Biology 3102 – Microbial Eukaryotes
Supplementary Course Material #2, Chapter 3
Fall 2020

CYSTS

Cysts are probably the most common alternative life history stage in microbial eukaryotes, and they are produced by numerous species across virtually all major groups of microbial eukaryote life. Cysts are fundamentally a resistance/dormancy stage, in which a cell assumes a compact form, displays greatly lowered metabolic activity, and is surrounded by an extracellular cyst wall, which is often quite thick and/or morphologically complex. As a result, cysts often remain viable for long periods of time (>>10 years in some cases), and are resistant to a wide variety of physical and/or chemical insults that would be fatal to active cells. For example, many (but not all) cysts will remain viable following desiccation. Cysts very often also have a dispersal function, because of their longevity and their ability to pass through otherwise lethal environments (e.g. blown through the air), and may also be used in seasonal persistence (e.g. overwintering). Unsurprisingly, the ability to form cysts is especially common in species that live in environments prone to drying, such as soils (unsaturated soils typically contain more cysts than active cells, by orders of magnitude). Cysts are also commonly used as a means of transmission by parasitic forms, especially intestinal parasites with fecal-oral transmission that must persist in the external environment between hosts, and/or pass safely through the chemically harsh conditions of the stomach. Cysts are also incorporated into the sexual cycles of some protists, or the feeding/'digestion' cycles of some species that consume large prey items.

Given this description, it would be reasonable to ask what the difference is between 'cysts' and 'spores' (e.g. the spores of slime molds, for example). It is not obvious that there is a clear biological difference at the level of an individual spore or cyst: The term 'spore' tends to be used when a large organism or aggregation produces numerous much smaller protected cells. The term 'cyst' tends to be used when a single independent cell transforms into a single protected cell (and thus the process looks more like 'transformation' than 'reproduction') (but see footnote 1).

Cysts are extremely diverse, and vary tremendously in the structure and composition of the cyst wall, and in the triggers and developmental processes of encystation and excystation (i.e. converting from an active form to a cyst, and *vice versa*). These notes summarise some general concepts, but it should be borne in mind that there will be exceptions to almost all of them.

Structure and composition of cyst walls.

Cyst walls may be relatively simple in overall structure, or composed of multiple layers. The comparatively well studied cysts of *Giardia* (Metamonada; Diplomonadida) and *Entamoeba* (Amoebozoa; Archamoebae) have a single cyst wall, albeit different layers within this wall can have different compositions (see below). By contrast, the cysts of some amoebozoan amoebae (e.g. *Acanthamoeba*) and heterolobsean amoebae (e.g. *Naegleria*) include distinct 'endocyst' (inner) and 'ectocyst' (outer) layers, often with a space between them in the mature cyst, such that the ectocyst fits quite loosely while the endocyst is tight to the cell membrane. The ectocyst is synthesized before the endocyst in the well-studied amoebozoan *Acanthamoeba* (see below for general comments about cyst wall formation during encystation). In addition, cyst walls may be continuous (as in *Giardia* and *Entamoeba*, for example), or may have one or more pores, which are plugged by a material that is different to that of the rest of the cyst wall. The latter arrangement is common in heterolobosean amoebae (for example), and is also seen in *Acanthamoeba*. The pores likely function to allow the cell to escape easily during excystation (see below).

Much of the cyst wall is composed of polysaccharides. These polysaccharides are primarily glucose polymers (e.g. cellulose and β-1,3 glucans), or chitin, which is an N-acetyl glucosamine polymer. These are the same linear polymers that are major components of the cell walls of many macroalgae (and plants), and fungi. However, there are only a small number of species for which the sugar component of cyst walls is known for certain, and there are known exceptions. For example, the human intestinal parasite *Giardia* uses an N-acetyl galactosamine polymer as the major cyst wall polysaccharide, not chitin. In *Entamoeba*, which does employ chitin as its cyst wall polysaccharide, a large minority of the subunits are chemically modified by an enzymatic process (de-acetylation, via chitin deacetylase). This modification presumably makes the cyst wall less susceptible to attack by chitinases produced by 'decomposer' organisms and thereby enhances the survivability of the cysts in the environment.

Cyst walls also have a protein component. In at least some cases (*Acanthamoeba*) the protein content of the cyst wall is similar by mass to the polysaccharide content (in *Acanthamoeba*, which has distinct a ectocyst and endocyst – see above – the ectocyst seems to be predominantly protein). *Giardia* cyst walls are known to contain several distinct 'cyst wall proteins'. Three main cyst wall proteins form aggregates that show binding *in vitro* to the naked polysaccharide component of the cyst wall. Because of this, it is proposed that one of their main roles is to stabilise and/or crosslink the cyst wall polysaccharides. Meanwhile, the two main cyst wall proteins in *Entamoeba*, called 'Jacob' and 'Jesse', both bind to chitin, which as discussed above is the primary cyst wall polysaccharide in this taxon. It is thought that 'Jacob' functions to cross-link chitin fibrils. 'Jesse', meanwhile, is believed to bind to the outer surface of the chitin matrix, and to form a shell that reduces the permeability of the cyst wall to small molecules:

'Jessie' appears on the surface of the cyst late in the encystation process, and (only) after this has happened, there is a large reduction in the permeability of the cyst wall.

Some cyst walls contain silica components as well (i.e. are partly biomineralised). The 'stomatocysts' that are characteristic of Chrysophyceae (a group within Stramenopiles) represent one example. Stomatocysts, also known as 'statospores', have a spherical silica wall, except at one point where there is a small pore (porus) closed by a plug of organic material. Silica biomineralisation of the cyst wall imposes a constraint on the development during encystation, as discussed below.

Encystation.

Encystation, the process of transforming from an active cell form to a cyst, can be triggered by a wide variety of chemical or physical conditions. Typical triggers for encystment include large changes in the osmolarity of the medium, desiccation and nutrient/food source deprivation. One interesting observation is that encystation in certain free-living amoebae can been rapidly triggered by exposure to toxin-producing bacteria.

Encystation usually involves dramatic morphological and physiological changes to the cell, in addition to a process of cyst wall formation. Almost all cysts are spherical or oval-shaped and are much smaller in volume than the active cells from which they develop (e.g. 80% smaller in some ciliates; Hausmann et al. 2003, Verni & Rosati, 2011). Cells with complex cytoskeletal systems will typically undergo dramatic simplification; for example, most flagellates will resorb their flagella during encystation, and flagellar microtubular roots will shorten or be lost entirely. Cell volume is reduced through degradation of many cell components, such as a reduction in the number and size of mitochondria; concomitantly, there can be substantial autophagic activity. Freshwater protists exhibit increased contractile vacuole activity during encystation, presumably to reduce the water content of the cytoplasm (see supplementary notes #1; section 1.2.3. for a brief discussion of contractile vacuoles).

Cyst wall precursor material needs to be exported to the cell surface. Typically, large populations of unusual small endomembrane vesicles are observed in encysting cells, and these contribute sugar and protein components to the cyst wall via exocytosis, with final assembly of the cyst wall occurring extracellularly. The best known of these systems is the Encystation-Specific Vesicle (ESV) system of *Giardia*, which is remarkable because active cells (trophozoites) of *Giardia* lack an obvious post-ER endomembrane system. ESVs bud off from the ER and act as the transport mechanism for both polysaccharide and cyst wall proteins. The ESVs also serve as the site where cyst wall polysaccharide is assembled in the first place, from N-acetyl galactosamine that is synthesized in the cytoplasm.

In a few taxa the cyst (or spore) wall is produced intracellularly within the encysting cell. The cysts of dinoflagellates are produced intracellularly, such that, in armoured dinoflagellates, the thecal plates are discarded during encystment. Interestingly, however, the cyst wall contains ornamentations that (generally) mirror the overlying thecal plates, forming a so-called 'paratabulation' that closely corresponds to the 'tabulation' pattern of the cell's thecal plates. The silicaceous 'stomatocysts' of Chrysophyceae are another conspicuous example. As discussed elsewhere, silicaceous

structures in microbial eukaryotes, such as the valves and girdle bands of diatoms, and the costal strips that form the loricae of acanthocoecid choanoflagellates, are produced within endomembrane compartments known as Silica Deposition Vesicles, or SDVs. This is the case with chrysophycean stomatocysts as well, but unlike these other structures, the stomatocyst is a single-piece silica structure that will enclose the entire cyst cytoplasm when mature. This means the some of the encysting cell's cytoplasm must remain exterior to the cyst wall during its formation. This cytoplasm either disintegrates after the cyst wall is complete (i.e. is abandoned by the cell), or is withdrawn back into the cyst through the single pore (porus), after which the organic pore plug is formed. Interestingly, in some of the former cases the silicaceous cyst wall initially forms as a complete sphere, and the porus is generated afterwards by resorption of the silica at one point in the cyst wall.

Maintenance and excystation.

The encysted form is often described as dormant. In fact, most cysts are probably weakly metabolically active. This is suggested by the months-to-years that they typically remain viable (rather than indefinitely). It is also consistent with a greater time taken for excystment of older cysts compared to younger cysts (observed in a ciliate – Fenchel, 1990). The cysts of *Giardia* reportedly show 10-20% of the metabolic activity of trophozoites (i.e. of active, feeding cells).

Excystation may be triggered by a variety of factors. At least some free-living forms can be induced to excyst by exposure to small organic molecules, presumably indicating nutrient-rich conditions suitable for growth. Excystment in *Giardia*, which is a parasite of the small intestine, can be triggered by low pH (as found in the stomach). Interestingly, higher excystment percentages in old cysts vs. young cysts have been observed in a couple of free-living species.

Excystment is typically quite rapid and involves swelling of the cell, and escape from the cyst wall, as well as development of the active cell morphology. Production of both proteases and enzymes to degrade polysaccharides (e.g. cellulases in *Acanthamoeba*, whose cyst wall contains cellulose) is known or inferred in a few species, suggesting that enzymatic degradation of the cyst wall is an important element in excystation.

Footnote 1:

Uses of the terms 'spore' vs 'cyst' has additional complications: For example, in many 'protosteloid' amoebae, a solitary active cell will transform into a single protected cell that is on the end of a long secreted stalk. This stalked protected cell tends to be called a 'spore', because of its similarity-in-miniature to the spore-holding fruiting bodies of cellular and acellular slime molds (e.g. *Dictyostelium* and *Physarum*). Yet, when the same process occurs in other amoebae, where the protected cell has no stalk, the protected cell is almost invariably called a 'cyst'.

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