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> ONE MONTH, ONE PROTEIN <

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mouths, enemies and spit

Vivienne Baillie Gerritsen

Our mouths are teeming with inhabitants of the most diverse origin. Bacteria and fungi for one, but all sorts of various-sized peptides too, each of which carry out various tasks. In fact, our mouths are like a metropolis, with its underlying complexity of continuous bonds and exchanges between its individuals and compartments. As in any society, things need to be kept balanced and regulated to avoid unrest and chaos. Likewise, the bacteria and fungi that nestle down in the nooks and crannies of our mouths must not be left to multiply unrestrained, which would only bring about a full-blown infection. This is why Nature has provided our saliva with an assortment of antimicrobial peptides, just in case things get out of hand. Histatins are antimicrobial peptides found in primate saliva. One histatin, called histatin 5 or Hst5, specifically fights off infections by *Candida albicans*, a yeast naturally harboured in our mouths. Hst5 does this by using crossing – unscathed – the yeast's membrane to reach the cell's cytoplasm. Hst5 then goes on to meddle with the yeast's ion homeostasis, gradually leading it to its death.

Hinko Smrekar (1883-1942)

Candida albicans is a commensal yeast, meaning it is a eukaryotic cell that is happy to share its life with another species. In particular, it likes to spend time with humans because its ideal growth temperature is 37°C. Consequently, though *C.albicans* can survive on its own, many of us carry the yeast around in the lining of our mouths, our genitourinary tract and on our skin, without it causing much trouble – or us them for that matter. However, when feeling under the weather or stressed, or in a situation where our immune system is weakened, *C.albicans* can switch into an aggressive mode and multiply, ultimately infecting our cells. Most of the time, infections are kept at bay. Occasionally, however, they become invasive, spreading to other parts of our body – sometimes even causing death.

Secreted by glands situated in our mouths, our saliva is stashed with components which each have a purpose. There are those needed for lubrification, those required for cleaning or for digestion, those that are vital for creating protective barriers and those that are called up for self-defence. Over 200 proteins and peptides are estimated to make up our saliva but there is also a lot of water, minerals and metals – all of which interact with each other to create a very lively environment. Our saliva is so rich with peptides that scientists have divided them into different families, depending on their composition mainly. Among these peptides are histatins – so named because they are full of the amino acid histidine.

Histatins are involved in maintaining the soundness of our mouths, such as the enamel on our teeth for instance, by forming a protective pellicle on the smooth surface. They emerged as important factors of our immune system when their capacity to fight off

yeast – and to a lesser extent bacterial – infection was discovered. This finding coincided with the AIDS outbreak in the 1980s when immunocompromised patients suffered from *Candida* infection – an ailment known as thrush. In these patients, histatins were unable to perform their usual microbicidal activity. There are about a dozen histatin family members, each generated from a parent histatin and all structurally related. One histatin can be singled out: the metal-binding histatin 5 (Hst5) cleaved from its parent histatin Hst3. Hst5 is not only one of our saliva's major peptide components but also the most potent with regards to antifungal activity.

Histatins are small in size, varying between 7 and 38 amino-acid residues. Like many other antimicrobial peptides (AMPs), the composition of histatins is amphipathic – meaning that they have a head and a tail which have opposite charges. Lipids that form the lipid bilayer of cell membranes are made in the same way. This is no coincidence since, thanks to their similar amphipathic structure, AMPs can slip into cell membranes, locally rearranging the membrane's make-up to create harmful pores, for example, or to facilitate their entry into the host's cytoplasm. Which is exactly how Hst5 enters *C.albicans*. Though its mechanism of action is not yet fully understood, we know that Hst5 crosses the yeast's cell wall and then its membrane to finally reach the cytoplasm. Here, Hst5 meddles with the flux of ATP, ultimately causing the cell's vital energy to flow out. At the same time, Hst5's capacity to bind metals – such as copper, zinc, iron, nickel, calcium and magnesium – produces intracellular reactive oxygen species (ROS) which, among other things, can damage the yeast's DNA. Taken together, the outflow of ATP and the production of ROS end up killing the yeast cell.

Why is it that Hst5 is so free to cross the yeast's membrane? You would expect its passage to be more difficult. You would also expect Hst5 to be degraded the moment it enters the host cell. The thing is, Hst5 is probably recognised as a familiar polyamine by transporters that are lodged in the yeast cell membrane. Hst5 can then simply steal a polyamine's seat and hitch a ride into the cell. Besides the loss of cellular ATP that follows, scientists discovered that there was also an outflow of K^+ ions – which only contributes more to the dysregulation of the yeast's global homeostasis. How are K^+ ions lost? Polyamines are known to modulate the passage of potassium through potassium channels. Perhaps Hst5 takes over by blocking or opening these channels, thereby dysregulating the flow of intracellular K^+. On the other hand, by binding to the channels, Hst5 could distort them in such a way that larger anions, such as ATP, can leak through. So far, it is only a model – albeit an elegant one.

AMPs are thought to have been around for about 2.6 billion years – long before the human species was even thought of – which, when considering the complexity of the human immune system for instance, is one of the reasons they are so "crude" in a way. Despite their crudeness, the moment AMPs were discovered, researchers saw their biomedical potential, and many are currently used in medicine to combat infections. What about antibiotics you may ask? Antibiotics continue to be used, naturally. However, microbes are becoming more and more resistant to them, which is why scientists are looking for alternative solutions. Fine-tuning AMPs is one. As an illustration, depending on the metal Hst5 binds, it behaves differently. Would it not be possible then to influence Hst5's activity by modulating its interactions with metals? No doubt. The thing is, *C.albicans* is a eukaryotic cell – like all human cells. If you change the nature of Hst5, it may make them harmful to other microbes – which is good – but it could also become harmful to our own cells too. It is all a question of balance.

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