ubiquitous	Sakaue-Sawano <i>et al.</i> (2008), Abe <i>et al.</i> (2013)
Kinase signaling	ERK FRET sensor	Ubiquitous	Kamioka <i>et al.</i> (2012)

K14, Keratin 14 promoter; H2B, histone H2B; GFP, green fluorescent protein; EMTB, ensconsin microtubule-binding domain; NMIIC, nonmuscle myosin IIC; ZO-1, zonula occludens 1; DPI, desmoplakin I; NuMA, nuclear mitotic apparatus; ERK, extracellular signal regulated kinase; FRET, Forster resonance energy transfer.

**TABLE 1:** Partial list of fluorescent genetic transgenic mouse lines for cell biological research.

do not yet have the diversity of reporters found in flies and worms, the advent of genome editing technologies is expected to yield an ever-increasing set of reporters for the mouse. This is part of a larger collection of genetic tools made by researchers and global projects such as the Knockout Mouse Project (KOMP) that allow the tissue and cell type-specific disruption or mis/overexpression of genes with existing alleles. In addition to these more traditional approaches, lentiviral infection of epidermal progenitors by in utero electroporation allows much more rapid analysis by both knockdown and misexpression analysis (Beronja *et al.*, 2010).

3. Large size. There are two major advantages to skin tissue being so large. First, there is an abundance of tissue when performing histological analyses, so there is more forgiveness for beginners (no fine dissections and limitations of materials). Of greater importance, the tissue abundance provides ample resources to facilitate biochemical studies. As an example, the initial identification of desmosomal cadherins was achieved through purification of desmosomes from a rich source: bovine muzzle epidermis (Drochmans *et al.*, 1978). Subsequent work would show that these are related to classical cadherins, that they form the adhesive core of desmosomes, and that some of them are targets for autoantibodies that disrupt desmosomes—a group of diseases known as pemphigus (Kottke *et al.*, 2006; Kowalczyk and Green, 2013). In addition to traditional biochemical approaches, the skin is amenable to biochemical interaction screens and proteomic analysis, promising to further spur our understanding of cell biology.
4. Cell culture systems. The importance of the ability to culture and passage epidermal cells cannot be overstated. Through the pioneering work of Howard Green in the 1970s, we can isolate epidermal progenitors (human and mouse), grow them for more detailed cell biological studies in vitro, and gain understanding of basic mechanisms. We can then take what we find back into the mouse to understand the physiology. Although care must be taken in interpretation (as cultured cells clearly exist in a very different environment from that in the tissue), such systems have proven to be invaluable tools in many studies. Cultured cells can

also be used to regrow stratified epidermis, a technique that can be used to generate a differentiated tissue from human cells as well as mouse cells. In addition, cultured cells can be engrafted back onto the mouse for analysis of genetically modified cells in a physiological environment.

5. Physiological readouts. The skin is an essential organ with diverse functions. As such, the different cell types require a multitude of fundamental biological processes to generate and maintain tissue function. In addition to readouts of tissue architecture, proliferation, and differentiation, we can also assess tissue function in a variety of ways, such as examination of the barrier activity of the epidermis. Connecting basic cell biological regulators to tissue functions often provides surprises, as discussed later.
6. Clinical insights. In some cases, basic cell biology can be advanced by the existence of human mutations (see examples in the next sections). The extensive descriptive work in dermatology, combined with new sequencing technologies, will allow the identification of genes and pathways that affect tissue architecture and function.

## EXAMPLES OF SUCCESS STORIES

### Desmosomes/hemidesmosomes/keratins

Determination of the composition, functions, and pathologies associated with these cell adhesion systems are among the greatest successes of research in the skin. Key to these studies were many of the advantages of the skin just listed. Reverse genetic studies were the first to demonstrate a functional role for keratins in mechanical integrity of the skin (Vassar *et al.*, 1991), studies that were subsequently supported by analysis of human patients with mutations in keratins (Coulombe and Lee, 2012). Continuing work has identified genetic lesions in many desmosomal proteins, hemidesmosomal proteins, and other keratins that lead to a spectrum of disorders not only in the skin, but also in the heart (Kottke *et al.*, 2006). In this case, the cell biology and the disease phenotypes have clear correlates—for example, the position of blistering (between cells, within cells, or between cells and the basement membrane)

depends on where in this network the protein is localized and functioning. Further studies on identification of components of the desmosomes, how they assemble, and how they connect to keratins have depended largely on experiments performed in cultured keratinocytes (Kowalczyk and Green, 2013). Although there are many questions left to answer, the combination of tools and the clinical relevance of the findings will make the process particularly exciting. As an aside, this work also made skin an early player in the mechanobiology field. Because this tissue experiences many physical insults, it is likely to continue to play an important role in understanding how cells/tissues sense and respond to force.

### Xeroderma pigmentosum

One of the earliest examples of patient-directed research leading to deeper understanding of cell biological mechanisms is the study of xeroderma pigmentosum (XP). The condition is a rare hereditary disease characterized by high sensitivity to ultraviolet (UV) radiation and increased susceptibility to cancers of the skin (Cleaver and Bootsma, 1975). Cultured fibroblasts isolated from XP patients had impaired responses to the repair of double-stranded DNA lesions after UV irradiation, a finding that was confirmed *in vivo* using tissue sections (Cleaver, 1968; Epstein *et al.*, 1970). Further studies revealed that these cells did not initiate the first step of nucleotide excision repair and that XP is caused by a failure to excise pyrimidine dimers after exposure of DNA to UV (Setlow *et al.*, 1969; Cleaver and Trosko, 1970). Before these discoveries, the mechanisms that controlled DNA repair had only been fully characterized in microorganisms (Cleaver, 1968, 1978). Consequently, the examination of XP patient samples provided the first functional evidence for nucleotide excision repair in humans and on how defects in this process are associated with a clinical phenotype. Mutations in other nucleotide excision repair genes lead to Cockayne syndrome and trichothiodystrophy, disorders with both overlapping and distinct clinical features (Kraemer *et al.*, 2007). Thus the genetics and cell biology continue to teach us about the roles of this pathway.

### EXAMPLES OF UNEXPECTED *IN VIVO* ROLES OF BASIC CELL BIOLOGICAL MACHINERY

The genetic analysis of core cell biological regulators often unveils unexpected or absent phenotypes. These studies can reveal unexpected additional functions for these proteins, uncover redundancy between different machineries, or expose additional levels of regulation that were previously unanticipated. Some examples, of many from the literature, are as follows.

p120-catenin is a well-studied adherens junction protein that functions to regulate cortical levels of E-cadherin (Davis *et al.*, 2003; Xiao *et al.*, 2003). Analysis of mutants lacking epidermal p120, however, revealed phenotypes not associated with cell adhesion. Instead, epidermal hyperplasia and chronic inflammation were induced by activation of the Rho/NF- $\kappa$ B axis (Perez-Moreno *et al.*, 2006). This function could not have been predicted from work in cultured cells alone.

The Arp2/3 complex generates branched actin networks that play diverse roles in cell migration, vesicle trafficking, and cell adhesion (Rotty *et al.*, 2013). Surprisingly, loss of Arp2/3 in the epidermis did not result in notable defects in cell architecture or adhesion. Instead, Arp2/3-complex mutant epidermis was hyperproliferative and showed defects in expression of both early and late differentiation genes. Yap1 activity was increased, and inhibition of this pathway rescued the differentiation defects. In addition, tight junction proteins showed decreased cortical organization in mutant tissue and did not generate robust resistance to ion flow in cell culture

(Zhou *et al.*, 2013). Again, neither biochemical studies nor studies in cultured cells predicted these phenotypes. Further, the phenotypes are nonoverlapping in different tissues. For example, in intestinal epithelia, the Arp2/3 complex regulates vesicle trafficking and the endolysosomal system (Zhou *et al.*, 2015). This highlights that different lessons will be learned by analysis of core cell biological regulators in diverse tissues.

Whereas Par3 is believed to be a master regulator of epithelial polarity, the epidermis develops normally in its absence, highlighting our incomplete understanding of how polarity is generated in a mammalian tissue. Loss of Par3, however, does have opposing effects on different tumor types, suppressing papillomas and promoting keratoacanthomas (Iden *et al.*, 2012). Although the underlying mechanisms are not fully understood, this suggests that the epidermis will be useful for studying polarity signaling pathways and their role in both homeostasis and tumorigenesis. It also demonstrates that comparative studies of simple and stratified epithelia will identify conserved and divergent regulators of epithelial function.

Examination of organelle functions is also providing novel insights into tissue biology. Loss of reactive oxygen species through mutation of a transcription factor required for expression of electron transport chain components caused unexpected differentiation defects in the epidermis, presumably through defects in Notch and Wnt signaling, revealing additional roles for metabolic enzymes in tissue function (Hamanaka *et al.*, 2013).

We have highlighted the utility of the skin as a model system, the different methodological approaches that can be used, and the types of information that can be gleaned from working with the skin. Together these examples demonstrate that the skin is a powerful tool in which one can examine a wide array of cell biological phenomena. Through continued collaborative efforts between cell biologists and skin biologists, we expect that additional fundamental insights into both basic cell biology and skin biology will continue to emerge.

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