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> ONE MONTH, ONE PROTEIN <

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more to it

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Multitasking is not limited to computers. On a day-to-day basis, humans frequently deal with more than one thing at a time – for the sake of speed, convenience and no doubt productivity. We brush our teeth while taking a shower, eat a sandwich while answering mails, wash the dishes while calling a relative. Though we are pretty good at it, humans are far from the only multitaskers on this planet. We also harbour a few inside us. Just consider cells… One cell can synthesize proteins, while secreting others, repairing its cytoskeleton and maintaining its membrane. And we now know that the odd protein is also able to juggle with more than one task – thus applying yet another layer of obsolescence to the not-so-old "one gene, one protein, one function" hypothesis. Such is the case for a protein known as dual function macrocyclase-peptidase, or POPB. POPB is involved in making amatoxins, which are very small cyclic peptides found in some mushrooms and particularly poisonous when ingested.

Black Relationship (1924)

Wassily Kandinsky (1866-1944)

Poisonous mushrooms have been known – and avoided – by animals and human beings for thousands of years. Among them: *Galerina marginata*, a small-sized and rather plain brownish mushroom that feeds off decaying softwood and hardwood in forests of the Northern Hemisphere – from North America to Europe and Asia. *Galerina marginata* was first described in 1789 by August Batsch, a German naturalist who was a renowned mycologist. Batsch discovered almost 200 new species of mushroom, which he described in a book, "Discussion of Fungi" – a reference in the field to this day. Why are mushrooms toxic in the first place, you may ask? The most obvious answer would be for their protection: by keeping animals away, they have time to disperse their spores and proliferate. But why, then, are some mushrooms toxic while others are not? Chance, no doubt. Evolution seems to have given some mushrooms the opportunity to develop toxins, which means that they have an advantage over others.

The toxins found in *Galerina marginata* are known as amatoxins. These are small cyclic eight amino-acid peptides, whose varying side groups define the variety of toxins found within a given mushroom. Despite their modest size, amatoxins are invariably lethal. That is because their structure is particularly rigid and stable, and they are able to squeeze through membranes with surprising ease while being resistant to proteases. When ingested, amatoxins are rapidly absorbed into the bloodstream which they use to reach the liver. There, they inhibit RNA polymerase II – a polymerase directly involved in translating DNA into RNA, and hence in protein expression. This is bad news for the liver, whose vital activities are gradually hindered and shut down, leading to death unless the poison has been countered.

Where does POPB come in? *Galerina marginata* synthesizes α-amanitin. Like all

amatoxins, α-amanitin is a cyclic octapeptide. It begins as a 35 amino-acid precursor peptide that undergoes two processes – proteolysis and cyclization, and in that order – both of which are accomplished by POPB though, surprisingly, not in two successive steps. So, in effect, POPB is a sort of deferred multitasker… The intermediate severed peptide is released before it binds again to POPB – and not necessarily the same molecule – to be cyclized. Why would it do this? Researchers think it is a question of space, and that the intermediate peptide doesn't have enough room to move and present the part that needs to be cyclized. So it leaves the peptidase altogether, to come back and position itself in the right way.

POPB has two structural domains – a catalytic domain, and a seven-bladed ß-propeller domain. In the absence of substrate, the two domains rest in an open conformation, similar to the way the two shells of an open oyster would stay apart. In the presence of substrate, the propeller domain moves towards the catalytic domain, positions itself on top of it while clamping the substrate inside. Once bound to POPB, the N-terminal 10 amino-acid leader is removed from the 35 amino-acid peptide precursor and discarded. This produces a 25 amino-acid peptide with a newly exposed N-terminal and the original C-terminal tail, which is subsequently released from POPB. When the 25 amino-acid intermediate binds again to POPB, the N-terminal 8 amino acids are cyclized. As a result, both the 35 amino-acid precursors and the 25 amino-acid intermediates bind to POPB via their C-terminal tails, which sink deep into POPB's propeller domain. Which begs the question: how does POPB know that it has to cyclize part of the 25 amino-acid intermediate and not simply cut a bit off, as it does with the 35 amino-acid precursor? Because there is a short linker region between the C-terminal tail and the N-terminal octapeptide that angles the two substrates differently in POPB's active site, thus promoting proteolysis or cyclization.

The subtle and clever ways Nature has of performing various activities has inspired many a life scientist. Cyclic peptides, like amatoxins, are small, structurally varied, sturdy, resistant to proteases and oblivious to membrane permeability thus making them great candidates for designing drugs. Associated with antibody drugs, cyclic peptides can be used as powerful warheads in targeting specific molecules; as an example, when associated with antibodies against colorectal and prostrate cancer, amanita has proved to be particularly effective in this way.

In the past ten years, nine cyclic peptides have actually been approved in the fight against bacterial infection, fungal infection, cancer and gastrointestinal disorders. And there are more on the way. The thing is, cyclic peptides are more expensive to synthesize than linear peptides are and, to date, the only source for amanita is still in the wild. But if POPB could be expressed in *S.cerevisiae* – which seems to be kinetically possible – then it could cyclize all sorts of novel cyclic peptides in which could also be included unusual amino acids which would add yet other chemical properties, structures and functions to potential drugs. The possibilities seem to be not only promising, but seemingly boundless.

Cross-references to UniProt

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