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> ONE MONTH, ONE PROTEIN <

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either you, or me

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The planet is replete with organisms of all shapes and sizes fighting for their survival. Humans included. The best part of an organism's life is spent looking for the right nutrients and conditions to thrive, and over time Nature has designed a variety of subtle mechanisms to sense them. In this way, plants bind photons from sunlight to transform it into energy, while their roots absorb nitrogen to synthesize amino acids. Animals on the other hand harness oxygen to consume their fuel, and bacteria pick up iron for vital metabolic processes. Two organisms may find themselves having to share nutrients. Or, on the contrary, having to fight for them. During the process of infection, for example, neither the host nor the pathogen are out to share, and common nutrients can actually lay the foundations for ruthless conflict. Take copper for instance. *Cryptococcus neoformans* is a fungal pathogen which infects cells by using the copper it finds in them for its own metabolism, while the host retaliates by raising the concentration of metal to create a toxic environment for the pathogen. In *C.neoformans*, a protein known as Cuf1 is at the heart of this quarrel over copper.

"Tug O' War", by Aneurin Jones

Courtesy of Meirion Jones

Copper is found in the Earth's crust in its native form or else in a variety of minerals, such as the visually appealing azurites and malachites. It was mined during the Roman era in Cyprus – hence *aes cyprium*, meaning 'ore from Cyprus' from which the word 'copper' stems. Because of attractive physical and chemical properties, copper has been used for thousands of years and its presence, today, in human activity is manifold: photographic technology, roofing, plumbing, electrical wiring, power generation, power transmission, electromagnets, vacuum tubes, radio frequency shielding, lightning protection, welding... The list seems endless. Copper has also been widely used to line the hulls of ships as a protection against barnacles and mussels because it is biostatic, and any form of life is unable to grow on it. This is also why copper is added to alloys or fabrics to make them antimicrobial – such as door knobs, handrails, computer keyboards, shopping cart handles or t-shirts.

Besides the astounding variety of the use of copper outside living organisms, it is perhaps hardly surprising that many metabolic pathways inside us also rely on its presence. The human body, as an illustration, contains between 1.4 to 2.1 mg of copper per kg of body mass, where it is involved in events as vital as electron transfer, energy production and transcription regulation for example. In fact, its biological role will probably have begun with the appearance of oxygen in the Earth's atmosphere. However, like the presence of other trace elements in organisms – such as cobalt, zinc and manganese for example – it is very important to keep the concentration of copper checked since large quantities in cells can cause irreversible DNA and protein damage, which is why copper is used as a fungicide.

Copper levels in cells is under tight regulation. *Cryptococcus neoformans* is an encapsulated yeast, i.e. a cell encased within a wall composed mainly of polysaccharides. It is found on plants, decaying wood and in bird guano, and is easily inhaled by animals in the form of desiccated yeast cells or airborne spores. If *C.neoformans* enters a host successfully, it can infect our lungs and cause pneumonia, or even reach the brain

via our bloodstream and cause meningitis. Copper plays a crucial part in *C.neoformans* infection because both *C.neoformans* and the host need copper to perform essential metabolic pathways. Furthermore, while *C.neoformans* infects host cells and helps itself to their copper, the host answers by sending macrophages which sequester yeast cells in phagosomes full of excess copper – thus creating a toxic environment for the pathogen. As such, a sort of complex tug of war for copper takes place, and this unique way of fighting infection has been coined 'nutritional immunity'.

This said, *C.neoformans* is also armed against copper bombardment and, besides lapping up copper for its own metabolism, it can also drive off excess copper. How? The answer is: copper-dependent transcription factor 1, or Cuf1. Upon infection, Cuf1 orchestrates the expression of various enzymes – namely copper transporters and copper exporters. The former bind host copper so that the yeast can grow, while the latter export excess copper to shun toxicity perpetrated by host defense. To date, this dual role – copper acquisition and copper detoxification – carried out by the same transcription factor is unique. How exactly it occurs is still not understood. Does, for example, Cuf1 bind directly to the promoters of the enzymes' genes? Or does it trigger expression by way of accessory proteins which themselves bind to the promoters?

Structurally, the C-terminal part of Cuf1 has two cysteine-rich regions which are known to be copper sensitive. It has been suggested that, in the presence of copper and by way of these two regions, Cuf1 could stimulate – directly or not – the transcription of genes whose products transport copper for *C.neoformans* growth. The N-terminal part of Cuf1 has been described as bearing no known homology with any other protein and devoid of cysteines. However, it is methionine-rich which may indicate some kind of participation in copper coordination. Such observations therefore hint that the C-terminal part of Cuf1 is involved in copper uptake while the N-terminal part would be involved in copper export. It is a very neat theory, but which still needs to be demonstrated.

Much in the way a jeweller delicately balances gold on a weighing machine and keeps it from tipping too much to one side, Cuf1 orchestrates the expression of various enzymes all of which work hard to keep the levels of copper optimal for *C.neoformans* and detrimental to the host. It is a fascinating example of how one potentially toxic trace element is used by living beings both as a means to survive and to destroy. Fungal pathogenesis is responsible for about 1.5 million deaths per year, and *C.neoformans* is sadly among the most devastating, with over one million infections per year and a high mortality rate, especially in individuals whose immunity is suppressed. Historically, until recently iron and manganese had been the two metals researchers studied to shed light on the host-pathogen axis, but today their attention is turning to copper. The more we get to know Cuf1, the more we will be able to develop means to fight fungal infections such as *C.neoformans*. Intriguingly, too, copper excess has also been associated with diseases such as Alzheimer and Parkinson which could suggest the involvement of a mechanism such as that described for Cuf1.

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