Biology 3102 – Microbial Eukaryotes
Supplementary Course Material #2, Chapter 4
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CONTRACTILE VACUOLES

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Overview

The contractile vacuole (CV) is an osmoregulatory organelle found broadly across eukaryotes, most prominently in protists from freshwater ecosystems. As fresh water is hypoosmotic relative to the cytoplasm of the cell, it is necessary to remove excess water that diffuses across the cell membrane for the cell to maintain homeostasis and cell size. Despite the widespread distribution of contractile vacuoles across eukaryote diversity, the data concerning this organelle are highly fragmentary, with most knowledge coming from a few model organisms, and little study of how well this information can be extrapolated to the rest of eukaryotes. In fact, it is currently unclear whether the CV is the same single, homologous organelle across the breadth of eukaryote diversity, or whether there have been multiple evolutionary origins of an organelle to deal with osmoregulation. In this chapter, we treat all CVs as a single, homologous organelle, but draw attention to the source of the data, and where the models agree or disagree, because they do not necessarily apply across the CVs of all eukaryotes.

Why is osmoregulation necessary?

Freshwater protists are generally hyperosmotic relative to their environment. While fresh water generally has an osmolarity below 7 mOsm/L (Allen and Naitoh, 2002), the intracellular osmolarity of freshwater protists has been measured between 45 and 117 mOsm/L (Prusch, 1977). One exception is the giant amoebozoan amoeba *Chaos diffluens*, which has a measured osmolarity of 5 mOsm/L. For freshwater organisms, this hyperosmolarity comes with a challenge—plasma membranes are semi-permeable, and allow water to pass through, travelling down osmotic gradients. This influx of water means organisms are required either to prevent the entry of that water or to continually remove it, otherwise they will swell and potentially explode (lyse). Many protists overcome this challenge through rigid external coverings (e.g. the frustules of diatoms, or the encasing cell walls of desmids). These coverings prevent the cell from expanding, which in turn prevents excess water from entering in the first place. This balance of forces—the cytosol pushing the cell membrane against the cell wall—is known as turgor pressure. However, for a cell to be capable of phagocytosis or movement using pseudopodia or flagella there needs to be regions of the plasma membrane that are not covered by a rigid cell wall (e.g. the flagellar membrane in the case of flagellates). In

these organisms, intracellular osmolarity is instead regulated by an organelle known as the contractile vacuole, or contractile vacuole complex. Though these terms are often used interchangeably, the contractile vacuole (CV) is the central vacuole that functions in excretion of water, while the contractile vacuole complex (CVC) explicitly includes the extended network of tubules, canals, and/or vacuoles that is involved in water collection, in addition to the central vacuole.

That cell walls and CVs are different solutions to the same water-influx problem is nicely illustrated by cases where contractile vacuoles are seen in only some stages of species with complex life histories. Many freshwater organisms have a dominant life stage with an enclosed cell wall that lacks a CV, but will have contractile vacuoles in the unicellular, wall-less, reproductive stages. Examples of such CV-bearing stages include the gametes of conjugating desmids and the zygotes that result from conjugation (Ling and Tyler, 1972), as well as the flagellated zoospores of the fungi-like oomycetes (Hardham, 2008).

Although CVs are generally associated with freshwater organisms, they are also visible and undergo continual functioning in some lineages of marine ciliates. The CVs of marine ciliates still function in osmoregulation, as when these ciliates are exposed to salinities below that of typical seawater, the CV activity increases in tempo (Kitching, 1934). CVs are also found in a few true animals (Metazoa), specifically freshwater sponges and *Hydra* (Benos and Prusch, 1972; Brauer and McKanna, 1978). These representatives of early diverging animal lineages are the only animals without specialized excretory tissues (i.e. protonephridia, nephridia, kidneys) to be found in fresh water.

The general form and function of contractile vacuoles

There is a wide array of CVC forms across the diversity of eukaryotes. Nonetheless most, if not all, CVCs share two common components: the bladder (or contractile vacuole, in the narrow sense) and the spongiome (Figure 1-A). The spongiome is composed of membranous tubules and/or vacuoles, and may be differentiated into the decorated spongiome and the smooth spongiome. The decorated spongiome surrounds the smooth spongiome (when present) and is studded with vacuolar ATPases (V-ATPases) that appear as small projections on the outer (i.e. cytoplasmic) surface of the membrane in TEM images (Nolta and Steck, 1994). The spongiome continuously collects water from the cytoplasm and transfers it to the bladder. The bladder gradually swells as the contents are transferred from the spongiome, and eventually expulses its contents into the external environment. Perhaps the most complex form of this system is seen in some ciliates (Figure 1-B). Here, the CVC is supported by microtubules that anchor it to a specific location in the cell with a permanent "pore" where the bladder empties, and radial canals are positioned around the contractile vacuole. The spongiome in these organisms in turn surrounds the radial canals, and transfers water to the canals before it is finally transferred to the contractile vacuole. In other lineages, the bladder may be anchored and/or reoccurring in the same area of the cell, often near the bases of the flagella (e.g. *Chlamydomonas reinhardtii*) or at the posterior end of the cell. Alternatively, it may be transient, occurring in and moving throughout many areas of the cell, with this being typical of large amoebae, for example (e.g. *Amoeba proteus*).

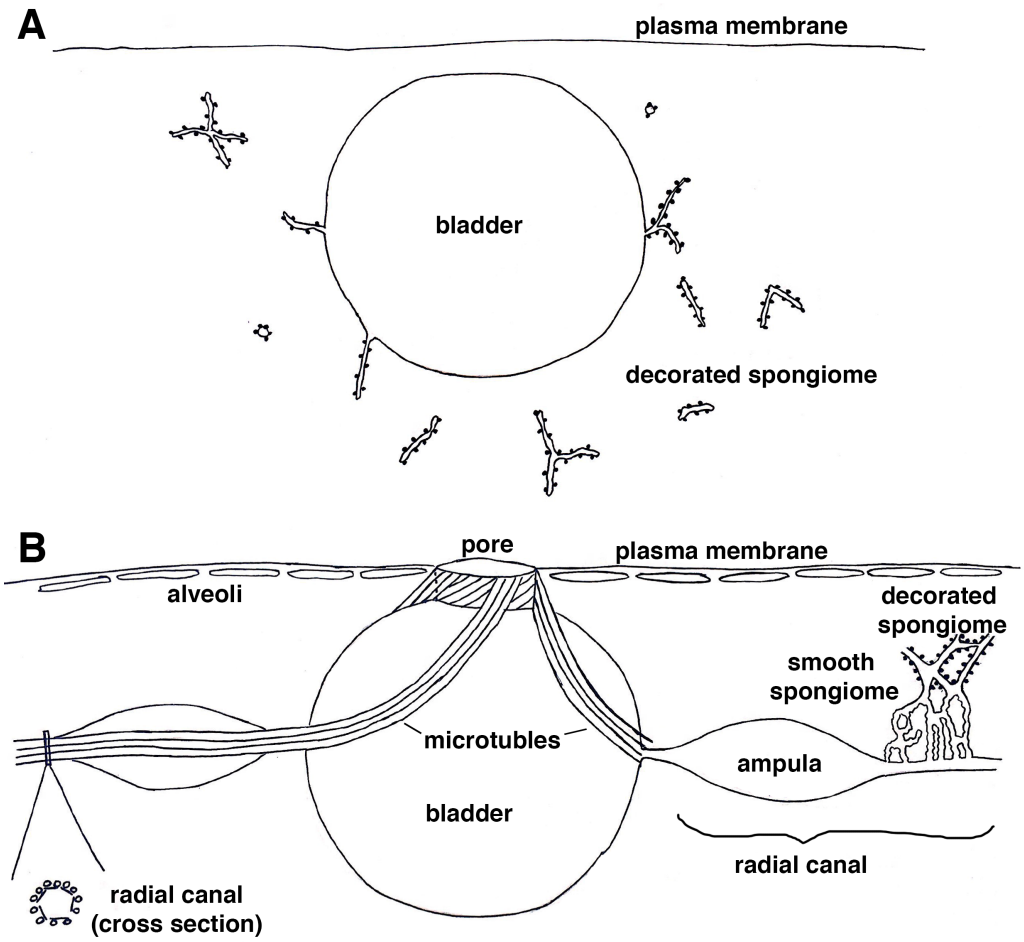

Figure 1. General morphology of two types of contractile vacuole complexes: the "typical" contractile vacuole, with a central bladder and tubular spongiome (**A**; e.g. *Tetrahymena, Trypanosoma*, etc.), and a complex contractile vacuole found in some ciliates, with a permanent pore and a system of radial canals for water collection (**B**; e.g. *Paramecium* spp.).

There are two main phases in contractile vacuole function: the collection of water from the cytoplasm into the contractile vacuole via the spongiome (enlargement of the bladder) and the expulsion of water from the contractile vacuole to the outside environment (evacuation of the bladder) (Figure 2). This *enlargement—evacuation cycle* of the bladder is usually visible with light microscopy. The tempo of this cycle varies dramatically by organism (e.g. 10-15 s in the green alga *Chlamydomonas reinhardtii*, 30-60 s in the ciliate *Rhabdostyla brevipes*, and 300 s in the amoebozoan *Amoeba proteus*) (Docampo et al., 2013; Kitching, 1934). In colpodid ciliates, the total amount of water expelled per unit time increases with the volume of the cell, and decreases with the osmolarity of the environment (Lynn, 1982); presumably this is typical across protists more generally.

A clear question that arises from observing this process is "How is the CV membrane recycled?" One possibility is that this happens by continuous generation and fusion of the spongiome with the bladder until the contractile vacuole reaches its maximum size, at which point the bladder undergoes conventional exocytosis and fuses with the plasma membrane. However, this is unlikely, because there would be a large continuous influx of CV membrane to the external plasma membrane, which would: a) have to be regenerated inside the cell for the contractile vacuole to continue to function; b) continually change the composition of proteins in the external plasma membrane; and c) require an equal amount of membrane to be internalized into the cell to maintain the cell shape and volume. Instead, bladder evacuation happens through a process called *kiss-and-run exocytosis*: rather than a complete fusion with the plasma membrane and subsequent endocytosis to counter the addition to the plasma membrane, the contractile vacuole fuses with the plasma membrane only until its contents have been evacuated, at which point the membranes part and the integrity of the contractile vacuole membrane is maintained. Depending on the form that the contractile vacuole complex takes, the CV bladder may remain entire, albeit in a collapsed state, or it may fragment into many smaller vesicles (e.g. Figure 2-A, C vs. Figure 2-B).

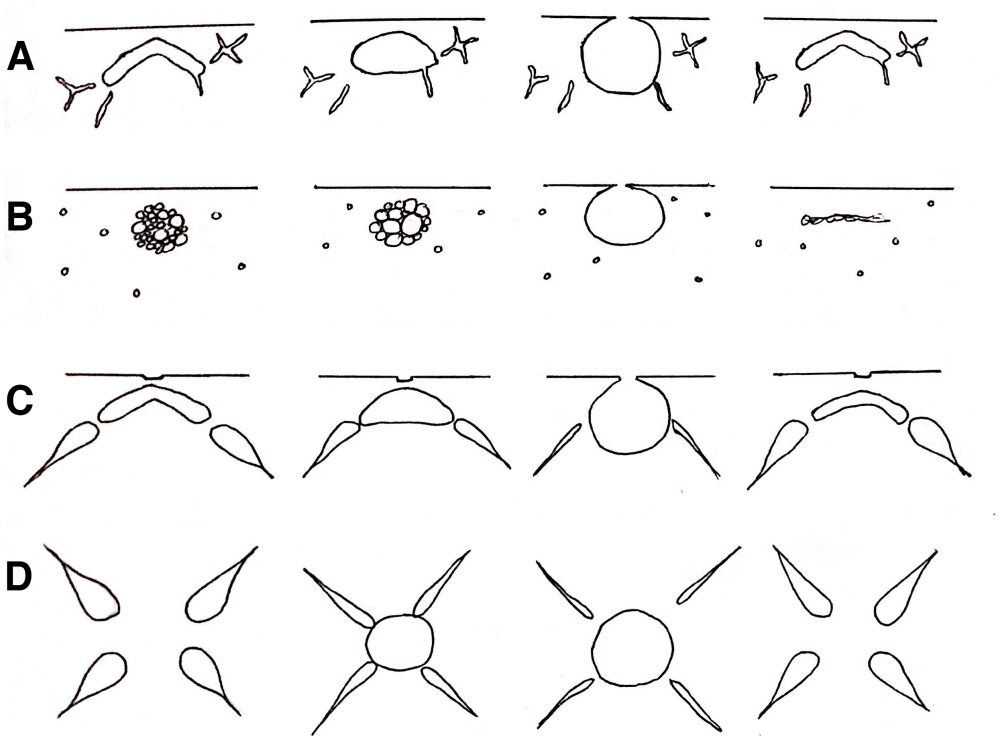

Figure 2. The enlargement—evacuation cycle of three contractile vacuole complex morphologies: a CV with tubular spongiome and central bladder (e.g. *Tetrahymena, Trypanosoma*, etc.) viewed from the side (**A**); a CV with the vacuole-type spongiome, which coalesces into the CV bladder and then collapses and fragments (e.g. *Amoeba proteus*) viewed from the side (**B**); and a CV with radial arms and pore (e.g. *Paramecium* spp.) viewed from the side (**C**) and above (**D**). Note that the bladder in (**D**) is only visible from above when filled, which is typical of what is observed with light microscopy. Modified from Figure 2 of (Patterson, 1980).

Molecular mechanisms of contractile vacuole function

If the contractile vacuole simply expelled unaltered cytoplasmic fluid, we would expect the pools of dissolved molecules in the cell to rapidly dilute, which would presumably be lethal. If the contractile vacuole is to be hypoosmotic to the rest of the cell, water transported into the CV must move against the concentration gradient, or ions must be returned from the CV to the cytosol; in either case requiring an input of energy. Two major hypotheses have been proposed for how this works: either using 1) facilitated transport to simultaneously transport water and ions into the contractile vacuole; or 2) transport of osmolytes into the contractile vacuole, which then, through the increased osmotic gradient, attracts water to the CV. Since CVs are not necessarily all the same organelle from an evolutionary standpoint, it is entirely possible that both models are correct, and that there are even more mechanisms of CV function.

The simplest model, Model 1, relies on a water-solute co-transporter, such as a cation chloride co-transporter (CCC). Under this hypothesis, the CCC co-transports Cl^-, a cation (e.g. K^+), and up to 500 molecules of water into the contractile vacuole (Figure 3-A) (Raven and Doblin, 2014). Chloride ions are then recycled in the cell through an H^+-Cl^- symporter transporting a proton and chloride ion from the contractile vacuole to the cytosol (Figure 3-B). The H^+ gradient driving this symporter is created by a vacuolar ATPase (H^+V-ATPase), which, powered by the hydrolysis of ATP, transports H^+ from the cytosol across the CV membrane (Figure 3-C). Potassium ions are returned to the cytosol through a K^+ transporter (Figure 3-D). Note that under this model the movement of all ions is balanced, and the only net movement is that of water into the CV. It should also be noted that although this model has been proposed for green algae, it is largely speculative. Out of the proteins suggested to be involved, only the H^+V-ATPase has been definitively associated with a green algal CV (*Chlamydomonas reinhardtii*; Ruiz et al., 2001). Early studies of the amoebozoans *Pelomyxa carolinensis* and *Amoeba proteus* found that their CVs were hypotonic to the cytoplasm, which provides evidence for this mechanism of CV enlargement (Riddick, 1968; Schmidt-Nielsen and Schrauger, 1963).

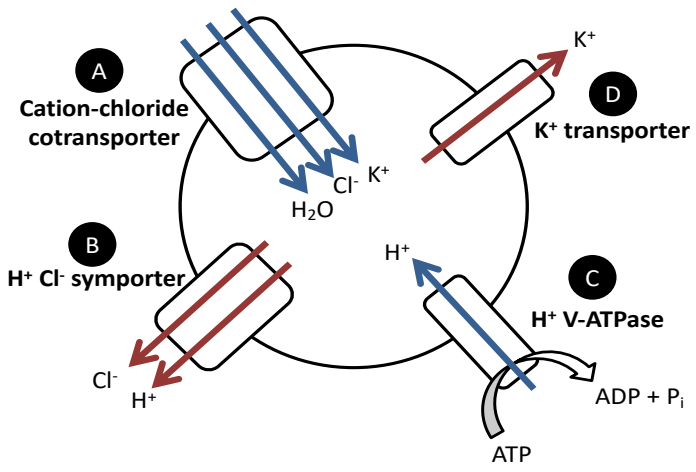

Figure 3. The water-solute co-transporter model of contractile vacuole water transport, proposed in green algae (after Raven and Doblin 2014).

Model 2 relies on expendable osmolytes inside the contractile vacuole raising the osmolarity inside the contractile vacuole, such that water now flows down the concentration gradient into the contractile vacuole. Around evacuation of the bladder, this model reverses the flow of the osmolytes from the CV to the cytoplasm, such that they may be recycled. As proposed using proteins found in *Dictyostelium discoideum* (Docampo et al., 2013), this is accomplished by a H^+V-ATPase or a pyrophosphatase (H^+PPase). Both enzymes are thought to pump protons into the contractile vacuole, using either ATP (in the case of the H^+V-ATPase) or pyrophosphate/diphosphate (in the case of the (H^+PPase) as an energy source (Figure 4-1). Despite this, the contractile vacuole is not an acidic compartment. Instead, the H^+ combines with ammonia (NH_3), which freely diffuses into the contractile vacuole and forms ammonium (NH_4^+). The ammonium is unable to diffuse across the CV membrane, and is retained. An anion exchanger imports bicarbonate (HCO_3^-) into the contractile vacuole in exchange for chloride (which passively diffuses into the contractile vacuole through a chloride channel). The bicarbonate and ammonium act as osmolytes and allow water to diffuse into the CV through aquaporins, which are water channels (Figure 4-2). Prior to the emptying of the bladder, a switch takes place (Figure 4-3) and the osmolytes are transported back into the cytoplasm of the cell to be recycled. Ammonium (NH_4^+) is transported directly by an NH_4^+ transporter, while HCO_3^- is first converted to CO_2 by a carbonic anhydrase and transported back into the cell by a CO_2 transporter. Like the first model, this results in the net transport of water into the contractile vacuole. The CV of the ciliate *Paramecium multimicronucleatum* is hyperosmotic to the cytoplasm during the enlargement phase, which implies a similar mechanism of creating an osmotic gradient as the one described here, except the major osmolytes involved are K^+ and Cl^- (Stock et al., 2002).

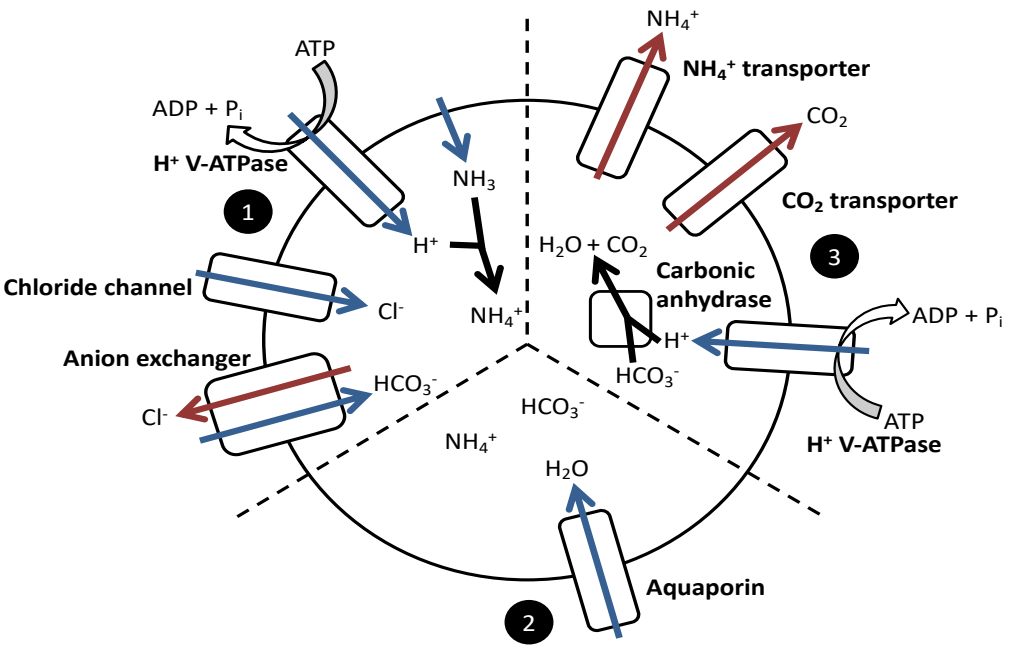

Figure 4. The osmolyte-driven model of contractile vacuole water transport proposed for *Dictyostelium discoideum* (after Docampo et al. 2013).

The molecular machinery of contractile vacuoles

Current knowledge of the molecular machinery of contractile vacuoles is fragmentary, with the majority of information coming from amoebae of the cellular slime mold *Dictyostelium discoideum* (Amoebozoa), the trypanosomatid parasite *Trypanosoma cruzi* (Kinetoplastea), *Paramecium* species (Ciliophora) and the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophyta, Archaeplastida). There are (as of yet) no distinctive contractile vacuole-associated proteins identified that are found in all the protists studied in detail. Only a handful of proteins have been identified in more than one model species (compiled in (Docampo et al., 2013; Plattner, 2013)). These include the above-mentioned H^+V-ATPases and H^+PPases, which pump protons into the contractile vacuole. Other common components include: aquaporins, which allow for passive transport of water across the cell membrane (though whether this is the same aquaporin in all CVs is yet to be determined); the calcium signaling protein Calmodulin; and a number of membrane trafficking proteins. These common membrane trafficking proteins include Rab GTPases that act as 'switches' to recruit and direct other proteins for proper localization and fusion of vesicles (specifically Rab11 and Rab14), and adaptor proteins that bind to cargo proteins and recruit the coat proteins responsible for deforming membranes to form vesicles (specifically AP180, which recruits clathrin, a vesicle coat involved in trafficking from the PM or Golgi body to the recycling endosome when paired with different adaptor proteins). Additionally, many other membrane trafficking proteins have been identified in one or more systems, including a variety of other Rab GTPases and adaptor proteins, clathrin, and SNAREs. These last proteins are present on the surface of both membranes to be fused, and interlock to bring the membranes into close enough contact to fuse. The involvement of many membrane trafficking proteins is perhaps to be expected, as the function of the CV requires, at minimum, two instances of fusion and scission of membrane-bound compartments: (1) the spongiome with the contractile vacuole bladder, and (2) the bladder with the plasma membrane (see above text and Figure 2).

Other functions of contractile vacuoles

In addition to being the major mechanism of osmoregulation in the studied protists, the contractile vacuole has been proposed to be involved in a number of other cellular functions. Based on research in at least two of the CV model organisms (mentioned above), the other potential roles identified are (1) the trafficking of proteins to and from the plasma membrane, and (2) calcium storage and signaling. Additionally, the presence of CVs in some marine ciliates has led to the hypothesis that they play a role in metabolite excretion (Kaneshiro et al., 1969; though the CVs of marine ciliates likely still function in osmoregulation as well, since, as discussed above, their activity increase when the ciliates are exposed to lowered salinities). These other functions have not been studied broadly, however, so it is uncertain whether they are universal features of CVs or are, instead, lineage-specific.

In contrast to the canonical path for trafficking proteins to the cell surface from the endoplasmic reticulum through the Golgi body, some cases have been found where the proteins are trafficked through the CV. In the trypanosomatid *Trypanosoma cruzi*, the contractile vacuole seems to play a role in trafficking glycosylphosphatidylinositol-anchored proteins (GPI-AP) to and from the cell membrane (these GPI-APs are linked

the lipids of the outer leaflet of the cell membrane, and sit 'above' the lipid bilayer of the membrane). Intriguingly, in *Trypanosoma brucei*, which lacks a contractile vacuole, the variant surface glycoproteins (a type of GPI-AP) that are in part responsible for host immune system evasion are trafficked to the plasma membrane in a pathway that also uses Rab11 (i.e. one the 'CV-associated' proteins identified in some model systems, including *T. cruzi* – see above). Protein trafficking via the CV is also reported in *Dictyostelium discoideum*, which traffics the calcium transporter PAT1 and the cell adhesion molecule DdCAM-1 to the plasma membrane through this system (Moniakis et al., 1999; Sesaki et al., 1997). Interestingly, Rab11 is involved in CV function in *D. discoideum* as well, but it is unclear whether it is involved in protein trafficking in addition to the process of membrane fusion (Harris et al., 2001).

There are a number of lines of evidence that suggest that the CV can be involved in Ca^{2+} storage and signaling. In *Dictyostelium discoideum*, isolated CVs take up and release Ca^{2+} (Malchow et al., 2006). Additionally, when CV function is impaired in *D. discoideum* through gene knock-outs, its ability to deal with high extracellular calcium levels decreases, implying a role for the CV in calcium regulation (Moniakis et al., 1999). Conversely, there is also evidence that calcium is an important regulator of CV function in *D. discoideum*, and that efflux of Ca^{2+} from the CV triggers the downregulation of a Rab GTPase, initiating the fusion of the CV with the plasma membrane (Parkinson et al., 2014). Similar Ca^{2+} sequestering is seen in *Paramecium multimicronucleatum* (Stock et al., 2002), while Ca^{2+} release in proximity to the spongiome has been observed in *P. tetraurelia* (Ladenburger et al., 2006), but in both *Paramecium* species the proteins involved seem to differ from those identified in *D. discoideum*.

Contractile vacuoles and their patchy distribution

Contractile vacuoles are found in freshwater species of almost all major eukaryotic lineages. They are, for the most part, absent from parasitic, marine, or hypersaline lineages, although some parasitic trypanosomatids (e.g. *Trypanosoma cruzi*) and some marine ciliates (both briefly discussed above) are notable exceptions. Most cellular features that are found in such a broad distribution of protists are confidently inferred as present in the last eukaryotic common ancestor. However, CVs are both extremely varied in morphology and patchily distributed, which opens the possibility that they are not strictly homologous across eukaryotes, and have arisen multiple independent times. As marine, parasitic, and freshwater species of many lineages seem to be closely related, a huge number of CV losses would have to be invoked in order to explain its distribution in freshwater species alone. On the other hand, there are reports that some marine protists have contractile vacuoles which are induced by introduction of the cell to lower osmolarities (e.g. the amoeba *Vahlkampfia calkensi*; Hogue, 1923). This has yet to be studied systematically, but if this is a widespread phenomenon, CVs may be a more universal feature of eukaryotic cells than is generally assumed at present.

The dinoflagellate "pusule"

The only major lineage of freshwater-inhabiting protists that lacks a complete cell wall, and *also* completely lacks reports of a CV is the dinoflagellates. Most—but not all—dinoflagellates are covered by thick thecal plates, which could mitigate the need for a CV (as discussed above); yet, most dinoflagellates also have flagella, which is expected to

confer the need for a CV. However, near to the flagellar pocket, there is often a membrane-bound organelle composed of tubules and chambers called the "pusule", which has been proposed by some researchers to be analogous to CVs (Cachon et al., 1983; Dodge, 1972). Interestingly, the pusule is bounded by two separate biological membranes, one inside the other, rather than the single bounding membrane of a CV bladder. The volume of the pusule varies with the salinity of the media the dinoflagellates are living in; however, this is the opposite phenomenon than expected if it were analogous to CVs, as the volume decreases with decreasing salinity (Klut et al., 1987). Additionally, the pusule does not have the enlargement—evacuation cycle observed in CVs, and it is routinely found in both marine and freshwater dinoflagellates. As such, there are a number of plausible proposed functions for this organelle other than osmoregulation, such as the uptake of macromolecules or excretion of ions (reviewed in (Kalinina et al., 2018)). More evidence is needed to determine the function of the pusule, and what role (if any) it plays in osmoregulation in freshwater dinoflagellates.

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