

# Revisiting the Cutaneous Epithelium: Insights from a Nontraditional Model System

## Das Hautepithel überdenken: Erkenntnisse von einem einfachen Modellsystem

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### Abstract

In the last 10 years, biology has made revolutionary advances from century-old debates about the relative importance of non-pathogenic bacteria. New applications of sequencing technologies are transforming our understanding of the biology of the skin. Similar to all other epithelia, the skin is colonized by an assemblage of microorganisms which, for the most part, peacefully coexist with their hosts. Alterations to microbial communities are associated with, and likely contribute to, a number of cutaneous disorders. This review focuses on the host factors that shape and maintain skin microbial communities, and the reciprocal role of microbes in pathogen defense and tissue

homeostasis. We highlight how work in the non-traditional model system Hydra is uncovering new mechanisms to old cell biological problems that provide a foundation for understanding, preventing and in the long-term even treating skin diseases. We demonstrate that the cutaneous epithelium is an ecosystem hosting a complex microbiome and that components of the innate immune system as well as transcriptional regulators of stem cells are involved in maintaining homeostasis between epithelia and their resident microbiota. We conclude that beneficial bacterial-host interactions should be considered an integral part of skin biology and that nontraditional model systems can provide a holistic understanding of the cutaneous epithelium.

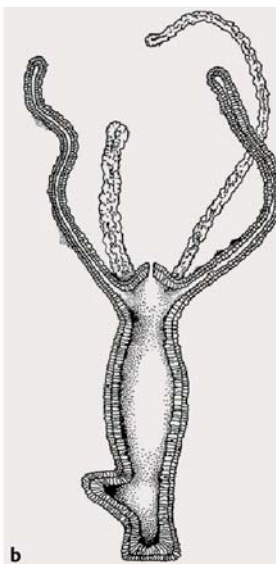
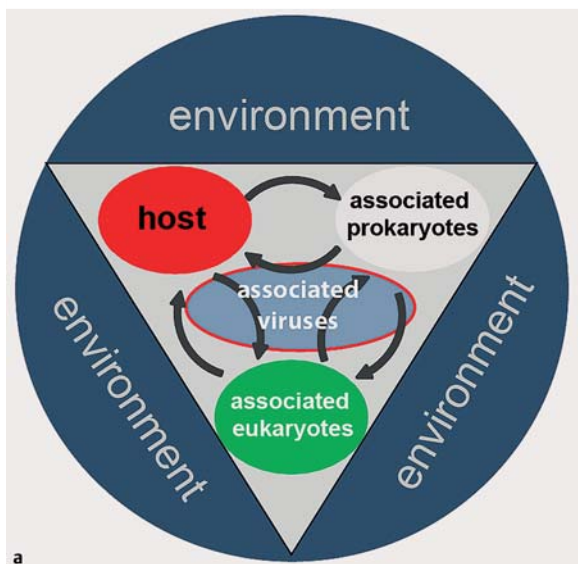
*“Nothing in biology makes sense except in the light of evolution.” [1]*

### Introduction: The cutaneous epithelium is an ecosystem and host to complex microbial communities

Our understanding of the biology of the skin is in the midst of a major transition. Extraordinary recent progress in molecular genetics in a number of model systems, novel sequencing technologies and comparative bioinformatics is revealing details about the cutaneous epithelium that undermine prior conceptions, and highlight the value of an evolutionary perspective. The naïve conception that the skin is an entity, with some ectodermal and mesodermal cell types interacting with each other, is fading as the complex and dynamic nature of organisms as metaorganisms is becoming better understood. All animals, ranging from simple invertebrates to humans, are host to complex microbial communities and, therefore, must be considered a meta-organism, i.e. the macroscopic host in synergistic interdependence

with bacteria, archaea, viruses, fungi, and numerous other microbial and eukaryotic species (Fig. 1 a) [2,3,4]. These resident microbes influence fitness and thus ecologically important traits of their hosts [5,6].

Since 150 years bacteriologists, microbiologists and immunologists have focused on bacteria as pathogens. This approach has led to enormous insights in the battle between the invading harmful microbes and the host and also opened up the opportunity to develop efficient strategies to fight infections. Today we know that most bacteria are not harmful but beneficial and are playing a key ecological role. In an updated literature survey, only about 200 of the millions of bacteria that interact with humans are regarded as emerging or reemerging pathogens [7,8]. Inexpensive, high throughput sequencing has uncovered a new world of relationships between skin cells and their colonizing microbes [9,10,11,12]. Using mass spectrometry and DNA sequencing, the bacteria and chemical compounds found on human skin have been sampled and mapped across the body in a series of 3-D images [13]. The chemical signature (secretome) found on the skin is



**Fig. 1** a Multicellular organisms are metaorganisms comprising the macroscopic host and its synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species including algal symbiont (modified from [4]). b *Hydra*, a basal animal with a simple gross anatomy (tentacles, head and foot) and a simple epithelio-muscular bilayer, has emerged 550 million years ago. The fresh water cnidarian is a close relative to corals and jellyfish with their enormous impact on environment on earth. In recent years this non-traditional model has shown to be useful answering fundamental questions in biology, concerning development, immunity and host-microbe interactions (modified from [39]).

thought to be unique to an individual and is distinct for certain body parts. It harbors specific combinations of bacteria and a distinct mix of molecules from foods eaten and even medicines taken. This newfound awareness of the skin as an ecosystem colonized by a diverse milieu of microorganisms presents additional layers of complexity for dermatologists and raises many questions that are being addressed by new and interdisciplinary research programs. The field of ecological evolutionary developmental biology (Eco-Evo-Devo) attempts to study and model this new view of nature by organizing concepts such as developmental symbiosis and developmental plasticity into evolutionary theory [14]. “Biology has entered a new era with the capacity to understand that an organism’s genetics and fitness are inclusive of its microbiome” [15]. For dermatologists this means that they must approach their field with an entirely new concept of nature.

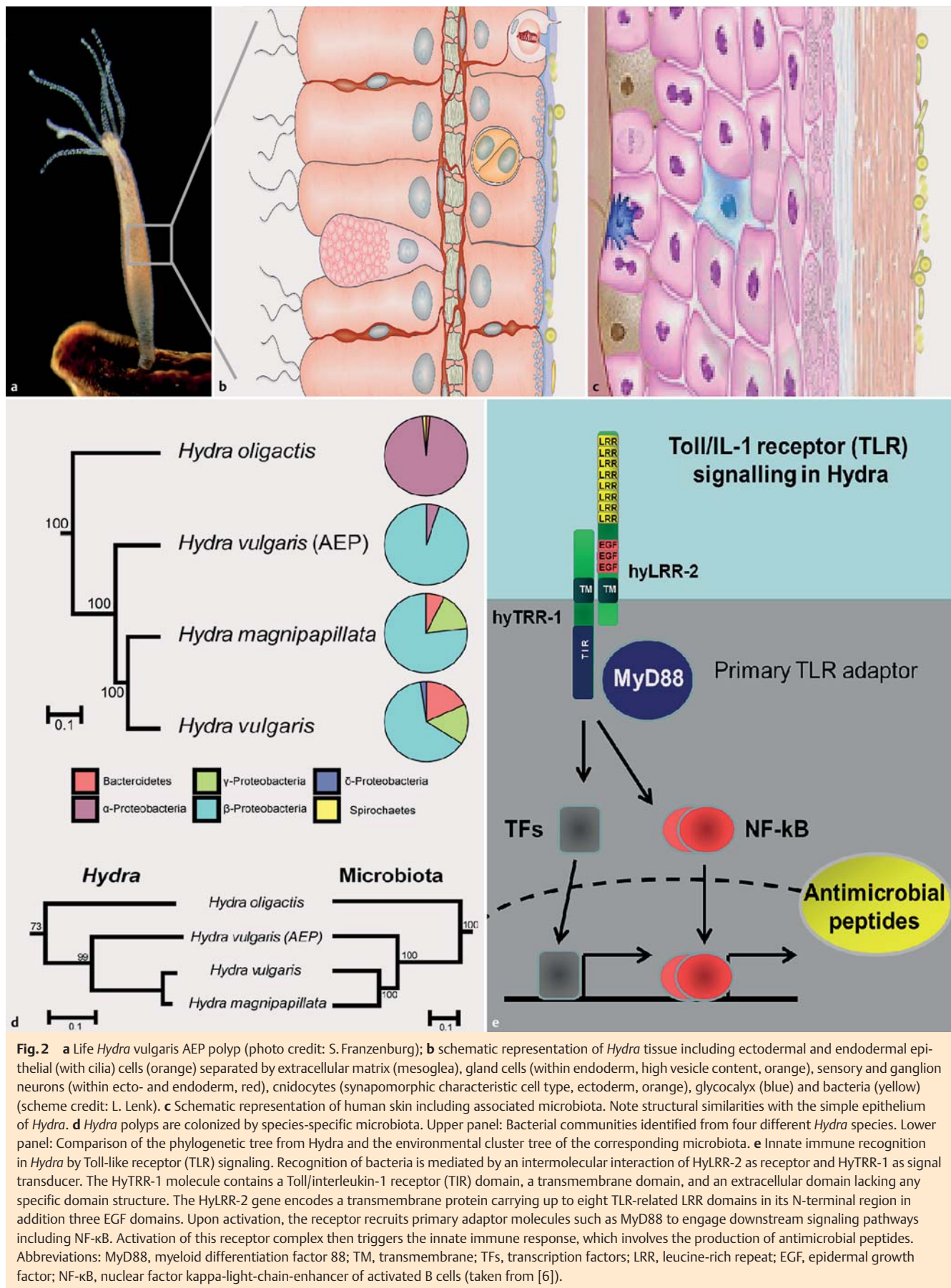
### Bacteria matter: recent lessons from *Haemophilus*

Symbiotic microorganisms occupy a wide range of skin niches [9, 10, 11, 12] and may even protect against invasion by more harmful or pathogenic organisms. Recent evidence supporting this view comes from a study focused on *Haemophilus ducreyi* [16]. This bacterium causes chancroid, a relatively common form of sexually transmitted genital ulcers that is endemic in certain parts of Africa and Asia and facilitates the transmission of HIV-1. The bacterium has also been implicated in nonsexually transmitted cutaneous ulcers in children in the tropics. Interestingly, infected individuals can either clear the infection or develop pustules that eventually form abscesses. What is the reason behind these differences? Taking advantage of a unique human skin infection model, researchers at Indiana University have found evidence to suggest that the makeup of the skin’s microbiome plays a major role in whether an individual can clear the *H. ducreyi* bacterial infection without intervention [16]. The investigators compared the skin microbiome in patients who resolved their *H. ducreyi* infection to those who did not. Strikingly, preinfection skin microbiomes of pustule formers and resolvers have distinct community structures that change in response to the progression of *H. ducreyi* infection. In people who progressed to an active infection, the microbiome was much more dispersed from the beginning of the experiment to the end than in those people who spontaneously resolved their

infections. The results highlight an association between the skin’s microbial inhabitants and resolution of infection. But do the bacteria that normally colonize our skin directly help to clear the pathogenic bacteria? Or is the microbiome only another indicator, but not the cause of bacterial infection? While we have to wait for answers to these questions, the *Haemophilus* study provides a convincing example of how the ecology of human skin can influence health and disease. Microbes therefore matter! Intriguingly, one of the abundant bacterial taxa among the infection-resolvers was *Propionibacterium acnes*, a microbe associated with skin acne. Thus it seems that under some circumstances bacterial species or strains such as *Propionibacterium acnes* may cause skin disease and under other conditions may guard the skin and keep it healthy. Principles of ecology appear to determine the homeostasis between skin-dwelling bacteria and their host tissue. Recent studies highlight that in addition to chancroid, the pathological outcome of many human and animal diseases is influenced by the co-existence with the residing microbial communities. Disturbed host-microbial interactions and related inflammatory signaling play an important role in the etiology of a number of chronic inflammatory disorders affecting barrier organs (e.g. inflammatory bowel diseases, psoriasis), but also in metabolic diseases and certain forms of cancer [17, 18, 19, 20, 21].

### How *Hydra*, a non-traditional epithelial model system, can help us to understand the complexity of cutaneous defense

Defining the individual microbe-host conversations in a given metaorganism (● Fig. 1 a) is a challenging but necessary step on the path to understanding the function of the associations as a whole. Untangling the complex interactions requires simple animal models with only a few specific bacterial species. Such models can function as living test tubes and may be key to dissecting the fundamental principles that underlie all host-microbe interactions. Here we introduce *Hydra* (● Fig. 1 b; ● Fig. 2 a and ● Fig. 2 b) as such a non-traditional model with one of the simplest epithelia in the animal kingdom, with only two cell layers, with few cell types derived from only three distinct stem cell lineages, and with the availability of a fully sequenced genome and numerous genomic tools including transgenesis.



**Fig. 2** **a** Life *Hydra vulgaris* AEP polyp (photo credit: S. Franzenburg); **b** schematic representation of *Hydra* tissue including ectodermal and endodermal epithelial (with cilia) cells (orange) separated by extracellular matrix (mesoglea), gland cells (within endoderm, high vesicle content, orange), sensory and ganglion neurons (within ecto- and endoderm, red), cnidocytes (synapomorphic characteristic cell type, ectoderm, orange), glycocalyx (blue) and bacteria (yellow) (scheme credit: L. Lenk). **c** Schematic representation of human skin including associated microbiota. Note structural similarities with the simple epithelium of *Hydra*. **d** *Hydra* polyps are colonized by species-specific microbiota. Upper panel: Bacterial communities identified from four different *Hydra* species. Lower panel: Comparison of the phylogenetic tree from *Hydra* and the environmental cluster tree of the corresponding microbiota. **e** Innate immune recognition in *Hydra* by Toll-like receptor (TLR) signaling. Recognition of bacteria is mediated by an intermolecular interaction of HyLRR-2 as receptor and HyTRR-1 as signal transducer. The HyTRR-1 molecule contains a Toll/interleukin-1 receptor (TIR) domain, a transmembrane domain, and an extracellular domain lacking any specific domain structure. The HyLRR-2 gene encodes a transmembrane protein carrying up to eight TLR-related LRR domains in its N-terminal region in addition three EGF domains. Upon activation, the receptor recruits primary adaptor molecules such as MyD88 to engage downstream signaling pathways including NF- $\kappa$ B. Activation of this receptor complex then triggers the innate immune response, which involves the production of antimicrobial peptides. Abbreviations: MyD88, myeloid differentiation factor 88; TM, transmembrane; TFs, transcription factors; LRR, leucine-rich repeat; EGF, epidermal growth factor; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells (taken from [6]).

For analytical purposes, *Hydra* is a premier model organism, which in the laboratory is propagated and mass-cultured. The ectodermal epithelium provides a permanent protection barrier to the environment and resembles in several aspects the anatomy of the cutaneous epithelium in vertebrates (● Fig. 2b and ● Fig. 2c). Since the genome content [22] and the ectodermal epithelial organization is remarkably similar to that of the human skin, these animals offer unique insights into the biology of a cutaneous epithelium.

### Microbial colonization

Bacteria are an important component of the *Hydra* metaorganism and colonize the mucus layer, which is coating the ectodermal epithelium (● Fig. 2b). The 36 identified bacterial phylotypes represent three different bacterial divisions and are dominated by Proteobacteria and Bacteroidetes [23,24]. Disturbances or shifts in any of these partners can compromise the health of the whole animal [25]. Because *Hydra* have been cultivated tens of years under standard conditions at constant temperature and identical food, it came as a surprise that examinations of the microbiota in different species kept in the laboratory for more than 20 years under controlled conditions revealed an epithelium colonized by a complex community of microbes, and that individuals from different species but cultured under identical conditions differed greatly in their microbiota. Bacteria in *Hydra*, therefore, are specific for any given species [24]. In line with this, the composition of the microbiome parallels the phylogenetic relationships of the *Hydra* species (● Fig. 2d). The microbiome, therefore, reflects an ancestral footprint of evolution, a pattern termed phyllosymbiosis [26]. This finding strongly indicates that distinct selective pressures are imposed on and within the *Hydra* epithelium and that the host cells actively shape the composition of its colonizing microbiota [27]. Microbiota colonization can depend on the genetics of the host, and there is an intensifying interest today in resolving the relative contributions of the environment and host genes on the assembly of host-associated microbial communities. In humans, along with evidence for the influence of environmental factors, there is clear support for a host genetic component in structuring of microbial communities [28]. In addition to candidate gene approaches, researchers have used host genome-wide genetic variation to find interactions with the microbiome. For example, in a recent genetic association study focused on *Psoriasis*, a chronic autoimmune disease with complex genetic architecture, evidence was provided that a number of susceptibility genes are involved in innate and adaptive immunity and skin barrier functions [29]. The microbiota, therefore, is a complex trait that is under strong host genetic control. The host genome may filter environmental microbes into host tissues as a form of symbiont domestication, and reciprocally, environmental microbes may prefer to occupy specific lineages of hosts [30].

### Microbial recognition and regulation

For microbial recognition, *Hydra* uses the Toll-Like Receptors (TLRs) with MyD88 as signal transducer (● Fig. 2e) and the nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs). Engagement of these receptors leads to a fast induction of protective programs. Prominent effector molecules downstream of the conserved TLR cascade are antimicrobial peptides (AMPs) (● Fig. 2e). AMPs in vertebrates and invertebrates act by disrupting the structure or function of the microbial cell membranes. Our work has shown that in contrast to previous assumptions these peptides are not simply killing microbes but

function as host-derived regulators of the symbiotic microbiota [24]. In humans, there are three important groups of AMPs: the defensins as cationic non-glycosylated peptides containing six cysteine residues; the histatins, which are small, cationic, histidine-rich peptides present in human saliva; and cathelicidin LL-37 which is derived proteolytically from the C-terminal end of the human CAP18 protein [31]. In the human genome there are at least 33  $\beta$ -defensin genes. In *Hydra* the situation is much more simple. Up to now three families of potent AMPs have been identified: the hydramacin, periculin and arminin peptides. Constitutive high level expression and species-specific variability made particularly the arminin peptide family an excellent candidate for investigating the role of AMPs in shaping the host-specific microbiota. Arminin-deficient *Hydra* have a decreased ability to select suitable bacterial partners from a pool of foreign potential colonizers as they are colonized differently than control polyps, which select for bacterial types partially resembling their native microbiota [24]. We conclude from these results that AMPs are shaping the stable associated microbiota and function as host-derived regulators of microbial diversity rather than being protecting agents against pathogenic infection only.

### Microbial function

Recent studies in germ-free animals have shown that shifts in the microbiome can have a strong effect on host traits and could be causal in disease phenotypes [32]. Similarly to studies in mice, we have used gnotobiotic *Hydra* to analyze the functional importance of commensal bacteria [25]. Bacterial colonizers in *Hydra* inhabit the outer layer of the glycocalyx and therefore, have no direct contact to the ectodermal epithelium (● Fig. 2b). While control cultures very rarely show signs of fungal infection, germ-free *Hydra* cultures are regularly infected by fungi. Fungal hyphae are growing on the surface of germfree polyps closely attached to the ectodermal epithelium and can cause the death of the animals. Restoring the specific microbiota in gnotobiotic polyps prevents fungal infection [25]. Bacteria found to significantly inhibit fungal outgrowth *in vivo* include *Acidovorax sp.*, *Curvibacter sp.*, *Pelomonas sp.* and *Undibacterium sp.*. Most importantly, none of the tested bacterial colonizers alone is able to provide full antifungal resistance. Mono-associations with distinct members of the microbiota are not efficient or fail completely to provide protection. Resistance is only achieved in polyps recolonized by a complex bacterial community. Multiple members of the microbiota act synergistically to confer resistance against the pathogenic fungus indicating that functional diversity within the ommensal microbiota is central to pathogen clearance from the epithelium.

### Stem cell proliferation is linked to innate immunity and microbiota composition

As always, the unexpected is the most fascinating. In an effort to uncover the molecular logic behind *Hydra's* unlimited life span by an unbiased transcriptome analysis, we found that the transcription factor forkhead box O (FoxO) is strongly expressed by all three stem cell lineages, whereas it is absent in differentiated cells [33]. By gain-of-function and loss-of-function analysis we subsequently could show that FoxO indeed is a critical component of the mechanisms controlling stem cell behavior in immortal *Hydra*. Interestingly, silencing of FoxO activity not only affects developmental and differentiation genes but also causes changes in the expression patterns of antimicrobial peptides (AMP) which represent the immune status of *Hydra* (● Fig. 3a). FoxO knock-

down polyps showed significant changes in expression of AMPs of the hydramacin, periculin and arminin family. In line with this, *in silico* analysis revealed multiple FoxO-binding sites on the promoter sequences of the corresponding antimicrobial peptide genes. The unexpected link between FoxO and components of the innate immune system (► Fig. 3) has shed at least some light on the age-old problem of how developmental pathways are linked to components of innate immunity. Taken together, it seems that beneficial microbes represent a major factor whose activities are linked to both tissue homeostasis, illustrated as stem cell factors, and immunity (► Fig. 3b).

### The immune system as hardware for a functioning interspecies network

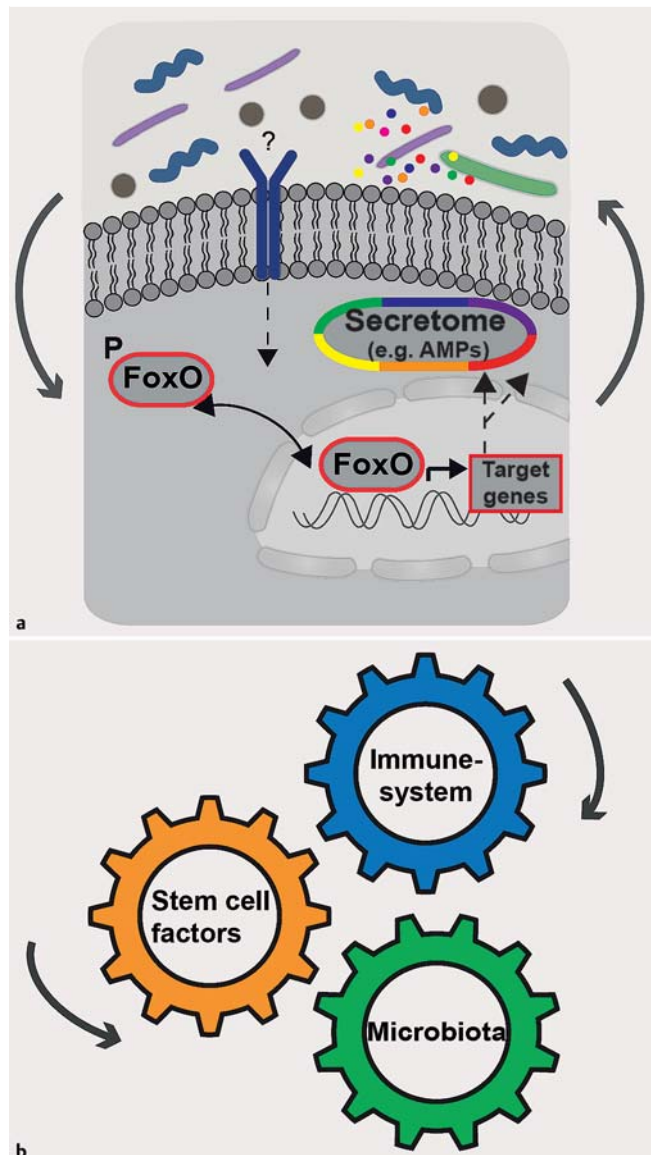
Numerous observations in *Hydra* indicate that immune systems evolved as much to manage and exploit beneficial microbes as to fend off harmful ones [34]. Evidence for this view comes from the discovery that individuals from different species differ greatly in their microbiota and that individuals living in the wild are colonized by microbiota similar to that in individuals grown in the lab, pointing to the maintenance of specific microbial communities over long periods of time. As a result of the finding that interactions between animals and microbes are not specialized occurrences but rather are fundamentally important aspects of animal biology and that antimicrobial peptides and other components of the immune system are key factors for allowing the right microbes to settle and to kick the less desirable ones out, the view of the role of the immune system has changed radically in the last decade and is seen now as door-opener for symbiotic interactions [34].

### Conclusions

The increasing awareness that cutaneous epithelia exist only within a partnership with symbionts has led to two important realizations. Firstly, the health and fitness of the skin appears fundamentally multi-organismal, therefore secondly, an in-depth understanding of the physiology, evolution and development of the epithelium must not be studied on isolated host cells only. Those unexpected insights ask for new research initiatives to systematically evaluate the critical position that microbes have in the cutaneous epithelium. However, in spite of all these insights we have still not been able to coherently integrate the accumulated abundance of information into a truly mechanistic understanding of host-microbe interactions in the skin. It is particularly striking that we do not even know yet what defines a healthy state of microbiota. Furthermore, how does the host control the symbiotic community composition? How stable are these host-associated species communities and how robust are they to environmental perturbations?

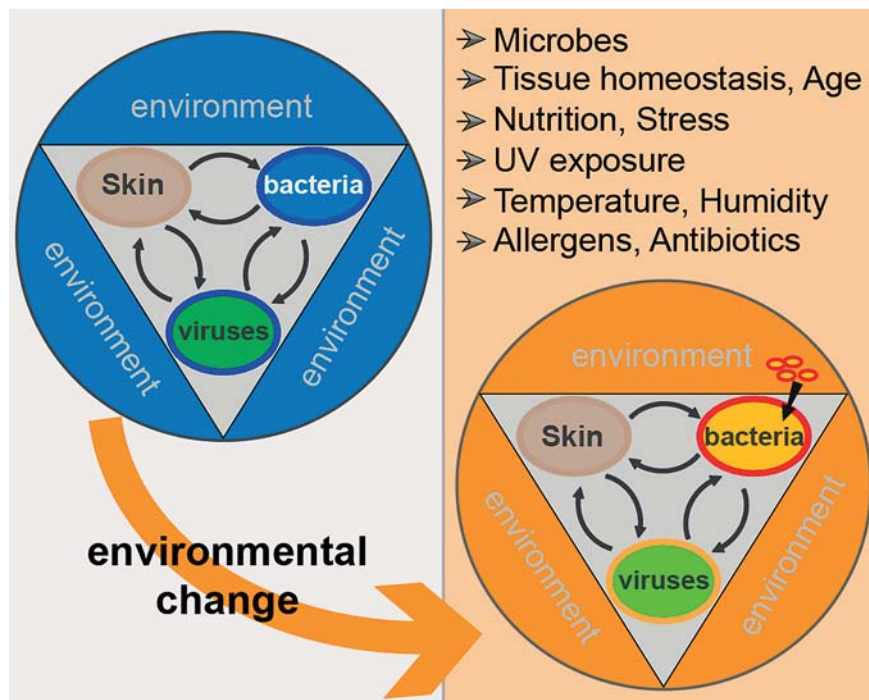
### Six important areas of future research

► Important areas of future research include developing approaches to examine at a mechanistic level how a complex microbiota interacts as a spatially and temporally dynamic network. Here, a key point is to understand to what extent the overall function of the microbiota is influenced by individual as well as synergistic contributions of community members.



**Fig. 3** a A scheme illustrating the current scenario of host gene environmental interactions. In response to changes in the microbiota FoxO activity is altered, which results in a change in expression of stem cell and immune genes, which has an impact on the maintenance of stem cell and immune system (secretome change, e. g. AMP's) and thereby on the composition of the microbiota as well as on the aging process of an organism. Young, non-aged individuals, and potentially immortal organisms such as *Hydra*, have high numbers of active stem cells and an effective immune system. Old, aged individuals, and foxO-deficient *Hydra* are characterized by a decline in stem cell number and functionality as well as an increasingly ineffective and unspecific immune system. b In the *Hydra* holobiont, beneficial microbes represent a major factor whose activities are linked to both tissue homeostasis, illustrated as stem cell factors, and immunity (modified from [6]).

- More generally, current microbiota research crucially requires us to be able to manipulate particular microbes within the community [35]. Tools to modify the presence of particular microbes are rare. Novel tools are needed to tag particular species or strains and/or identify molecules that can impact a specific taxon.
- Important areas of future research also include metagenomic screening of the skin associated virome, including both DNA and RNA mammalian viruses and bacteriophages, and its correlation with disease.



**Fig. 4** A model that summarizes and illustrates how changes on environmental conditions influence the partner choice within the metaorganisms. The quick exchange of partners leads to a fast alteration of the functional abilities of the holobiont and allows, without immediate change of the host genome, an adaptation to the changed environment.

- ▶ What is urgently needed is to integrate information across numerous organisms and from multiple levels of organization, portraying the ecological and genetic interaction networks of entire systems, moving away from a linear cause and effect perspective. Such integrative and multi-level research approaches are required to systematically evaluate the critical role of the microbe-host assemblages in the cutaneous epithelium as units of selection during evolution.
- ▶ With a deeper understanding of the interdependent networks and interfaces that define host-microbiota interactions, we will hopefully be able to determine whether microbial community dynamics result from disease or are implicit in instigating disease.
- ▶ This will be a key future development in guiding therapeutic strategies, including those based on engineering microbial genomes and synthetic communities. Since functional studies are crucial both for elucidating the causal mechanisms whereby microbes affect host fitness and human genetic variation impacts the microbiome, and for identifying novel treatments for inflammatory skin diseases, such as atopic dermatitis and acne vulgaris, nontraditional model systems such as *Hydra* may serve as an informative experimental tool in rethinking paradigms in medical research.

### Seeking a holistic understanding of the cutaneous epithelium

Epithelia are ecosystems carrying a myriad of microbes with them. Current efforts to understand the association between microbes and host cells treat the interacting partners as separate entities, rather than parts of one holistic system. The properties of a given symbiosis system, however, cannot be determined or explained by the specific features of its separate constituents. We have seen above that the microbiota associated with a given cutaneous epithelium might change in response to changes in tissue homeostasis or environmental conditions. That makes it exciting to ask whether the ability of the cutaneous epithelium to adapt to stresses, to function under different environmental

conditions, and resist a pathogen infection, is dependent not only on the genome of the epithelial cells, but on the genomes of its symbionts. In principle, the modularity and interoperability of the components of the metaorganism allows rapid adaptation to changing environmental conditions by altering the associated microbiota. This view was conceptualized by the “holobiont concept” [36,37,38] which predicts that changes in the microbiome – from a shift in the ratio of different microbes to the acquisition of new ones – can allow the holobiont to adapt quickly to changing circumstances and even acquire new abilities during its lifetime. Depending on the variety of different niches provided by the host, which can change with developmental stage, diet or other environmental factors, a more or less diverse microbial community can be established within a given host species. The dynamic relationship between symbiotic microorganisms and environmental conditions results in the selection of the most advantageous holobiont. A living system such as the cutaneous epithelium is nothing but a network of self-coordinating parts, which can bolster its resilience. It may be this kind of modular structure that provides the human skin with resistance against certain pathogens (such as *Haemophilus*) enabling it to fast adapting to novel environmental conditions (● Fig. 4).

Accordingly, and in more general terms we see host-microbe interactions as significant drivers of animal evolution and diversification [14]. The forces that shaped and still are shaping the colonizing microbial composition are the focus of much current investigation, and it is evident that there are pressures exerted both by the host and the external environment to mold these ecosystems. Understanding the diversity of such genome-microbiome-environment interactions requires integrative, multidisciplinary, and modelling-based approaches [14, 34].

The newness of all of this microbiome research and the implications of these discoveries revolutionizing many aspects of biology and medicine are truly exciting. And the lessons are clear: Over decades we have learnt about the toothed wheels (● Fig. 3b), but we still do not understand the clock. The time has come for a holistic understanding of the cutaneous epithelium.

## Acknowledgement



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## Conflict of Interest



The authors declare no conflict of interest.

## Zusammenfassung

### Das Hautepithel überdenken: Erkenntnisse von einem einfachen Modellsystem



In den letzten 10 Jahren hat sich die Biologie aus Jahrhunderte alten Debatten über die relative Bedeutung der nicht-pathogenen Bakterien rasant fortentwickelt. Neue Sequenzierungstechnologien verändern unser Verständnis der Biologie der Haut. Ähnlich wie andere Epithelien ist das Hautepithel von einer Vielzahl von Mikroorganismen kolonisiert, die zum größten Teil friedlich mit dem Wirt koexistieren. Änderungen in den Mikrobengemeinschaften sind korreliert und wahrscheinlich auch ursächlich beteiligt an einer Vielzahl von kutanen Erkrankungen. Dieser Übersichtartikel konzentriert sich zum einen auf die Wirts-Faktoren, die an der Entstehung und Beibehaltung der mikrobiellen Gemeinschaften der Haut beteiligt sind. Wir beleuchten ferner die wechselseitige Rolle der Mikroben in der Erreger-Abwehr und Gewebe-Homöostase. Wir unterstreichen dabei insbesondere, wie die Arbeit an nicht-traditionellen Modellsystemen neue Mechanismen zu alten zellbiologischen Grundfragen aufdecken kann, die eine Grundlage für das Verständnis, die Verhütung und langfristige möglicherweise auch für die Behandlung von Hauterkrankungen darstellen. Jüngste Beobachtungen in *Hydra* zeigen, dass Komponenten des angeborenen Immunsystems wie auch transkriptionelle Regulatoren an der Aufrechterhaltung der Homeostasis zwischen Epithelien und Mikroben beteiligt sind.

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