

In like a shot

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Making use of a tubular structure to inject something into something else is a widespread practice. Doctors use syringes to inject medicine into patients. Mammals use their reproductive organ to supply their female counterparts with semen. Wasps use their sting to insert venom into their enemy. And *Encephalitozoon cuniculi* uses a polar tube as a means to infect. *E.cuniculi* infects species throughout the animal kingdom. It does this by inserting a long tube into the host cell's membrane and injecting directly into the cytoplasm what it needs to proliferate. *E.cuniculi* is a parasitic unicellular eukaryote and thus cannot survive on its own. But the pathogen has to be able to recognise its host first. Scientists have discovered one protein – known as 'spore wall and anchoring disk complex protein EnP1' – which is found in the area from where the polar tube is thrust, and which is capable of binding to surface molecules on the host cell's membrane. Thus creating the cell to cell contact needed to trigger off infection.



'Purple glove' 1986 , oil on linen, 48" X 48"
by Ilona Sochynsky

Collection of Dr. Walter Hoydysh
Courtesy of the owner

Why is it that *E.cuniculi* cannot survive on its own? Researchers are still not sure whether microsporidia are highly primitive organisms, or indeed far more advanced than believed. The fact remains, however, that *E.cuniculi* has no mitochondrion and thus cannot provide itself with the energy it needs. So its only option is to live at the expense of another organism. The notion of *E.cuniculi* inserting its insides into a host cell does not sound like ground-breaking news, since many tiny creatures have devised ways to inject their DNA into hosts with an end to multiply. The surprising fact with *E.cuniculi* though – and its fellow microsporidia – is that,

to date, no one has ever seen any other unicellular eukaryote infect by way of a tube. Many infectious microorganisms use endocytosis to move substances from one cell to another for instance. This said, some scientists think that the polar tube could have been formed by the aggregation – many years ago – of two or more original vacuoles.

Like all microsporidia, *E.cuniculi* goes through a number of stages before it becomes a spore and is ready to infect. A full grown spore is surrounded by a rigid wall which protects the protist from the extracellular environment. The wall is made up of an outside layer, known as the exospore whose main constituent is protein, and an inner layer known as the endospore, whose major constituent is chitin, which surrounds the protist's plasma membrane. At one end of *E.cuniculi* is a thickened area – the anchoring disk – onto which is moored one end of the polar tube. The polar tube is long and one very practical way to fit into the space it has been given is to coil it, as many as thirty times! When *E.cuniculi* is ready to attack, the bit of wall situated where the polar tube is attached breaks down and the tube inserts itself into the host's membrane. While whether it does this by brute force or a more subtle mechanism is unknown, the fact remains that the inside of *E.cuniculi* is then propelled through the tube and shot into the host's cytoplasm.

In order for *E.cuniculi* to infect its prey, a number of events must take place. The protist has to recognise its host. It has to get close to it,

if not bind to it. And then it has to trigger off the process which will have its sporoplasm poured into the host's cytoplasm. In fact, it is highly probable that all these events are dependent on one another, and EnP1 may well have a central role. This particular protein is not only found in *E.cuniculi*'s endospore but also its exospore, and, more interestingly, at high concentrations in the vicinity of the anchoring disk. What is more, EnP1 is very likely to recognise and bind to glycosaminoglycans on the host's cell surface thus bringing both cells into contact.

How does EnP1 recognise glycosaminoglycans on a host's cell surface? The EnP1 sequence is full of domains known as heparin-binding domains. These are domains which are known to bind to glycosaminoglycans. With EnP1 highly expressed at the anchoring disk, there is a fair chance that it will recognise glycosaminoglycans present on another cell's membrane thus bringing the two cells closer to one another and perhaps even orienting the anchoring disk in such a way that the polar tube has all its chances to be fired properly. Recognition and adherence could in turn activate infection *per se* and what seems to be an explosive reaction – brought on by a very high osmotic pressure – where the polar tube literally crashes through the host's membrane as it evaginates much in the same way as you would reverse a glove's finger.

Besides EnP1's role in the process of polar tube evagination and the onset of infection, this

adherence protein also probably has a structural role in the spore. EnP1's heparin-binding motifs not only recognise external glycosaminoglycans, but they can also bind to the chitin-related glycosaminoglycan entities in the endospore. This would help to make the spore wall rigid. Furthermore, EnP1 is riddled with cysteine residues making it more than likely that there are numerous inter- and intra-EnP1 cysteine bridges which would help to make the spore wall even more solid.

The very first description of the polar tube dates back to the late 1800s and was made by a marine scientist, Henneguya Thélohan, who was studying freshwater fish in Chad. Little did he know that he had depicted an organelle that was quite a singular occurrence in protists. What is more, over 100 years later, scientists have discovered EnP1, intimately involved with the evagination of the polar tube and which does seem to be essential not only in spore formation but also in host adherence and infection. Aiming antibodies against EnP1 should stop cell adherence and hence infection; EnP1, on the other hand, could also be used to develop vaccines. However, further studies are still needed to understand the mechanisms in detail so that scientists can find ways to treat microsporidiosis – an illness which afflicts many people whose immune system is weak, such as HIV positive patients or patients who have to take immunosuppressive drugs following tissue transplant.

Cross-references to UniProt

Spore wall and anchoring disk complex protein EnP1,
Encephalitozoon cuniculi (Microsporidian parasite) : Q8SWL3

References

1. Southern T.R., Jolly C.E., Lester M.E., Hayman J.R.
EnP1, a microsporidian spore wall protein that enable spores to adhere to and infect host cells in vitro
Eukaryotic Cell 6:1354-1362(2007)
PMID: 17557882
2. Weiss L.M., Xu Y.
The microsporidian polar tube: a highly specialised invasion organelle
International Journal for Parasitology 35:941-953(2005)
PMID: 16005007
3. Taupin V. Méténier G., Delbac F., Vivarès C.P., Prensier G.
Expression of two cell wall proteins during the intracellular development of *Encephalitozoon cuniculi*: an immunocytochemical and in situ hybridization study with ultrathin frozen sections
Parasitology 132:815-825(2006)
PMID: 16469199