

PROBLEMS & PARADIGMS

Prospects & Overviews

Hydra and the hair follicle – An unconventional comparative biology approach to exploring the human holobiont

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Funding information

Monasterium Laboratory GmbH; Deutsche Forschungsgemeinschaft; Canadian Institute for Advanced Research; Giuliani Spa

Abstract

The microbiome of human hair follicles (HFs) has emerged as an important player in different HF and skin pathologies, yet awaits in-depth exploration. This raises questions regarding the tightly linked interactions between host environment, nutrient dependency of host-associated microbes, microbial metabolism, microbe-microbe interactions and host immunity. The use of simple model systems facilitates addressing generally important questions and testing overarching, therapeutically relevant principles that likely transcend obvious interspecies differences. Here, we evaluate the potential of the freshwater polyp *Hydra*, to dissect fundamental principles of microbiome regulation by the host, that is the human HF. In particular, we focus on therapeutically targetable host-microbiome interactions, such as nutrient dependency, microbial interactions and host defence. Offering a new lens into the study of HF – microbiota interactions, we argue that general principles of how *Hydra* manages its microbiota can inform the development of novel, microbiome-targeting therapeutic interventions in human skin disease.

KEYWORDS

antimicrobial peptides, microbial ecology, microbiome, model organisms

INTRODUCTION: CLINICAL IMPORTANCE OF STUDYING HOST-MICROBIOME INTERACTIONS IN HUMAN HAIR FOLLICLES

The human hair follicle (HF) is a mini-organ composed of several cell layers that arise from neuroectoderm- and mesenchyme-derived cell lineages (Figure 1A). As the result of an invagination of the skin surface, these structures extend the epithelial tissue for microbial colonisation. The HF microbiota is thought to be ubiquitously distributed throughout this mini-organ, forming a unit within the human holobiont. However, its precise distribution, regulation and interactions within this human host (mini-) organ remain to be clarified.

Although HFs enjoy immune-privilege characteristics within and beyond their stem cell compartment, the bulge,^[1] they are home to

many immunocyte populations and antimicrobial peptides (AMPs).^[1] These, not only participate in the defence against pathogens but also manage the HF microbiota.^[2,3] This microbiota has previously been reported as being ubiquitously and differentially distributed throughout the mesenchymal and epithelial compartments of these mini-organs, being dominated by members of the Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria (Table 1). Among these the most predominant members are *Cutibacterium acnes*, *Staphylococcus epidermidis*, *S. aureus*, *Corynebacterium* spp. However, other less abundant genera, such as *Pseudomonas*, *Acinetobacter* and *Dietzia* also populate this mini-organ along with fungi belonging to the *Malassezia* genus.^[3] Despite their physiological tight regulation, dysbiosis of these resident commensals has a known pathological role in many HF-associated diseases, including acne vulgaris, folliculitis

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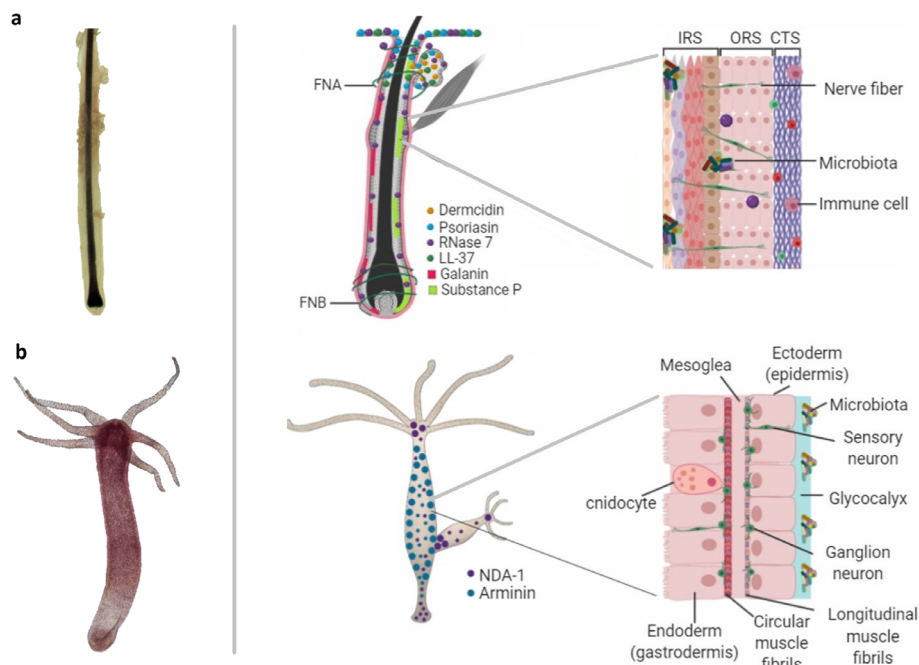


FIGURE 1 Comparative physiology of the HF and *Hydra*. (A) Macroscopic image (left) and schematic representation (right) of the human HF. The HF is composed of two layers of epithelial tissue, the IRS and the ORS, surrounded by a mesenchymal layer, the CTS. Besides fibroblasts and endothelial cells, immune cells (e.g. T cells, mast cells and macrophages) are embedded into the extracellular matrix-enriched CTS, which are differentially distributed throughout the human HF CTS. Similarly, there is a differential presence of AMPs (e.g. RNase 7, LL-37, psoriasin and dermcidin) throughout the human HF epithelium.^[52,53,84] In a similar pattern of expression to these AMPs, the HF has a variety of neuropeptides distributed throughout its epithelium. In its distal areas, the HF is wrapped by two neuronal networks: the follicular neural networks A and B (FNA and FNB). Although the exact location of microbial communities in the human HF as well as differences in the HF microbiome between different skin sites (e.g. scalp, axillae, pubes, chest, beard) remain to be fully characterised, it is thought that these are localised throughout the entire HF epithelium. (B) Macroscopic (left) and schematic representation (right) of the *Hydra*. The *Hydra* body is composed of two layers of epithelial tissue that provide a barrier to the outside and establish a communication gradient with its microbiota, in similar with the more complex HF epithelium. These epithelial layers, the endoderm and ectoderm, are separated by the mesoglea, a layer of extracellular matrix, and a network of nerve cells, which spread throughout the body.^[85] Within these two layers of cells there are sensory cells which are linked to the neural network that runs across the body of this organism. The ectoderm is surrounded on the outside by a mucus layer (glycocalyx), which is colonised by microbiota, and might loosely be compared to the CTS of HFs.^[42] The differential release of arminin, and other AMPs from the epithelial cells and release of neuropeptides with AMP functions (NDA-1) from the neuronal cells into the glycocalyx are thought to regulate microbial communities.^[75] AMP, antimicrobial peptide; CTS, connective tissue sheath; HF, hair follicle; IRS, inner root sheath; ORS, outer root sheath

TABLE 1 Comparison of the microbiomes between *Hydra* and the hair follicle

Bacteria	<i>Hydra</i>	Hair follicle	References
Actinobacteria	<1%	20%–30%	[2,3,7,29,58,101–103]
Actinomycetota	<1%	20%–30%	
Alphaproteobacteria	<1%	1%–10%	
Bacteroidetes	1%–10%	<1%	
Betaproteobacteria	>50%	10%–20%	
Firmicutes	<1%	20%–30%	
Gammaproteobacteria	40%–50%	1%–10%	

and hidradenitis suppurativa, and potentially in both androgenetic alopecia (AGA) and alopecia areata (AA).^[3] These imbalances are often associated with changes in the immune and metabolic landscape of the HF microenvironment, including AMP dysregulation, reactive oxygen species (ROS) production and inflammatory cytokine and immune cell induction, which result in induction or exacerbation of inflammatory

processes.^[4–8] However, whether this HF dysbiosis plays a primary pathogenic role or represents a secondary consequence of disease-related environmental and/or intra-tissue changes remains to be clarified. For example, in AA, the initial stimulus recognised by the immune cells that triggers the accumulation of a dense perifollicular inflammatory cell infiltrate remains unknown. However, it is conceivable that this stimulus may also originate from the HF microbiota.^[3] It has been hypothesised that the dysbiosis observed in the pathobiology results in activation of T-cells and the development of the inflammatory cascade observed.^[9] Similarly, acne vulgaris is accompanied by shifts in *C. acnes* phylotypes and/or increased bacterial abundance; however, the initial stimulus driving these shifts remains unclear.^[10] Despite, it is thought that this imbalance results in the production of more porphyrin, which generates ROS and keratinocyte inflammation.^[11]

In addition, the HF microbiota plays a role as a reservoir for re-colonisation of the skin surface following disinfection.^[12] Hence, it is reasonable to assume that HF dysbiosis may not only promote hair diseases but may also impact on epidermal pathologies associated with skin microbiome dysbiosis, such as in psoriasis, rosacea, seborrheic

dermatitis and atopic dermatitis.^[3,13,14] Understanding these complex microbe-host interactions, including nutritional availability and regulation is therefore important for the development of microbiome-targeting therapeutic interventions in dermatology, including prebiotics and probiotics.^[15] Moreover, in principle, topically applied antibiotics or AMPs might even impact on human hair growth by altering HF microbiome composition.^[7]

Herein, it is hypothesised that important open general questions in human HF microbiome research may be instructively addressed by using an early evolutionary model, the freshwater polyp *Hydra*, as a very unconventional, but instructive and accessible model to dissect fundamental principles of microbiome regulation by the host, emphasising therapeutically targetable host-microbiome interactions within the holobiont.^[16–18] Specifically, proposing that this comparative biology approach to exploring the human holobiont within an exemplary human mini-organ, the HF, by drawing lessons from *Hydra*, will facilitate the elucidation of general principles of how to therapeutically manage host-associated microbiota with the overall aim to facilitate the development of novel therapeutic interventions in human skin disease.

STUDYING HF-MICROBIOME INTERACTIONS AND WHY EXPERIMENTAL MODEL SYSTEMS MATTER

Routinely, preclinical HF studies use the organ culture of scalp skin punches or isolated HF.^[19] This *ex vivo* method preserves general HF morphology, growth modulators, basic gene expression and metabolic patterns, thus allowing defined experimental manipulation of HF biology, including hair growth, metabolism, pigmentation and immune status. However, cultured HF lack sebum in the hair canal and are kept at constant environmental conditions in supplemented media.^[19,20] Further, they require the use of antibiotics/antimycotics so as to suppress rapid bacterial and fungal over-colonisation from the rich HF microbiome.^[7,21] Therefore, neither conservation of the natural HF microbiota, nor HF organ culture under germ-free conditions are as yet feasible. This severely limits the use of scalp skin or HF cultures to study human host-microbe interactions *ex vivo*. Similarly, the study of HF disease states is challenging, given the very limited availability of patient material.

For these reasons, animal models have been used as a powerful strategy to investigate the mechanisms by which the HF regulates its microbiota as well as their role in pilosebaceous physiology and the skin immune system (e.g.,^[22–24]). Despite profound differences between the murine and human immune system, given that their complex microbiota can be manipulated in a reasonably well-controlled fashion, murine models have been widely used in skin microbiome research (e.g.,^[25–27]). These models facilitate the study of microbial interactions with the immune system and how specific HF markers, which contribute to the dynamic process of the hair cycle, are impacted by their microbiota. Meaning, how dysbiosis of the HF microbiome affects HF function (i.e., hair growth and HF cycle); characterisation of the regulation of HF markers by the microbiota and their impact on skin homeostasis.^[23]

However, despite their deceptively similar anatomy and general functional characteristics, there are important interspecific differences in HF development, cycling pattern, pigmentation, metabolism and molecular controls.^[28] Further, generating germ-free or transgenic animals and their manipulation is not only time-consuming but, as in humans, the HF microbiota is difficult to access. Subsequently, environmental and nutritional cues have a higher degree of variability and represent a challenge to targeted microbial manipulation. Thus, the study of nutrient dependency of the holobiont and the general principles of their regulation would benefit from the use of much simpler models, in which greater experimental control can be attained.

In the fresh water polyp *Hydra*, the microbiota is located on the outside of the organism in a mucus-like layer, where it is easily accessible for environmental and microbial manipulation experiments.^[29,30] This mucus-like layer (termed glycocalyx, Figure 1) is densely colonised by a stable multi-species bacterial community.^[31] This community is characterised by members of *Curvibacter*, *Pseudomonas*, *Acinetobacter* and *Duganella* (Table 1), and is shaped by means of the *Hydra* innate immune system and a rich repertoire of AMPs.^[32] This is very similar to the situation in humans, where AMPs also are involved in regulating the microbiota.^[33] This pattern, therefore, may be a conserved principle and points to the epithelium as a crucial regulator of microbial homeostasis.^[30]

As both in *Hydra* and in humans the microbiota is controlled by the innate immune system, these organisms are therefore ideal to model regulatory mechanisms (Figure 1B).^[34] Particularly, this model offers a simpler system that can easily be experimentally manipulated and made germ-free to disentangle basic principles of the likely considerably more complex interactions between host-habitat, nutrient dependency, host defence and bacteria interactions of the human HF.

Although *Hydra* differs from the well-established aforementioned animal models that allow detailed studies of the HF cycle,^[21,35] in similarity with the HF, *Hydra* feature a dynamic changing environment during development, with changes in the immunological landscape, which influences the associated microbiota.^[22,36,37] Moreover, while *Hydra* can be easily cultured in essentially unlimited quantity,^[38] obtaining a sufficient number of human scalp HF from transplant or facelift surgery is notoriously difficult.^[21] Thus, it deserves careful consideration which host-microbiota interactions relevant to the human HF might as well be studied in *Hydra* to elucidate relevant principles that apply to mammalian skin biology, despite the massive interspecies differences over an evolutionary divide of approximately 193 million years.^[39]

GENERAL PRINCIPLES OF THE HYDRA HOLOBIONT THAT CAN BE TRANSLATED TO HUMAN HF MICROBIOME RESEARCH

Host environment

The close proximity of HF to sebaceous glands provides a source of triglycerides, squalene, cholesterol and wax esters, and even HF

themselves are substantial lipid sources.^[40] Together with a moist environment and moderately acidic pH, these benefit colonisation by triglyceride-hydrolysers.^[11] For this reason, it is not surprising that lipophilic microbes such as *C. acnes*, *Corynebacteria* and *Malassezia* spp. dominate these mini-organs,^[3] which are themselves greatly impacted on by lipid metabolism.^[41,42] Shifts within the lipid-degrading microbiota often coincide with skin diseases. For instance, dandruff and seborrheic dermatitis are associated with an overgrowth of *Malassezia*,^[14] while increased abundance of certain *C. acnes* strains participates in the pathobiology of acne vulgaris, though probably less than often assumed.^[43] These changes in abundance of triglyceride-hydrolyser are relevant, since they alter the environmental conditions in the HF. The resultant production of free-fatty acids promotes microbial adherence, has antimicrobial properties and contributes to the regulation of the acidic pH of the skin,^[44] which in turn regulates other microbes.

Yet, the reciprocal nutritional benefits between the HF and its microbiota remain underexplored, namely the complex nutritional network between the different colonising microbes and the regulation of the microbiome by HF-derived nutrients. Given that dysbiosis is observed in several HF-associated diseases,^[3] while antibiotic manipulation of these microbiota may modulate hair growth,^[7] understanding the extent of microbial regulation by the immune system and nutrient availability is essential to inform therapy development. Whether the introduction of new microbes or the change in environmental conditions, for example by use of pre- or pro-biotics, is sufficient to alleviate the pathobiological symptoms, or if the baseline microbiota remains dominant despite these external manipulations, requires elucidation. Hence, analysing the nutrient dependency of commensal microbes is important to understand the regulation of the microbiome composition, its contribution to disease progression and its potential for therapeutic manipulation.

Similar to the environmental conditions of the HF there is a clear nutrient dependency of commensal microbes in *Hydra*. However, in contrast to HFs, *Hydra*'s environmental conditions are more accessible and experimentally controllable, given the easily accessible position of the glycocalyx layer (Figure 1B). In similarity to the intestinal mucus in mammals,^[45] the constant renewal of this mucus-like layer, which covers the epithelial surface layer of *Hydra*,^[30] most likely allows *Hydra*'s rapid adjustments to a constantly changing environment.^[30] The surface-secreted polymeric compounds of the glycocalyx protect the epithelial cell layer from environmental stress and form a defined habitat, which is colonised by a host-specific bacterial community.^[31,32] This host-specific colonisation is maintained even under long-term cultivation of *Hydra* under well controlled laboratory conditions and the carrying capacity is limited to 100 000 bacterial cells per polyp.^[46,47] Interestingly, the culture water does not contain any source of nutrients. For this reason, *Hydra*-associated microbes solely depend on nutrients provided by their host.^[48]

Thus, the nutritional dependency of host-associated microbes can be investigated in *Hydra* in order to address the fundamental question whether and how the host shapes its microbiome by regulating the nutrient supply and/or control microbial colonisation by its immune

system and AMP production by epithelial cells. Due to the surface localisation and the ability to generate germ-free animals the chemical landscape of the host habitat can be precisely characterised in the presence and absence of microbes. Moreover, surface nutritional conditions can be modified by administration of selected nutrients to the surrounding water. This allows to study the impact of nutrients on the microbiota, addressing the questions if an uncontrolled proliferation of nutrients disturb the microbiota and if the host can compensate for this nutrient induced imbalance. Furthermore, the use of prebiotics in the resilience of the microbiome can be investigated. The principles deduced from such studies allow one to formulate concrete working hypotheses on similar host environmental parameters that may control the human HF microbiome, which can subsequently be tested, for example in antibiotic, antimycotic- and serum-free short-term scalp HF organ culture using defined media.^[21]

Microbe-microbe interactions

The interaction between different microorganisms is an important defence mechanism in the skin, as resident microbes are able to inhibit pathogen colonisation.^[49,50] In fact, many of the HF bacterial colonisers exhibit biosynthetic gene clusters and lipases with antimicrobial activity. For example, type I-2 *C. acnes* produces a thiopeptide active against *S. epidermidis*,^[51] whilst *Burkholderia* spp. is known for their production of antifungal compounds, possibly contributing to the regulation of the HF fungal population.^[52] However, not only competition can be expected within the HF microbiome. Since only few members of the HF microbiota are able to hydrolyse sebum-derived triglycerides, it is plausible that other commensals benefit from released fatty acids.^[53] Depending on the abundance and composition, the availability of triglycerides and products released by these other microbes multifaceted interactions can be expected in the HF microbiome.^[54] Disturbance of this complex network, for example by antibiotic treatment, can lead to dysbiosis. Elucidating the factors contributing to the restoration of a healthy microbiome is therefore fundamental for disease prevention and management. Equally important is the evaluation of the microbial founder communities and how these change over time, since, in skin, probiotic efficiency depends on the baseline community.^[55]

Again, *Hydra* offers a convenient model system for dissecting principles of microbe-microbe interactions, as these play an important role in the defence against pathogens but also in the regulation of the bacterial colonisation process in these polyps. In the absence of bacteria, germ-free *Hydra* gets easily infected by the filamentous fungus *Fusarium* sp.^[29] Interestingly, recolonisation of *Hydra* with only one bacterial isolate does not suffice to fully recover host protection, while a combination of two bacterial strains prevents fungal infection.^[29] The interplay between the associated bacterial partners seems to play an important role not only in host-resistance to pathogen infection, but bacteria also benefit from a diverse multispecies colonisation.^[47] For example, in recolonisation experiments mono-associated (with *Curvibacter* sp. or *Duganella* sp.) *Hydra* polyps only reached a reduced carrying

capacity for each of the introduced microbes. Multispecies colonisation, instead, reaches ten times higher abundances, which point to bacteria-bacteria interaction, in which bacteria benefit from inter-species metabolic exchange.^[47] Interestingly, the addition of bacteria to water polyps does not elevate the carrying capacity. Rather, it leads to an overall reduction of the bacterial load per polyp.^[47] Whether this is caused by disruption of bacteria-bacteria interaction or elicited host immune response has to be further investigated, particularly regarding the use and function of probiotics. How the founding community affects further colonisation and contributes to the resilience of the associated microbiota is another important question that can be addressed in *Hydra*.

These evolutionarily ancient, complex microbe-microbe interaction principles, which have also been identified in many evolutionarily younger species,^[56,57] should be conserved in the human HF microbiome, where they are much more difficult to study than in *Hydra*. Thus, principals from these polyps again could facilitate the formulation of simple working hypotheses that are testable under the HF organ culture conditions listed above.

Host defence

Different immune mechanisms are recognised as important players in microbiota management and upon microbial infection. Microbial recognition is made by pattern recognition receptors, including toll-like receptors (TLRs), which are differentially distributed in the HF,^[58,59] and nucleotide-binding and oligomerisation domain (NOD)-like receptors, present, amongst others, in keratinocytes.^[60] This recognition is imperative in the activation of an immune response to fight infections and for the development of tolerance to commensal microbes. Upon pathogen encounter, activation of the innate immune system, though TLRs are responsible for regulation of AMPs and induction of a further immune response, either by MyD88-dependent or independent pathways, resulting in transcription and release of inflammatory cytokines and immune cell recruitment.^[61] Similarly, neonatal mice skin and human gut microbiome studies have shown that proper induction of regulatory T cells (fundamental effectors of the adaptive immune response) is essential for immune tolerance to the commensal microbiome.^[62] Microbial recognition by keratinocytes and resident immune cells (including dendritic cells and T cells) and thereby regulatory T cell regulation is at the centre of this immune tolerance.^[63] Hence, TLR recognition of microbial products is crucial for proper immune induction. In HF diseases, such as AGA and AA, overexpression of these receptors is observed and correlates with dysbiosis.^[58,59] Therefore, understanding whether dysbiosis or the change in TLR expression is the primary stimulus, is crucial for comprehension of the pathobiology. Further, grasping how the microbiota exploit these receptors is important for finding potential targets and advance the treatment of inflammatory diseases where activation of TLRs elicits an inappropriate immune response.

Another line of defence for microbial regulation is AMP expression. HFs possess a range of differentially distributed AMPs gener-

ated by the HF epithelium and resident immune cells, which vary in their antimicrobial activity.^[64,65] These include dermcidin, cathelicidin LL-37, β -defensins, RNase7 and psoriasin^[5,64,66,67] (Figure 1A), and exhibit a broad spectrum of antimicrobial activity.^[5,64,66,67] Among the more abundant HF microbiome, dermcidin is active against and *C. acnes* and LL-37 is active against group A and C *Streptococcus*, while both exhibit antimicrobial activity against *S. aureus*, *S. epidermidis* and *Pseudomonas*.^[66,68–70] Similarly, RNase7 exhibits activity against *S. aureus* and *Pseudomonas*, as does psoriasin, as well as *C. acnes*, *Corynebacterium* spp. and *Acinetobacter*.^[67,71] In addition, they act against potential pathogens, such as *Trychophyton* and *Microsporum*.^[67,72] Unsurprisingly, modulation of these AMPs is common in dysbiotic events. Abnormal AMP expression is associated with loss of integrity of the epidermal barrier and increased infections in diseases that also affect the HF, such as atopic dermatitis.^[73] In addition to traditional AMPs, other peptides produced by HF keratinocytes or released by perifollicular sensory nerve endings possess antimicrobial activity, including the neuropeptides, α -melanocyte-stimulating hormone, neuropeptide Y and substance P.^[74–77] Yet, although their antimicrobial activities were shown in vitro their functional regulation of the HF microbial community remains to be explored. Further, understanding the extent of the communication between these neuropeptides and the commensal HF microbes could provide additional insights into the as yet essentially unexplored modulation of human hair growth by the microbiota. Specifically, whether this communication is unidirectional and whether the microbial communities modulate HF expression/release of these neuropeptides.

Here, too, *Hydra* offers an instructive model to dissect recognition and regulation of the microbiota. As a basal metazoan, *Hydra* lacks an adaptive immune system. Immune recognition of microbes is carried out by pattern recognition receptors (TLRs and NOD-like receptors). TLR signalling activates an innate immune response, through a MyD88-dependent pathway and contributes to the recognition of commensal bacteria as well as host defence against pathogens.^[78] As in humans, AMPs represent major effectors of this response. These include periculin,^[79] hydramacin^[80] and arminin^[81] (Figure 1B), displaying differential antimicrobial activity and are predominantly produced by epithelial cells^[32] or are secreted by (epithelial) gland cells, for example kazal-type serine protease inhibitor.^[82] Interestingly, some AMPs show strong antimicrobial activity against Firmicutes bacteria, which compared to HF are naturally underrepresented in the microbial community of *Hydra* (Table 1). Periculin is highly active against *Bacillus* spp., hydramacin predominantly targets *Staphylococcus hemolyticus* and *Acinetobacter* spp., and arminin exhibits activity against *S. aureus*.^[79–81] Despite their potential to hinder potential pathogenic bacteria from colonisation, *Hydra* AMPs manage the resident bacterial community similar to the HF.^[32] Thereby the ability to generate transgenic *Hydra* offers the unique opportunity to overexpress selected AMPs and to study their impact on the bacterial community composition. For instance, overexpression of periculin in ectodermal epithelial cells affects the composition and reduces the total load of associated bacteria compared to wildtype polyps.^[79] Selective manipulation of defined microbes to evaluate AMP expression and interaction with

the other immune and microbial players would further clarify their role in the holobiont and could also be easily achieved in *Hydra*, however much more challenging in human HFs. Manipulation of key signalling pathways such as TLRs by MyD88 knockdown animals can further contribute to a better understanding of host-microbe interactions,^[83] thus uncovering new signalling pathways involved in bacterial regulation, whose homologs can then be probed in human HF organ culture.

For example, downregulation of the transcription factor FoxO in *Hydra*, known in *Hydra* to be involved in stem cell growth and proliferation, negatively affects AMP expression leading to a disturbed bacterial colonisation after antibiotic perturbation.^[84] Interestingly, FoxO downregulation is also observed in skin and HF-associated diseases, such as psoriasis characterised by overexpression of HF AMPs (e.g., psoriasin, RNase7).^[85,86]

Microbial regulation of host physiology

Moreover, there is increasing evidence that microbes talk to their host. In the skin, the establishment of the HF microbiome and HF morphogenesis are pivotal for regulatory T cell migration and proper immune induction, a key process for the development of tolerance to the resident microbiota.^[22] Not only this cross-talk between the microbes and the immune system, namely with resident macrophages and Langerhans and mast cells stimulates memory T cells, but their interaction with keratinocytes and sebocytes stimulates production of constitutive AMPs.^[2,87] Dysregulation of these AMPs and microbial dysbiosis are often seen in HF diseases. For example, cicatricial alopecias are associated with increased abundance of Staphylococci and β -defensin expression.^[5] However, the initial stimuli are not clear and could represent a potential mechanism for microbiome-targeted therapeutic development.

In addition, a role of these communities in hair growth cannot be excluded.^[7] Indeed, oral supplementation of probiotics containing *Lactobacillus paracasei*, a producer of the short chain fatty acid lactate, promoted hair growth in a mouse model.^[88] In the skin, it is known that short chain fatty acids enhance TLR2 responses in sebocytes, suggesting that changes in the metabolic potential of the microbiome drive alterations in the physiological immune state of the host.^[89] However, the interaction between these metabolites and the HF epithelium requires further investigation.

In other more well studied human microbiomes, such as the gut and the skin, association between the microbiota and its metabolites with production and regulation of neuropeptides is also reported.^[77] In the HF, these neuropeptides, including α -melanocyte-stimulating hormone, neuropeptide Y and substance P, are known modulators of immune status and hair growth.^[90] Further, several members of the skin and HF microbiota, bacteria and viruses, are known producers of peptides that share high similarity with these eukaryotic neuropeptides.^[77] Therefore, the resident microbial communities might play a role in shaping follicular endocrinology and modulate the follicular immune and stress responses, as well as hair growth. However, the extent to which and how this communication occurs, is

maintained and how it is affected by dysbiosis remains unanswered. Substance P plays a pivotal role in maintaining immune function in the HF, and alteration of its expression is linked to the pathobiology of AA, where dysbiosis has also been reported.^[58,91]

In *Hydra* neuropeptides, such as NDA1, possess antibacterial activity and play a functional role in the regulation of the *Hydra* microbiome composition.^[92] Microbial metabolites not only stimulate *Hydra*'s immune response but also influence behaviour.^[93] For example, interaction of the microbiota with the enteric nervous system, particularly pacemaker cells, drives peristalsis and can be further investigated in *Hydra*.^[94] This can be done by microbial transplantation experiments of germ-free polyps using either single bacterial strains or different combinations of *Hydra* specific bacterial isolates.^[93] Moreover, metabolites produced and secreted by bacteria after transplantation when living in the mucosal surface environment of *Hydra* can be analysed by conducting metabolomics of the surrounding water. By this, potential neuro-activating compounds can be identified and further tested for causality by applying them directly to the *Hydra* culture water.

Elucidating the impact of microbial metabolites on immune and neuronal communication systems in the *Hydra* model organism will extend our understanding how targeted microbial manipulation can be used to alleviate the altered expression of specific metabolites in inflammatory skin and HF diseases.

CONCLUSIONS AND PERSPECTIVES

'For a large number of problems there will be some animal of choice on which it can be most conveniently studied ... I am afraid that most of them are unknown ... Zoologists need to find them and lay their hands on them.'^[95] In the years after this insight by Krogh, much has been learned about host-microbiota interactions through the study animal models.^[96-98] At first glance, *Hydra* and human HF do not have much in common (Tables 1 and 2). However, when looking at the basic and experimentally testable mechanisms by which *Hydra* controls its microbiome, the highly informative nature of this model system is uncovered. Although evolutionarily, anatomically and functionally diverse from human HFs, from a holobiont perspective, the *Hydra* model system can provide a general understanding of the tightly linked interaction between host environment, nutrient dependency of host-associated microbes, microbial metabolism, microbe-microbe interactions and host immunity. Investigation of these could identify the extent to which environmental factors contribute to dysbiosis and disease development in inflammatory skin and HF diseases (e.g., scalp psoriasis^[99]) and facilitate the elucidation of the triggering stimulus in HF-associated inflammatory diseases, such as AA (Table 3).

Thus, understanding the regulatory factors of the host immune system, such as TLR stimulation and AMP production, in *Hydra* invites concrete working hypotheses on comparable, evolutionarily conserved mechanisms by which human HFs regulate their microbiome and vice versa. Further, understanding if the host can compensate for environmental stress-induced changes of the microbiome and if genetic

TABLE 2 Main differences between *Hydra* and the hair

<i>Hydra</i>	Hair follicle	References
Composed of two epithelial cell layers: epidermis and gastrodermis, separated by extracellular matrix (mesoglea)	Composed of seven concentric epithelial layers (inner and outer root sheaths) surrounded by a mesenchymal layer (connective tissue sheath)	[104,105]
Has no dynamic remodelling but holds remarkable regeneration capacity	Has dynamic remodelling: undergoes cycles of growth (anagen), apoptosis driven regression (catagen) and relative quiescence (telogen)	[104,106]
Has innate immune system only	Has both innate and adaptive immune systems	[105,107]
Microbiota coat the exterior and are located within the glycocalyx	Microbiota are ubiquitously distributed, predominantly within the epithelial layers	[2,3,105]
Germ-free model and microbiome and nutrient manipulation possible, as well as generation of transgenic animals	Germ-free model not yet possible and microbiome and nutrient manipulation have limitations, as well as transient gene knockdown	[7,108,109]

TABLE 3 *Hydra* as a model organism to address HF microbiome open questions

Topic	Open question	<i>Hydra</i> model
TLR genetic modification	How changes in TLR expression affect the hair follicle microbiome and how does the microbiome interact with TLR expression?	Modulation of the TLR signalling pathway by transgenesis. ^[83]
Uncoupling microbial-nutrient dependency	How the hair follicle manages its microbiota through nutrient availability? How nutrient dependency can be used to favour beneficial microbial growth?	Characterisation of the <i>Hydra</i> chemical landscape of germ-free and wild-type polyps by metabolomics. Modulation of the surface chemistry by the addition of nutrients to the environment.
Manipulation of microbial community	How disturbance of the microbiota composition affect the HF immunity	Selective manipulation of the bacterial community composition by selective recolonisation of germ-free polyps; Addition of selected bacterial strains and of certain nutrients, or removal of specific bacteria by phage therapy.
Epithelial-neuronal interaction manipulation	How the HF microbiota affects neuropeptides and influence neural-epithelial communication?	Combining microbiome manipulation with single cell transcriptomics ^[94] and calcium imaging of transgenic <i>Hydra</i> lines. ^[110]

HF, hair follicle; TLR, toll-like receptor.

predisposition contributes to a disturbed resilience of the microbiome provides a basis by which the *Hydra* model can guide the development of strategies for therapeutic intervention in skin diseases. In particular, in those that are associated with HF dysbiosis, providing valuable pointers to whether the administration of selected bacterial strains can compete with the resident community and restore homeostasis, or whether it is necessary to reduce the bacterial load prior to probiotic intervention.

Manipulating environmental conditions of the microbial habitat by supplementing specific metabolites, such as prebiotics, AMPs or nutraceuticals, to enhance the therapeutic efficiency of probiotics^[100] is another conceivable intervention strategy that can be explored first in *Hydra*. These would allow the translation of the core principles learned in this model to the design of corresponding HF organ culture studies that probe the increasingly highlighted role of the HF microbiome in hair diseases.^[3,87] Thereby, highlighting the importance of

learning from a very distant ancestral species that has long proven to be an exquisite teacher of host-microbiota interactions.

ACKNOWLEDGEMENTS

Tim Lachnit acknowledges funding from the Deutsche Forschungsgemeinschaft (DFG), the CRC 1182 'Origin and Function of Metaorganisms'. Thomas C. G. Bosch's research is supported by the Deutsche Forschungsgemeinschaft (DFG), the CRC 1182 'Origin and Function of Metaorganisms' and the CRC 1461 'Neurotronics: Bio-Inspired Information Pathways' (Project-ID 434434223 – SFB 1461). Thomas C. G. Bosch appreciates support from the Canadian Institute for Advanced Research. Writing of this review was further supported by a PhD fellowship to MBL, co-sponsored by Giuliani Spa, Milano, Italy and Monasterium Laboratory GmbH, Münster, Germany and an associated basic research grant. All manuscript images were created with biorender.com.

CONFLICT OF INTEREST

Marta B. Lousada and Janin Edelkamp are employees of Monasterium Laboratory GmbH, of which Ralf Paus serves as CEO.

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How to cite this article: Lousada, M. B., Lachnit, T., Edelkamp, J., Paus, R., & Bosch, T. C. G. (2022). Hydra and the hair follicle – An unconventional comparative biology approach to exploring the human holobiont. *BioEssays*, 44, e2100233. <https://doi.org/10.1002/bies.202100233>