Virus-like immune defense protein in mushrooms

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Fungi and plants do not have an adaptive immune system. Innate immunity serves as their sole defense, often based on carbohydrate recognition by lectins. In a twist of nature, as revealed by Sommer *et al.* (2018), a conserved fungal immunoprotein adopts the shape of a miniature virus.

When the flu strikes, we are glad that we have a well-functioning immune system that usually limits our agony to a few days. But how do other organisms deal with similar challenges? After all, bacteria, plants and fungi can also become infected. And in contrast to humans, their limited motility also puts them at significant risk of falling victim to predators. Innate immunity is the immune system all organisms have from their first day of life. In humans, it is based on Toll-like receptors, which recognize common pathogenic fingerprints called PAMPs, such as RNA and lipopolysaccharides (LPS), whereas our adaptive immunity is antibody-based and develops over a lifetime. Well-known examples of innate immunoproteins of bacteria and archea are restriction enzymes (Dussoix and Arber, 1962) – now a boon for

molecular biology – whose purpose is to cleave the DNA of invading viruses. Likewise, proteases feature prominently among immune defense proteins. Of more recent fame are antimicrobial amyloids (Kagan, 2011), prions that filamentous fungi use to distinguish self from non-self (Greenwald and Riek, 2010), as well as CRISPR (Marraffini, 2015), a bacterial immune defense system that has obtained celebrity status.

For plants and fungi, lectins serve as immunoproteins of choice. Their defense actions are manifold, from warning their kin to poisoning their enemies (De Hoff *et al.*, 2009). In this issue of *Structure*, Varrot, Künzler and colleagues describe a lectin called tectonin from the mushroom *Laccaria bicolor* (Lb-Tec2) (Sommer *et al.*, 2018). Like all lectins, it recognizes sugars. This particular lectin binds methylated sugar molecules, a rarity in the glycan world. In bacteria, they are found on LPS; and they are also present in nematodes (worms) – both common predators of mushrooms. It is likely that these methylated sugars represent exactly the type of epitopes that mushrooms need to avoid and hence represent ideal PAMPs (Wohlschlager *et al.*, 2014). The study by Sommer *et al.* (2018) includes two high-resolution X-ray crystal structures of O-methylated sugar complexes that reveal the details of PAMP recognition and selectivity by Lb-Tec2.

The quaternary structure of Lb-Tec2 is almost spherical. It adopts this shape by assembling four six-bladed β-propeller units on the vertices of a tetrahedron. Despite the high resolution (1.65 Å), solving the structure was a technical tour de force, since the "top" subunit lying on a three-fold crystallographic axis suffered statistic disorder. For crystallography enthusiasts, reading the Methods section of this publication is therefore highly recommended. The final structural model is flawless, with R and R_{free} values well below 20%, and the relevance of the tetrameric structure well supported by small-angle X-ray scattering (SAXS).

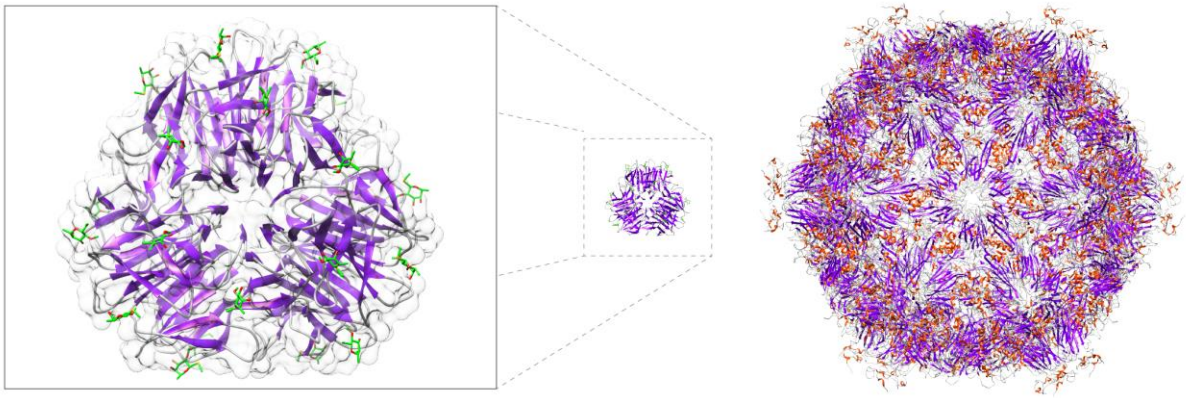

Figure 1: **Who's who?** The tetrameric multivalent glycan binder Lb-Tec2 resembles a miniature virus. Here compared to rhinovirus, the cause of common cold.

The assembled Lb-Tec2 tetramer is a highly compact nanoparticle, reminiscent of a viral capsid, and displays 24 sugar-binding sites on its surface (Figure 1). In this way, Lb-Tec2 can bind multiple PAMPs at once. Lectins are champions of multi-valency, but still this one is special: it achieves one of the highest valencies known for a lectin, and possibly *the* highest binding site *density*. For Lb-Tec2, Varrot and colleagues could show that multi-valency increases the protein's avidity by 60-fold compared to the millimolar mono-valent binding affinity that is typical for glycans. Lb-Tec2's toxicity may well result from this ability to cross-link multiple glycans, aggregating them and either agglutinating the cells or disturbing the underlying cell membranes. This process may resemble one of the mechanisms used by non-enveloped viruses to enter host cells (Kalia and Jameel, 2011). By clustering glycan receptors, viruses can induce membrane curvature through their multivalent capsid proteins, and hence enter the cells through endocytosis, a mechanism shared by several protein toxins. In the endosome, at lower pH, viruses often change their conformations and expose previously hidden hydrophobic segments. They may also shed parts of the capsid and open pores through which their genetic material can escape. Lectins like Lb-Tec2 do not harbor any genetic material, but may well exhibit similar conformational rearrangements when (and if) internalized. We note that Lb-Tec2 was crystallized at pH 6.5, which is similar to conditions

in early endosomes and above the lectin's pI (6.0). Late endosomes have a more acidic pH, at which the net charge of Lb-Tec2 would be positive, with potential effects on structural integrity and membrane interaction.

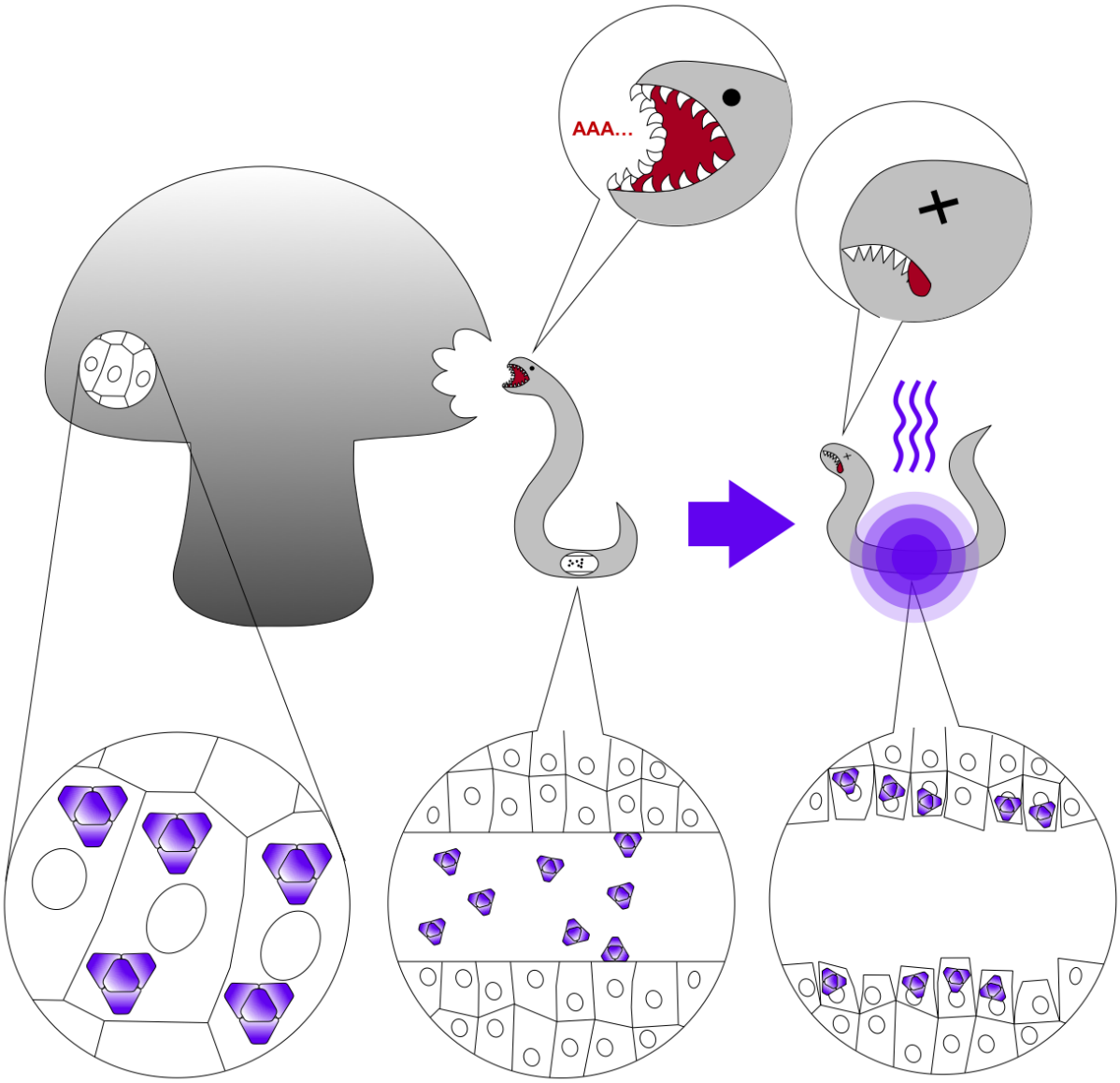

Figure 2: **Innate immune defense of mushrooms by tectonins.** The cartoon depicts the fate of a nematode worm feeding on a mushroom that in its cytoplasm contains the toxic Lb-Tec2 lectin. The toxic effect is likely caused by cross-linking the surface glycans of the worm's intestinal cells and disruption of the cell membranes. Note that in real life, most nematodes do not sport fangs.

Clearly, the interaction mechanism of Lb-Tec2 and its likes with cell membranes and host cells needs to be further explored. This will be an exciting endeavor. In addition, there are other intriguing questions. For example, Lb-Tec2 appears to have a preference for the surface glycans of nematodes (Wohlschlager *et al.*, 2014), but how does the lectin get there? Since the Lb-Tec2 gene lacks a secretion signal, it is assumed to code for a cytoplasmic protein that will be consumed by the fungal predators (Figure 2). Is it then excreted by the worms and in this way transferred to their mates, or does the nanoparticle stay intact when its victim rots, delivering post-mortem strikes? To find out, it would be interesting to study how stable these toxins are. Alternatively, maybe the partial degradation of mushrooms by pathogens and predators exposes Lb-Tec2 on the surface of the mushroom, enabling a double punch, internal and external? On a completely different note, Lb-Tec2 has been shown to be important for the formation of the mushroom's mycorrhiza roots. What role does it have in this process, and is its ball-shaped structure important for this function? For example, could it serve to mediate interactions between the mushroom roots and mycorrhizal symbionts, like plant lectins (De Hoff *et al.*, 2009)?

Tectonins appear to be widespread among different species. A search for structural and functional orthologues revealed a list of uncharacterized candidates spanning fungi, bacteria and metazoans, with varying degrees of sequence identity (Sommer *et al.*, 2018). Additionally, the tectonin domain is frequently associated with other protein modules. Fungi often use chimerolectins for self-defense, where the sugar-binding lectin domain is coupled to additional domains that exert a cytotoxic effect (Cordara *et al.*, 2017). Are such domains also added structurally to the tetrameric assembly? And what effect would this have?

The quaternary structure of Lb-Tec2 was unexpected. However, this protein is most likely not the only ball-shaped lectin out there. It is reasonable to assume that at least closely related family members adopt the compact, virus-like fold of Lb-Tec2 as first line of defense. So next

time we catch the flu or a cold, we may take comfort from knowing that hosts can use similar mechanisms to ward off invaders as viruses do to infect us.

Cordara, G., Manna, D., and Krengel, U. (2017). Family of papain-like fungal chimerolectins with distinct Ca^{2+}-dependent activation mechanism. Biochemistry *56*, 4689-4700.

De Hoff, P.L., Brill, L.M., and Hirsch, A.M. (2009). Plant lectins: the ties that bind in root symbiosis and plant defense. Mol. Genet. Genomics *282*, 1-15.

Dussoix, D., and Arber, W. (1962). Host specificity of DNA produced by *Escherichia coli* II. Control over acceptance of DNA from infecting phage λ. J. Mol. Biol. *5*, 37-49.

Greenwald, J., and Riek, R. (2010). Biology of amyloid: structure, function, and regulation. Structure *18*, 1244-1260.

Kagan, B.L. (2011). Antimicrobial amyloids? Biophys. J. *100*, 1597-1598.

Kalia, M., and Jameel, S. (2011). Virus entry paradigms. Amino Acids *41*, 1147-1157.

Marraffini, L.A. (2015). CRISPR-Cas immunity in prokaryotes. Nature *526*, 55-61.

Sommer, R., Makshakova, O.N., Wohlschlager, T., Hutin, S., Marsh, M., Titz, A., Künzler, M., and Varrot, A. (2018). Crystal structures of fungal tectonin in complex with O-methylated glycans suggest key role in innate immune defense. Structure *26*, Current issue.

Wohlschlager, T., Butschi, A., Grassi, P., Sutov, G., Gauss, R., Hauck, D., Schmieder, S.S., Knobel, M., Titz, A., Dell, A.*, et al.* (2014). Methylated glycans as conserved targets of animal and fungal innate defense. Proc. Natl. Acad. Sci. U.S.A. *111*, E2787-E2796.