The Skin Commensal Yeast Malassezia globosa Thwarts Bacterial Biofilms to Benefit the Host

Giuseppe Ianiri¹, Joseph Heitman¹ and Annika Scheynius²

Malassezia are abundant, lipid-dependent, commensal yeasts in the skin microbiome that also have a pathogenic lifestyle associated with several common skin disorders. Malassezia genomes encode myriad lipases and proteases thought to mediate lipid utilization and pathogenesis. Li et al. report the biochemical characterization of a unique secreted aspartyl protease produced by Malassezia globosa, MgSAP1, and demonstrate its active role in hindering biofilm formation of the bacterium Staphylococcus aureus. Because biofilms are an established virulence attribute of S. aureus, this study reveals a potential benefit to the host of the fungal aspartyl protease MgSAP1 and opens the door for the investigation of the roles of such molecules in microbial interactions and their possible effects on the host.


Fungi of the genus Malassezia

The skin of humans and animals is colonized by numerous microorganisms that constitute the skin microbiome. These include commensal bacteria and fungi that establish a nonpathogenic interaction with the host, but in certain circumstances, such as immune conditions within the host, or intrinsic microbial imbalance, some commensals can become pathogenic or beneficial. The study by Li et al. (2018) reports a previously unknown beneficial role for a secreted aspartyl protease produced by the fungus Malassezia globosa.

A secreted aspartyl protease from Malassezia globosa exhibits unexpected hydrolytic activity that acts on Staphylococcus aureus biofilms.

Malassezia species are the most abundant fungal skin inhabitant of humans (Findley et al., 2013). This genus includes a monophyletic group of yeasts within the basidiomycetes that is closely related to the smut plant pathogen Ustilago maydis and more distantly related to skin-infecting fungal species, such as Candida albicans and the dermatophytes. The unique Malassezia genus currently includes 17 species, of which M. globosa, M. restricta, and M. sympodialis are most commonly associated with humans (Sparber and Leibund-Gut-Landmann, 2017). A common feature is the inability of Malassezia to synthesize lipids due to the loss of the fatty acid synthase gene, and as a consequence, these species mainly colonize sebaceous skin. Comprehensive molecular and genetic research on Malassezia has highlighted unique features compared with other fungi, such as a reduction in genome size (as small as approximately 7–9 Mbp) with extensive loss of genes encoding hydrolyses and other enzymes involved in carbohydrate metabolism, and concomitant expansion of genes encoding secreted lipases and proteases thought to mediate lipid utilization and play roles in pathogenesis (Wu et al., 2015).

Is Malassezia a beneficial skin inhabitant?

Although Malassezia are commensal inhabitants of the skin, they are associated with several skin disorders, including pityriasis versicolor, dandruff, seborrhoeic dermatitis, atopic dermatitis (AD), and folliculitis (Saunders et al., 2012). It is presumed that Malassezia strains associated with these clinical conditions are derived from normal commensal strains, but this has not been experimentally proven. As a result, the pathophysiology of Malassezia and the mechanisms that govern its shift from commensal to pathogen are poorly understood.

It is reasonable to hypothesize that the immune system has adapted to the commensal nature of Malassezia and thus tolerates its presence on the skin, but the immune system can also recognize the fungus as a pathogen and respond. Malassezia can be recognized by the host, either directly through membrane-bound pattern recognition receptors or indirectly through production of inflammatory metabolites released by hydrolysis of sebum by
fungal lipases and proteases. These metabolites are thought to be responsible for clinical dandruff and seborrheic dermatitis (White et al., 2014).

Another way in which Malassezia is known to interact with the immune system is through the production of specific allergens. These include a set of nine conserved and four unknown proteins identified in the M. sympodialis genome (Giotti et al., 2013). Approximately half of adult patients with AD have allergen-specific IgE and T-cell reactivity to Malassezia, which are rarely observed in respiratory allergies, suggesting a host-microbe interaction related to the skin environment in AD (White et al., 2014). A clinical hallmark of AD is dry, itchy skin and increased permeability and water loss. There is a distinct reciprocal expression pattern of induced inflammatory genes and repressed lipid metabolism genes (Sääf et al., 2008). This results in an inhospitable environment for the lipid-dependent Malassezia, and there is an increased pH level on AD skin that stimulates the yeast to release more allergens compared with culture at normal skin pH (Selander et al., 2006).

Yet another way for Malassezia to communicate with the host is through the release of extracellular nanosized vesicles, designated MalaEx, that carry allergens and can induce inflammatory cytokine responses (Gehmann et al., 2011). MalaEx, which contain small RNAs as a cargo (Rayner et al., 2017), have the potential—like other fungal extracellular vesicles—to deliver functional mRNAs and microRNA-like RNAs to recipient host cells, thereby interfering with the host RNAi machinery to silence host immune genes and cause infection (Weiberg et al., 2013).

Although establishing the basis and the relationship between commensalism and pathogenicity is a recognized and active research field, Malassezia interactions with other microbes on the skin and their effects on the host are largely unexplored. Li et al. (2018) reported that a secreted aspartyl protease produced by M. globosa displays antibiotic properties against Staphylococcus aureus, which is responsible for skin and soft tissue infections and which is frequently associated with skin infections in AD. Previous genomic studies revealed a robust secretory repertoire for all species belonging to the genus Malassezia, consistent with the importance of these hydrolytic enzymes for Malassezia biology and pathophysiology (Wu et al., 2015; Zhu et al., 2017).

RNAseq analysis revealed that 2 of the 15 predicted aspartyl proteases of M. globosa, encoded by MGL_3328 and MGL_1932, were highly expressed in vitro during growth in both rich and minimal media. Through biochemical analyses, Li et al. (2018) characterized a specific aspartyl protease activity, revealing strong cleavage of specific substrates (RPKPYPvWM and RPKPVEvWR), increasing activity in the first 16 hours of growth, and a pH optimum between 4 and 5. Mass spectrometry and trypsin in-gel digest analyses identified MGL_1932 as the major aspartyl protease in culture, and it was named MgSAP1 (from M. globosa secreted aspartyl protease 1). Further analyses demonstrated that MgSAP1 has a cleavage preference adjacent to aromatic residues, in particular tryptophan, which distinguishes it from other known proteases.

Li et al. then aimed to establish if the secreted protease MgSAP1 was (1) produced in situ on human skin, (2) had any role in Malassezia biology and/or pathophysiology, and (3) could potentially mediate interactions with existing skin microflora. MgSAP1 was found to be expressed on the faces of 17 of 18 healthy male and female volunteers, indicating its ubiquitous production. Among the skin microflora, S. aureus coexists in many sites with M. globosa (Oh et al., 2014) and produces protein A (SpA), which is essential for biofilm formation, a major S. aureus virulence factor (Archer et al., 2011). Because SpA is rich in lysine and aromatic residues, the authors hypothesized that it could be hydrolyzed by MgSAP1, thus impacting S. aureus pathogenicity. Strikingly, MgSPA1 rapidly degraded recombinant SpA in vitro and strongly reduced the in situ volume of S. aureus biofilm formation without affecting bacterial viability. A representation of MgSAP1-mediated biofilm destruction is shown in Figure 1.

This study raises questions about the specific roles of Malassezia proteases, as their common in situ expression has been correlated with their involvement in pathogenesis (White et al., 2014). This predicted function as virulence factors is likely based on the role of analogous proteases in more well-studied fungi that can live on the skin such as the dermatophytes and C. albicans. In fact, their
genomes reveal a significant expansion of genes predicted to encode proteases in Malassezia, dermatophytes, and *C. albicans*, suggesting an important role of proteases in evolution and adaptation on the skin. In *C. albicans*, secreted asparyl proteases have long been recognized as virulence factors that act by promoting adhesion to, invasion of, and damage to epithelial cells and tissues and by inducing the secretion of proinflammatory cytokines independently from their proteolytic activity (Pietrella et al., 2010). With respect to dermatophytes, these fungi secrete an abundance of proteases for multiple functions, such as adherence on the skin, keratin digestion for penetration, and modulation of cell metabolism and the host immune system (de Hoog et al., 2017; White et al., 2014).

The study by Li et al. (2018) represents a paradigmatic example of a fungal-bacterial interaction that is mediated by a secreted molecule and that potentially results in a beneficial effect for the host. Fungal and bacterial interactions are common, although they are understudied and technically challenging to elucidate. However, there is an increasing awareness of their biological and pathophysiological importance (Peleg et al., 2010). As an example, Graham et al. (2017) recently described an interesting interaction between the bacterium *Enterococcus faecalis* and *C. albicans*. These two microbes occupy overlapping niches in the mammalian microbiome and display antagonistic activity resulting in reduced virulence for both microbes. In *C. albicans*, this reduced virulence was attributed to the reduction of hyphal development and biofilm formation due to the activity of the secreted *E. faecalis* bacteriocin, EntV (Graham et al., 2017).

**Conclusions**

The study of Li et al. (2018) reveals that the commensal and sometimes pathogenic fungus *M. globosa* produces an asparyl protease, unique in its evolutionary trajectory and substrate specificity, which impacts a recognized virulence attribute of a commensal and pathogenic bacteria, thus potentially resulting in unexpected benefits for the host. This finding underscores the importance of in vivo studies to determine the function of unknown proteins during microbial interactions, and the beneficial or detrimental impact that they might have on the host. For example, whether the characterized MgSAP1 protease also plays a role in *Malassezia* pathogenesis as described for other asparyl proteases is still unknown. There is a crucial need to develop and establish reliable host-pathogen models to study how *Malassezia* interacts with both cells in the skin and different immune cells, and with other microbes that live on the skin, as well as the induced immune responses in robust mouse and humanized-mouse models of infection. This coupled with the recent development of tools for targeted and insertional mutagenesis (Celis et al., 2017; Janiri et al., 2016) will help to characterize the mechanisms that establish the commensal, pathogenic, and beneficial lifestyles of *Malassezia* species on the skin.

**CONFLICT OF INTEREST**

AS has co-authored large collaborative publications with the senior author (Tom Dawson) of the present article commented on herein, published in 2013 (Griot et al., 2013), 2014 (White et al., 2014), 2015 (Wu et al., 2015), and 2017 (Zhu et al., 2017). This did not influence the views presented here with respect to the Commentary published in the *Journal of Investigative Dermatology*. JH has co-authored large collaborative publications with the senior author (Tom Dawson) of the article discussed, published in 2013 (Griot et al., 2013), 2015 (Wu et al., 2015), and 2017 (Zhu et al., 2017). This did not influence the views presented here with respect to the article published in the *Journal of Investigative Dermatology*.

AS has research support from the Swedish Research Council-Medicine, the Cancer and Allergy Foundation, the CHAMP (Centre for Allergy Research Highlights Asthma Markers of Phenotype) consortium that is funded by the Swedish Foundation for Strategic Research, the Karolinska Institutet, AstraZeneca and Science for Life Laboratory Joint Research Collaboration. AS is a member in the Joint Steering Committee for the Human Translational Microbiome Program at SciLifeLab/Karolinska Institutet together with Ferring Pharmaceuticals, Switzerland, during 2016–2018, outside the scope of this work and without any financial support. GI states no conflict of interest.
Epidermolysis bullosa (EB), a group of heritable blistering disorders, presents with a spectrum of phenotypic severity, with the primary manifestations relating to skin and mucous membrane fragility (Fine et al., 2014). In the most severe forms, patients affected with this disease die within a few weeks or months of life, whereas in the milder forms, affected individuals exhibit life-long blistering that does not affect longevity. This spectrum of phenotypic severity reflects the fact that mutations in as many as 20 distinct genes can underlie the EB phenotype, influenced by the topographic expression of the affected genes within the cutaneous basement membrane zone, the types and combinations of the mutations, and their consequences at the mRNA and protein levels (Uitto et al., 2016; Vahidnezhad et al., 2018). Based on ultrastructural demonstration of the level of blistering within the skin and clinical presentations, EB has been divided into four broad categories: EB simplex (EBS) with intraepidermal blistering; junctional EB with tissue separation within the dermo-epidermal basement membrane itself (primarily influenced by the lamina lucida); dystrophic EB demonstrating sublamina densa blistering within the upper papillary dermis; and Kindler syndrome with multiple levels of blistering. The treatment of these different forms of EB relies primarily on prevention of trauma and infections, and there is no specific cure for this, currently intractable group of disorders. However, over the past decade, and especially over the past few years, there has been considerable progress in understanding the mechanistic details leading from the genetic defects into blistering phenotypes, and preclinical work has suggested a number of potential treatment approaches, some of which have been extended to the level of early clinical trials.

One of the first early clinical trials for the systemic treatment of EB consisted of allogeneic whole bone marrow transplantation (BMT), reported in 2010, in patients with severe recessive dystrophic EB (RDEB) (Wagner et al., 2010). More than 40 children with either RDEB or junctional EB have now undergone BMT worldwide. Although the results of the clinical experience involving the full cohort of these patients have yet to be published, it appears that beneficial clinical response, but not cure, can be noted in some, but not all, children with RDEB after BMT. Although experience with BMT in junctional EB is limited, this approach does not appear to have any therapeutic value for this type of EB (Hammersen et al., 2016; Hook et al., 2017). In this issue of the Journal, two complementary studies now ask if BMT might be applicable to EBS characterized by intraepidermal blistering (Egawa and Kabashima, 2018; Huenefeld et al., 2018). As a disease model, Huenefeld and coworkers employ a desmoglein-3 knockout mouse (Dsg3−/−) that demonstrates blistering exclusively within the epidermis and mucosal membranes, clinically manifesting with perioral erosions and a runted phenotype, and no significant increase in mortality in the first 4–6 months of life. BMT was performed at 8–10 weeks after the birth using bone marrow cells from enhanced green fluorescent protein (EGFP) expressing wild-type Dsg3+/− mice that can be used to track the engraftment and migration of the transplanted cells. Ten weeks after BMT, the distribution of donor cells was...