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Book chapter: Review on the Cell Biology of Diatoms

Cell Biology of organelles

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Abstract. Diatoms are phototrophic, unicellular, and eukaryotic organisms. They originate from secondary endosymbiosis, a specific evolutionary process. Accordingly, their cells and organelles have a typical organisation, as revealed by ultrastructural investigations. Diatoms possess specific compartments and structures, including a silica shell surrounding the diatom cell, so-called silica deposition vesicles (SDVs), as well as complex plastids that are surrounded by four membranes.

Here we provide an overview of diatom organelles, and recapitulate recent information obtained from 3D imaging of whole diatom cells, focusing on the subcellular topology of the model diatom *Phaeodactylum tricornutum*. This chapter will not discuss issues of the cell wall and the SDVs, which are covered in chapters 13 and 14.

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Abbreviations

BTS: bipartite targeting sequence

cER: chloroplast endoplasmic reticulum

cERM: chloroplast endoplasmic reticulum membrane,

EE: early endosome

ER: endoplasmic reticulum

ERAD: endoplasmic reticulum-associated protein degradation

iEM: inner envelope membrane

MTOCs: microtubule organising centres

MVBs: multivesicular bodies

NLS: nuclear localisation signal

oEM: outer envelope membrane

PPC: periplastid compartment

PPM: periplastid membrane

PR: periplastid reticulum

PS: photosystem

SDVs: silica deposition vesicles

SP: signal peptide

TGN: trans-Golgi network

VN: vesicular network

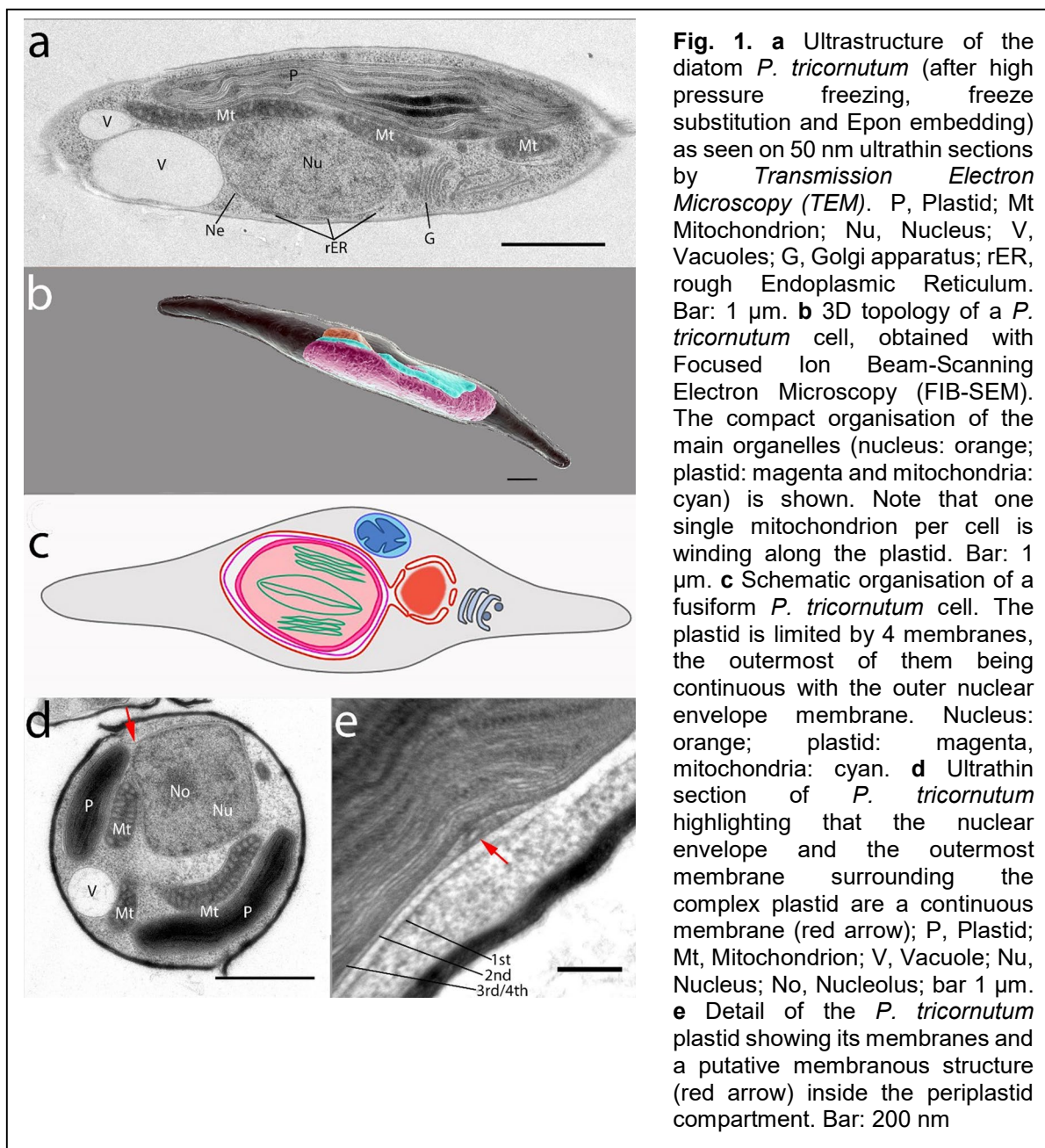
Introduction. Diatoms are key phytoplankton players. By converting CO₂, sunlight and nutrients into biomass and oxygen, these unicellular phototrophs contribute up to 20% of the primary ocean productivity (Field *et al.* 1998), are at the basis of food webs and of the biological CO₂ pump, i.e. the main carbon storage pathway in the oceans (Field *et al.* 1998; Smetacek 1985). These diverse microalgae can reach up to 100.000 species (Malviya *et al.* 2016) thriving in different freshwater and ocean environments, including the ice (Thomas and Dieckmann 2002) and turbulent waters (Margalef 1978). A possible reason for their ecological success is their high metabolic plasticity (Falciatore *et al.* 2020), which is illustrated, e.g. by the presence of chimeric pathways (Allen *et al.* 2011), peculiar photoprotective responses (Bailleul *et al.* 2010) and specific bioenergetic features (Bailleul *et al.* 2015).

While genomic studies (Armbrust *et al.* 2004; Bowler *et al.* 2008) and large scale genomic approaches (Malviya *et al.* 2016) have provided relevant insights on diatom evolution and biodiversity, we still miss comprehensive knowledge on the diatoms cell biology. This is a critical aspect, since previous work has suggested that, for instance, the physiology and metabolism of diatoms are determined by their peculiar cell organisations and more particularly by the morphology and arrangement of key energy-producing organelles machineries, such as the plastids and mitochondria (Bailleul *et al.* 2015; Flori *et al.* 2017).

In the following chapter, we first introduce the structure and function of important diatom subcellular compartments, namely nucleus, endoplasmic reticulum, Golgi apparatus, endosomes, vacuoles, peroxisomes, autophagosomes, cytoplasmic membranes, mitochondria, and plastids. Next, we illustrate recent advancement in understanding the subcellular architecture of a diatom cell (focusing on the plastid and its interaction with other organelles), based on 3D imaging techniques in the model species *Phaeodactylum tricornutum*. Finally, we discuss a particular case of symbiosis involving diatoms and dinoflagellates (Dinotoms), which are widely distributed in both freshwater and marine environments, displaying different levels (temporary or permanent) of interaction between the two organisms.

The cell nucleus. The nucleus of diatoms is surrounded by a typical nuclear envelope (just like in other eukaryotes), in which nuclear pores are visible in ultrastructural investigations (e.g. (Round *et al.* 1990)). Within the nucleus, heterochromatic and

euchromatic areas including, one or more nucleoli, are present. The nucleus can be visualised by dyes inserting into the genomic DNA such as DAPI (e.g. (Tanaka *et al.* 2015)) or Hoechst 33342 (Schellenberger Costa *et al.* 2013). The outer membrane of the nuclear envelope is not only connected to the endoplasmic reticulum (ER) membrane, but also continuous with the outermost membrane of the plastid (Fig. 1a, 1d, see also scheme in Fig. 1c), which has been termed chloroplast ER (cER) membrane (see below).



This cellular arrangement is difficult to visualize in ultrastructural studies with respect to the ER-nuclear envelope connection. However, it has been revealed by localisation

studies of ER proteins fused to GFP, in which the ER lumen and the lumen of the nuclear envelope are marked (e.g. (Apt *et al.* 2002)).

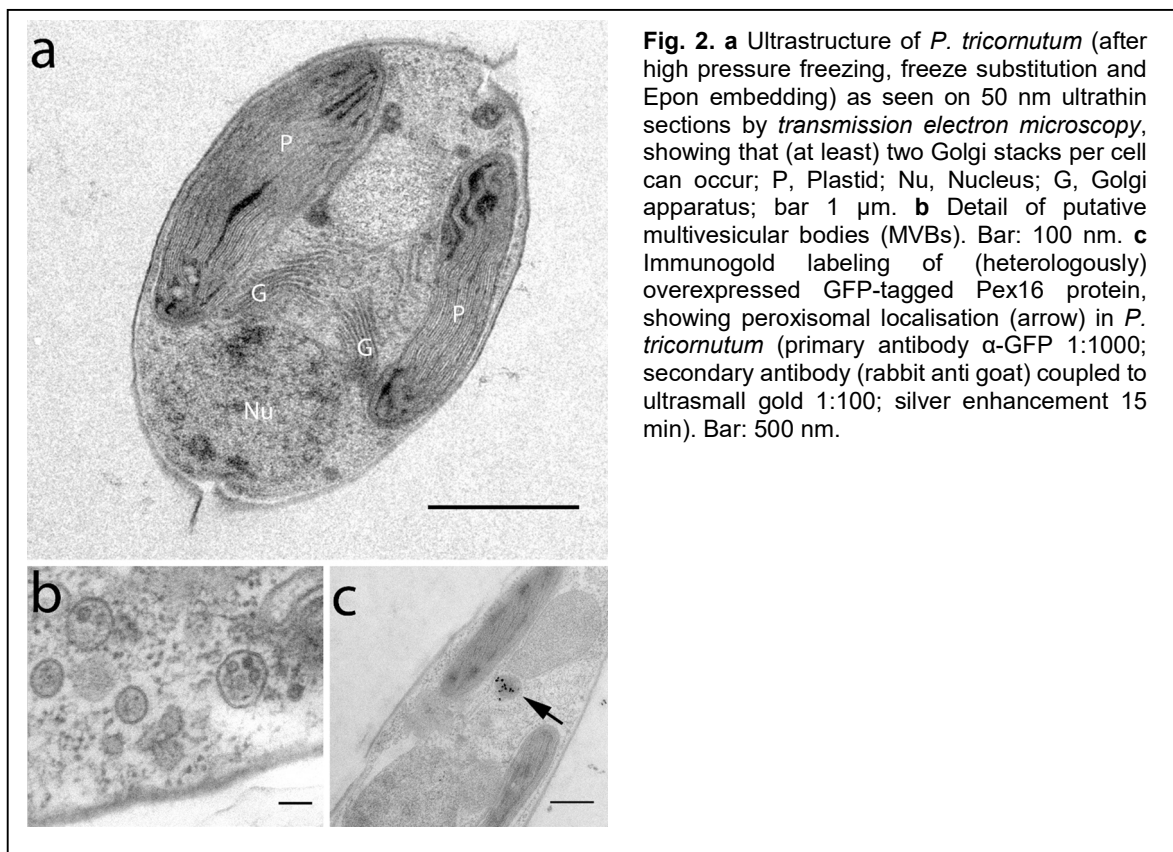
With respect to nuclear division, early investigations indicated the presence of microtubule-organizing centres (MTOCs) and at least a partial breakdown of the nuclear envelope in some diatoms (Manton *et al.* 1969; Tippit and Pickett-Heaps 1977; Tippit *et al.* 1980). However, a more recent investigation of the diatom *P. tricornutum* surprisingly showed that in the course of nuclear division, at least one nuclear membrane is maintained (Tanaka *et al.* 2015). In addition, no evidence was obtained in this study for a mitotic apparatus that includes microtubules. Instead, an ingrowing furrow is involved in the penetration and bisection of the nucleus. Import of many proteins into the nucleus seems to be equivalent to the canonical pathway of eukaryotes, in which proteins are exposing a nuclear localisation signal (NLS) that allows import with the help of importins. Protein targeting into the nucleus has been demonstrated for the aureochrome photoreceptor proteins (Schellenberger Costa *et al.* 2013).

Endoplasmic reticulum. The endoplasmic reticulum (ER) is composed of one highly branched cisterna that forms a network covering nearly all cytoplasmic regions (as shown by decoration with GFP fusion proteins, (e.g. (Apt *et al.* 2002)). Also, the ER-Tracker Blue-White DPX (Invitrogen) was recently applied for visualizing the ER of the diatom *P. tricornutum* (Shao *et al.* 2019). Beside its biochemical functions such as lipid synthesis (Zulu *et al.* 2018), the ER is the platform for synthesis of proteins of the endomembrane systems, of the cytoplasmic membrane, as well as of secreted proteins. Soluble proteins that either are restrained in the ER lumen or that are directed from the ER to other organelles, as well as some membrane proteins, possess an N-terminal signal peptide (SP), which is predicted by algorithms like SignalP (for the version 5.0 of SignalP see (Armenteros *et al.* 2019)). In other eukaryotes, several proteins, not possessing a SP, can also be integrated into a membrane either by an SRP-iNDependent (SND) targeting pathway (e.g. (Aviram *et al.* 2016)), or in case of proteins with only one C-terminal transmembrane domain, by a C-tail insertion pathway (Chio *et al.* 2017; Moog 2019). The presence of both mechanisms in diatoms still has to be investigated in detail.

N-linked protein glycosylation, which is an important function of the ER, has been studied in diatoms (e.g. (Baïet *et al.* 2011; Mathieu-Rivet *et al.* 2020)).

Like in other eukaryotes, transport of proteins from the ER to the Golgi apparatus and *vice versa* is supposed to take place via COPII and COPI vesicles, respectively. Soluble proteins of the ER lumen, targeted to the Golgi apparatus, have to be re-translocated to the ER. For this step a C-terminal signal, a so-called KDEL signal, is required. In case of the BiP proteins of *P. tricornutum* and *T. pseudonana* a C-terminal DDEL tetrapeptide might acts as an “ER retention signal” (e.g. (Apt *et al.* 2002; Sheppard *et al.* 2010)).

Golgi apparatus. The Golgi apparatus is an important organelle involved in several biochemical functions, including sorting of proteins with a post-Golgi destination. This organelle, as in other cells, can be identified on the ultrastructural level by its well-pronounced stacks of disk-like compartments called cisternae (Fig. 1, 2). Vesicles supposed to traffic from or to the Golgi apparatus were identified in the surroundings of this organelle in *P. tricornutum* (e.g. (Tanaka *et al.* 2015)).



A recent study proposes the Golgi apparatus of *P. tricornutum* to be a single stack of cisternae, positioned near the cell nucleus ((Tanaka *et al.* 2015), Fig. 1a, 2a). With regard to the cell cycle, the Golgi apparatus in *P. tricornutum* duplicates prior to

karyokinesis ((Tanaka *et al.* 2015), Fig. 2a), so that both daughter cells are equipped with a Golgi apparatus just after cytokinesis. The Golgi apparatus of diatoms can form complicated arrangements with other compartments. In some diatoms the Golgi apparatus has a tight association with the ER and the mitochondria (so-called Golgi-ER-Mitochondrion unit, G-ER-M unit), or it can encircle the nucleus forming a perinuclear shell (see: (Medlin and Kaczmarska 2004; Schmid 1988)).

With the Golgi-Tracker Red (Beyotime, Beijing, China), a specific dye is available for visualizing the Golgi apparatus in live diatom cells (Shao *et al.* 2019). Additionally, several proteins can be used as specific Golgi markers. For sub-compartmental localisation, a predicted β 1,2-Xylosyltransferase (XylT) can be used to specifically label the Golgi cisternae (Liu *et al.* 2016). This protein partially overlaps in its localisation with a putative retromer subunit (Vps29), indicating different functions in different cisternae of the Golgi apparatus of *P. tricornutum* (Liu *et al.* 2016). Also, the GDP-L-fucose transporter and the fucosyltransferase (FuT) are useful Golgi-stack specific markers in *P. tricornutum* (Zhang *et al.* 2019). Finally, the Rab-family small GTPase Sec4 might be involved in the formation of post-Golgi vesicles (Tanaka *et al.* 2015).

Endosomes. Endosomes are important organelles of eukaryotic cells as their functions include the transport of endocytosed material to organelles involved in molecule degradation (lysosome, vacuole), the recycling of proteins to be transported to the cytoplasmic membrane/extracellular space after endocytosis and directing newly synthesised proteins to a degradation organelle.

Diatom endosomes are not well studied. So far, several genes encoding endosomal proteins including Rab5 were identified in the genome of *P. tricornutum* (Zheng *et al.* 2016). To compare diatoms and land plants, it would be of interest to test if the trans-Golgi network (TGN) and the early endosome (EE) have overlapping functions, as is the case in higher plants (e.g. (Dettmer *et al.* 2006; Viotti *et al.* 2010)). Similarly, the presence of late endosomes/multivesicular bodies (MVBs), the latter having characteristic internal vesicles, are not well characterised in diatoms. One example for a putative MVB is shown in Fig. 2b.

Vacuole. Like most plant and algal cells, diatoms also harbour a vacuole. An enlargement of the vacuole volume was observed in a nitrate reductase knockout strain of *P. tricornutum*, propagated in NO_3^- -containing medium (McCarthy *et al.* 2017) and in WT cells under phosphate limitations (Dell'Aquila *et al.* 2020). One of the

pronounced functions of the vacuole is the storage of chrysolaminarin, a non-crystalline β -glucan that is used by diatoms as carbohydrate storage. Several reports focusing on chrysolaminarin synthesis and degradation indicated that a central glucan synthase is located in the tonoplast membrane and utilises UDP glucose (Roessler 1987) on the cytoplasmic side, secreting chrysolaminarin as a linear molecule into the vacuole (Hildebrand *et al.* 2017; Huang *et al.* 2018). Furthermore, three transglycosylases have been identified in *P. tricornutum* that reside in the tonoplast membrane (Huang *et al.* 2016). They possess N-terminal signal peptides and single transmembrane helices at the C-termini, followed by a short domain that may represent cytoplasmic tails. Accordingly, the enzymatically active moieties are supposed to be located on the vacuolar side of the tonoplast, where they may add carbohydrate side chains to the chrysolaminarin chain (Huang *et al.* 2016).

The tonoplast, the vacuolar membrane in diatoms, can be stained with the dye MDY-64 (Huang *et al.* 2016), and several tonoplast integrated proteins, involved in important functions of the vacuole including carbohydrate metabolism, were localised (e.g. (Huang *et al.* 2016; Schreiber *et al.* 2017)).

Two different targeting routes of proteins to the vacuole have been identified in higher plants. In the “canonical” route, newly synthesised vacuolar proteins first traffic via the secretory system to the trans Golgi/early endosome, where they are sorted for final targeting to the vacuole. Some vacuolar proteins are targeted on a second route from the ER to the vacuole, bypassing the Golgi apparatus (e.g. (Viotti *et al.* 2013)). Currently, we do not know if the latter route is present in diatoms, while there are strong indications for the existence of the “canonical” targeting route.

In the case of vacuolar 1,6- β -transglycosylases putative tyrosine-based and di-leucine based motifs were identified (Huang *et al.* 2016), which might act as targeting signals. Consistent with this assumption is the finding that a di-leucine motif has been shown to be essential for correct targeting of tonoplast proteins in *P. tricornutum* (Schreiber *et al.* 2017).

Peroxisomes. Peroxisomes are single membrane-bound organelles, which harbour a variety of pathways, including fatty acid degradation and detoxification of reactive oxygen species (ROS). Along with the majority of eukaryotes, diatoms also possess peroxisomes, as documented by both *in silico* and *in vivo* studies (Winkler and Stabenau 1995). Genes encoding typical peroxisomal biogenesis proteins, so-called

peroxins (Pex) have been identified in centric and pennate diatoms (Armbrust *et al.* 2004; Gabaldón *et al.* 2016; Gentekaki *et al.* 2017; Gonzalez *et al.* 2011; Mix *et al.* 2018). Most detailed information about peroxisome biology in diatoms exists for *P. tricornutum*. In this species, not only a comprehensive set of peroxins is encoded in the genome, but also several enzymes for peroxisomal metabolism (Gonzalez *et al.* 2011). Using confocal and electron microscopy, many of these proteins were localised as GFP fusion proteins in *P. tricornutum* peroxisomes, which in electron microscopy appear as round, single membrane bound compartments usually smaller than 500 nm (Fig. 2c) (Mix *et al.* 2018). Moreover, protein (co)localisation in diatom peroxisomes can be studied using additional fluorescent markers such as YFP, CFP and mRuby3

Among the peroxisomal proteins identified in *P. tricornutum* are a catalase for detoxification of reactive oxygen species, enzymes of the glyoxylate cycle, and a complete set of enzymes for fatty acid beta-oxidation (Gonzalez *et al.* 2011). These findings indicate that diatom peroxisomes contain the typical metabolic functions known from other eukaryotes. It is unknown yet whether there are unexpected biochemical pathways or reactions in diatom peroxisomes.

One peculiarity of diatom peroxisomes is the lack of one of the two classical import pathways for peroxisomal matrix proteins: *P. tricornutum* peroxisomal proteins contain peroxisomal targeting signals of type 1 (PTS1), a C-terminal tripeptide usually composed of the amino acids SKL (and derivatives of this motif). The less common PTS2 (an N-terminal nonapeptide) seems to play no role in peroxisomal protein import in diatoms (Gonzalez *et al.* 2011). This was concluded from the complete absence of peroxisomal proteins with PTS2 targeting signals, and by the missing PTS2 receptor (Pex7) and Pex7-binding motif in the PTS2 co-receptor (Pex5) of diatoms (Gonzalez *et al.* 2011; Mix *et al.* 2018). However, the lack of the PTS2 import pathway apparently is not a specific feature of diatom peroxisomes, and is found in other eukaryotes including *Drosophila*, *Caenorhabditis elegans*, *Cyanidioschyzon merolae* and haptophytes (see (Gonzalez *et al.* 2011; Mix *et al.* 2018) and references therein).

Autophagosomes. Autophagosomes, organelles involved in delivering cytoplasmic regions and/or organelles to the lysosome/vacuole, are hard to detect. Their occurrence under stress conditions and their role in clearance of defect organelles has been predicted by the expression of genes that encode putative autophagosomal proteins in *T. pseudonana* and *P. tricornutum* (Rastogi *et al.* 2018; Shemi *et al.* 2015).

Cytoplasmic membrane. The cytoplasmic membrane surrounds the cytoplasm and is involved in many cellular functions. Several reports are available dealing with the import and export of molecules, catalysed by proteins integrated into the cytoplasmic membrane, e.g. aquaporins, a bicarbonate transporter (SLC4) and the metal ion transporters ZIP (zinc transporter protein) (e.g. (Coale *et al.* 2019; Kazamia *et al.* 2018; Matsui *et al.* 2018; Nakajima *et al.* 2013). Another example of a cytoplasmic membrane protein is the plasma membrane intrinsic protein 1 (Liu *et al.* 2016). Furthermore, the so-called PDZ proteins (PDZ1, PDZ2), were found to be located in the plasma membrane. Tagging with eGFP has shown that cytoplasmic membrane proteins are not always evenly distributed as it is the case for PDZ2, but can be also enriched in defined spots in case of the eGFP fused PDZ1 protein (Liu *et al.* 2016). Membrane integrated receptors are essential for signalling at the cytoplasmic membrane. Genes and transcripts of G-protein coupled receptors were identified recently (Mojib and Kubanek 2020), and may indicate the presence of signalling pathways in diatoms, although an in-depth characterisation is still pending.

The existence of endocytosis is suggested by staining the cytoplasmic membrane with the dye FM4-64 (e.g. (Liu *et al.* 2016)) and additionally by application of endocytosis inhibitors in case of siderophore uptake studies (Kazamia *et al.* 2018). Very recently, internalisation of partially condensed silicic acid (oligosilicates) via endocytosis was proposed (Annenkov *et al.* 2020). In addition, an endocytosis motif (D/ExxxL) is present in case of the iron starvation-induced protein 1 (ISIP1), involved in the uptake of siderophores via endocytosis. This motif is known to interact with the adaptor protein-2 (AP-2) complex (Kazamia *et al.* 2018; Lommer *et al.* 2012). Thus, clathrin-mediated endocytosis seems to occur in diatoms.

Mitochondria. The mitochondria of diatoms vary in shape and quantities (e.g. (Schober *et al.* 2019)). Typically, diatom mitochondria show either an oval or tubular-like morphology, with sizes range from approximately 0.5 to 1 μm in diameter (Schober *et al.* 2019; Werner 1977). Like in other eukaryotes, mitochondria of diatoms possess two membranes, the innermost membrane forming tubular cristae. The genome in the diatom mitochondrial matrix has a size between 35 to 80 kbp, encoding typically less than 40 proteins (e.g. (Guillory *et al.* 2018)). In a *P. tricornutum* cell one large, branched

mitochondrion, winding along the plastid, can be found per cell (see 3D model in Fig. 1).

For specific localisation studies, “mitotracker” dyes (MitoTracker Orange or MitoTracker Green FM, Invitrogen, USA) were used (e.g. (Marter *et al.* 2020; Tanaka *et al.* 2015)). They are of special interest for co-localisation of mitochondrial proteins fused with a fluorescent protein, but also for studying mitochondrial dynamics under different physiological conditions or with respect to the cell cycle (e.g. (Tanaka *et al.* 2015)). A protocol for the isolation of a high-quality mitochondrial fraction for diatoms was recently published for *Thalassiosira pseudonana* (Schober *et al.* 2019). Proteome studies (Schober *et al.* 2019) might contribute considerably to the physiological and evolutionary understanding of mitochondria in diatoms.

Nucleus-encoded proteins transported into the mitochondrial matrix of diatoms are equipped with a matrix targeting signal at their N-terminus. These targeting sequences can be predicted according to (Gruber and Kroth 2014; Gruber and Kroth 2017), and be used for screening diatom genomes for mitochondrial matrix proteins in general. In parallel, identification of a matrix targeting signal in a protein of interest can lead to *in silico* localisation predictions, as it was shown, e.g., for a contiguous translational fusion encoding an N-terminal triosephosphate isomerase (TPI) and a C-terminal GAPDH of the diatom *P. tricornutum* (Liaud *et al.* 2000). Other targeting signals such as those for proteins of the outer and inner membrane have to be identified and studied in detail. In the genome of *P. tricornutum*, several genes were identified encoding proteins known to be essential for protein import into mitochondria. These include factors for the TOM- and TIM-complex in addition to components of the SAM-complex and for inner membrane targeting (Delage *et al.* 2011). Thus, there is striking evidence that in diatoms similar mitochondrial protein import mechanisms operate as in other eukaryotes.

Plastid. Whereas the organelles described above are present in nearly all eukaryotes (for exceptions regarding of peroxisomes in certain chromalveolates, see e.g. (Mix *et al.* 2018; Moog *et al.* 2017)), the plastid types found in diatoms are restricted to most organisms of the supergroup Chromalveolata (Cavalier-Smith 1999). Although the numbers and sizes of complex plastids vary in diatoms (Round *et al.* 1990), they all contain four surrounding membranes, at variance with plastids with two surrounding

membranes that are present in green and red alga, glaucocystophytes and land plants. Complex plastids of diatoms originated in the course of a higher order endosymbiosis (e.g. (Keeling 2009)), in which a red alga was engulfed by another eukaryote and reduced to a complex plastid (e.g. (Cavalier-Smith 2000; Douglas *et al.* 1991)).

Already in 1998, a protocol for the isolation of plastids from the diatoms *Odontella sinensis* (Greville) Grunow and *Coscinodiscus granii* Gough was published (Wittpoth *et al.* 1998). These plastid fractions were photosynthetically active and would be a highly suitable resource for studying the protein composition of diatom plastids. However, for neither diatom are genome data available yet. To overcome this limitation, a plastid isolation protocol for the model diatom *T. pseudonana* was published recently (Schober *et al.* 2019). The herewith-isolated plastid fraction was used for extensive proteome maps for both diatom plastid and mitochondria, though lacking stromal plastid proteins.

Of the four membranes of diatom plastids, the outermost membrane (chloroplast endoplasmic reticulum membrane, cERM) is facing the cytoplasm and believed to be the remnant of a phagotrophic membrane, which surrounded the red alga symbiont in the early stages of the evolution of complex plastids (e.g. (Cavalier-Smith 2000)). The second outermost membrane (membrane II), also termed the periplastid membrane (PPM), might be the remnant of the cytoplasmic membrane of the engulfed red alga, whereas the two innermost membranes (membrane III is also called outer Envelope Membrane oEM and IV, i.e. the inner Envelope Membrane iEM) are homologous to the plastid envelope membranes of the red alga symbiont.

In diatoms, the outermost membrane of the plastid is connected to the outer nuclear membrane (Fig. 1d, see also scheme in Fig. 1c). Therefore, the lumen between the outermost plastid membrane and the PPM, and the lumen of the host ER form a continuous space, although both have different functions. The adjacent lumen, which is delimited by the PPM and membrane III, is called periplastid compartment (PPC), and is considered to be the remnant of the cytoplasm of the engulfed red alga.

The diatom stroma harbours a plastid genome ranging from approximately 100 up to 200 kbp in different species (e.g. (Yu *et al.* 2018)) as well as 70S-type ribosomes. Finally, the stromal membrane system is typically composed of a so-called 'girdle lamella', which is a triple thylakoid structure encircling the central thylakoids. The central thylakoids form a network of internal membranes typically enriched in

galactolipids and contain the photosynthetic complexes devoted to photosynthetic electron transfer. In diatoms, these membranes have different topologies than in vascular plants and green algae (see below).

New interpretations of older ultrastructural data (see e.g. (Gibbs 1979)), discussed and summarised in (Cavalier-Smith 2018) as well as the results from a newer study (Flori *et al.* 2016) prompt again speculations of an additional compartment within the PPC, the “periplastid reticulum” (PR) (see Fig. 1d). The PR is surrounded by one membrane and one of its function might be delivery of proteins into the PPC for further sorting (Cavalier-Smith 2018). However, it has still to be shown that the PR is an independent compartment within in the PPC and not an invagination of the PPM (Zauner *et al.* 2019). The diatom PPC lacks a nucleomorph, a nucleus relic with only a small coding capacity, found in cryptophytes and chlorarachniophytes (Zauner *et al.* 2019). Therefore, in diatoms nearly all of the proteins of complex plastids are nucleus-encoded and have to be imported from the cytoplasm. Nucleus-encoded proteins delivered to the PPC and the PPM as well as to the stroma are expressed as preproteins with a characteristic targeting signal at the N-terminus (Apt *et al.* 2002; Gruber *et al.* 2007; Lang *et al.* 1998). This signal is composed of a signal peptide for co-translational ER import followed by a transit peptide-like sequence, together called bipartite targeting sequence (BTS). A BTS is sufficient to import a protein into the complex plastid, as shown for example by GFP targeting experiments (Apt *et al.* 2002). Furthermore, the first amino acid of the transit-peptide like sequence is indicative for the final location of the imported protein. The presence of an aromatic amino acid or a leucine at this position is characteristic for stroma proteins, whereas PPC located proteins show one of the remaining amino acids at the N-terminus of the transit peptide-like sequence (Gruber *et al.* 2007; Killian and Kroth 2005). These targeting sequences are expected to interact with translocons, for which one has to be present in each of the plastid surrounding membranes. The outermost plastid membrane is studded with 80S ribosomes and proteins imported into the complex plastid traverse this membrane via co-translational, Sec61-dependent transport into the lumen between membrane I and the PPM. It is supposed that the signal peptide of the BTS is cleaved off in this lumen, thereby exposing the transit peptide-like sequence for direct or indirect interaction with a translocator in the PPM. In the PPM, an ERAD (endoplasmic reticulum-associated protein degradation)-like machinery was detected (Sommer *et al.* 2007), and named symbiont-specific ERAD-like machinery (SELMA)

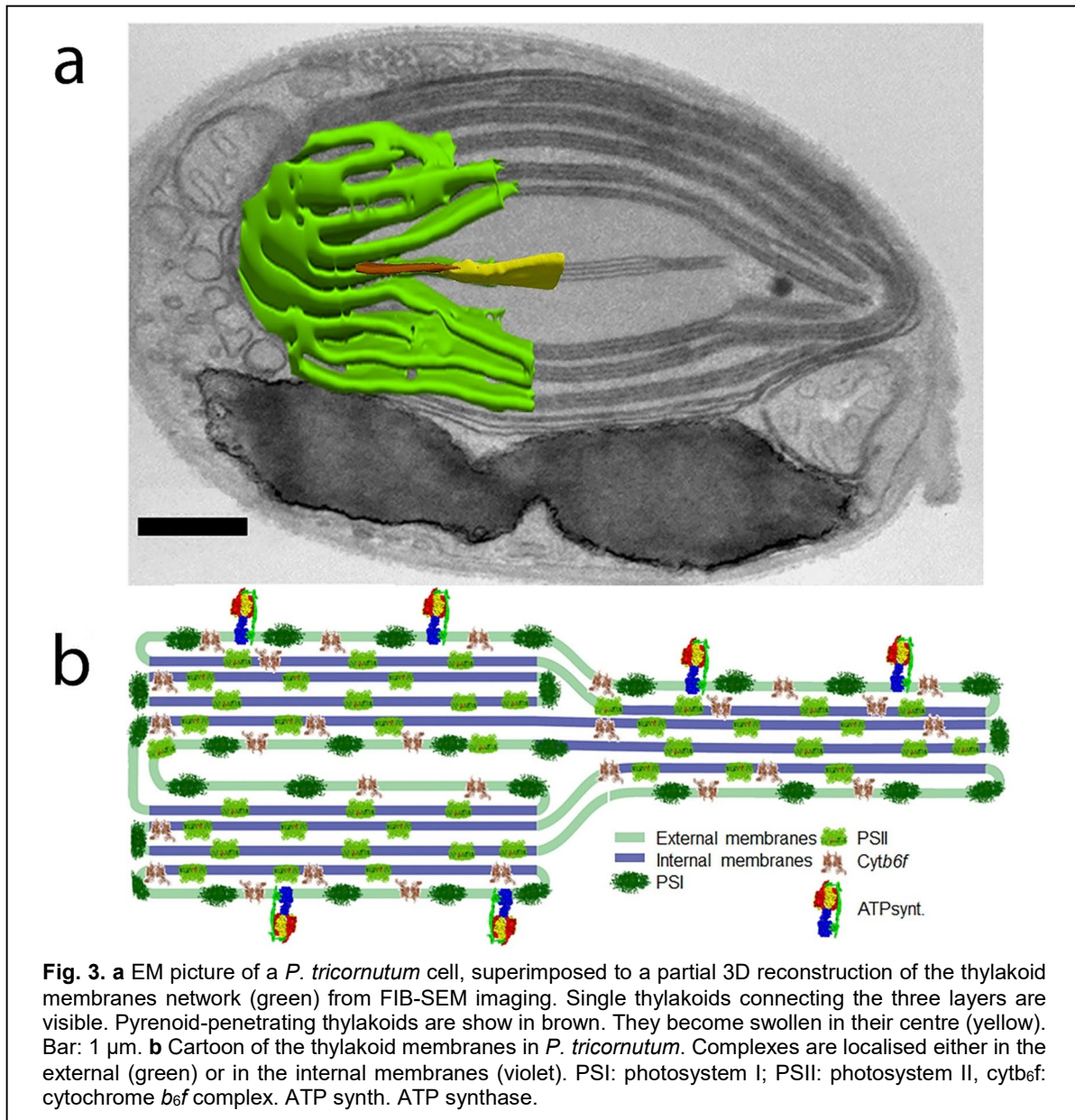
(Hempel *et al.* 2010; Stork *et al.* 2013). This complex still is the only known putative protein translocation machinery in the PPM of diatoms. Several components of the SELMA machinery were identified, which are at least functionally homologous to ERAD factors (Lau *et al.* 2016; Stork *et al.* 2013; Stork *et al.* 2012). These include as the membrane components homologous to the ERAD components Der1 (sDer1-1, 1-2), an E3 ligase, sUbx, a rhomboid protease, and the soluble proteins sCdc48-1/2, sUfd1 and sNpl4 in the PPC. According to a current model, proteins pass the PPM by the help of the SELMA components. During its import into the PPM, the N-terminal region of the protein is ubiquitinated, which is essential for the final transport into the PPC via Cdc48, Ufd1 and Npl4. After completion, ubiquitin can be eliminated from the transported proteins by the help of a de-ubiquitinase (Hempel *et al.* 2010).

An alternative import model has been proposed by (Cavalier-Smith 2018), suggesting that protein transport into the PPC starts via vesicles generated at the PPM, which deliver the proteins to the PR, from which they are transported into the PPC via a SELMA complex.

Non-PPC proteins have to be further targeted either into membrane III or IV, into the stroma or the thylakoids. For passing through membrane III, an Omp85 protein was identified, which is homologous to Toc75, the general import pore in the outer plastid membrane of land plants (Bullmann *et al.* 2010). Additionally, Tic components are encoded in the genome of diatoms (e.g. (Gruber *et al.* 2007)) indicating that a TIC complex should be present in membrane IV to enable protein import into the stroma. Proteins with a final destination in the thylakoid lumen have to pass an additional membrane. Proteins of this type might use an equivalent to the Δ pH-dependent protein transport system or a Sec-dependent pathway, both known from land plants (see (Grouneva *et al.* 2011)). In case of thylakoid membrane proteins, FCP proteins were characterised which require stromal factors as well as GTP for thylakoid membrane integration (Lang and Kroth 2001).

Plastid 3D organisation. As in other microalgae, the diatom plastid is organised in such a way that the electron transport reactions and the CO₂-fixing reactions (Calvin cycle) of photosynthesis are segregated in two distinct compartments: the thylakoids and the pyrenoid, respectively.

The thylakoids host the proteins performing the light-phase (electron transport reactions) of photosynthesis. In plants, these membranes form a continuous compartment (Mustárdy and Garab 2003; Staehelin 2003) characterised by a clear lateral heterogeneity between grana (stacks of thylakoids) and their connecting membranes, the stroma lamellae. No such clear distinction between grana and stroma lamellae is seen in diatoms (Fig. 3a), where membranes are generally organised in stacks of three layers, the 'girdle lamellae', which run parallel to the envelope membranes. Every thylakoid is bounded by a lipid bilayer enclosing an aqueous lumen. Thylakoids are densely populated in proteins, which occupy up to 70% of the total membrane area (Kirchhoff *et al.* 2002). In plants, extensive biochemical, fractionation and immunolocalisation studies have shown that the photosystem I (PSI) and the ATP-synthase (ATPase) accumulate preferentially in the stroma exposed membrane (the stroma lamellae and the grana margins) due to steric repulsion between their bulky stromal protrusions (Amunts and Nelson 2008; Dekker and Boekema 2005; Junge *et al.* 2009). Conversely, the photosystem II (PSII), which has flat stromal surface, is mostly localised in the grana. The cytochrome *b₆f* complex (Cyt *b₆f*), which functionally connects PSI and PSII, has a more homogeneous localisation in these compartments (reviewed in (Albertsson 2001; Kouřil *et al.* 2012)). Most likely, the main reason for segregating the photosystems in two different domains of the membranes is to prevent a physical contact between them, which would otherwise lead to excitation energy transfer (spillover) from PSII to PSI. Energy spillover may result in unbalanced energy distribution between the photosystems, as reported for cyanobacteria and red algae under particular growth conditions (Biggins and Bruce 1989).



Immunolocalisation experiments in diatoms have shown a topological segregation of PSI and PSII in different thylakoid domains (as in plants) despite the lack of apparent differentiation between the grana and stroma lamellae (Flori *et al.* 2017). PSI is found mostly within the peripheral membranes of the girdle, while PSII is located within the innermost thylakoid membranes (Fig. 3b). This segregation could be driven by the same bulkiness rules as in plants with ‘protruding’ PSI molecules being located in non-appressed peripheral membranes and ‘flat’ PSII being found in the core membranes. Alternatively, the proposed heterogeneous lipid composition of the two thylakoid

fractions could drive this segregation, if the core membranes are enriched in lipids that favour PSII stability and function (Lepetit *et al.* 2012).

3D reconstruction of the plastids has revealed that single thylakoids often bridge the contiguous girdle lamella (Fig. 3a), possibly enabling electron shuttling between photosynthetic complexes of different stacks. Moreover, it was found that the diameter of the thylakoid lumen (7.1 ± 1.5 nm, unpublished data) is two times larger than the size of the soluble electron carrier (cyt $c_6 \sim 3.3 \times 2.3$ nm, (Akazaki *et al.* 2009)). This feature should allow for largely unrestricted diffusion of this hydrophilic redox carrier inside the thylakoid membranes, thus enabling electron flow. Overall, the thylakoids topology of diatoms seems to be conceived to optimise their photosynthesis by avoiding unbalanced energy transfer to the photosystems and enhancing photosynthetic electron transfer. The stroma is the compartment where carbon fixation takes place. In most algae, the enzymes required for carbon fixation (i.e. RuBisCO) are located in a specialised region named the pyrenoid (Borkhsenius *et al.* 1998; Holdsworth 1971; Lacoste-Royal and Gibbs 1987). Different diatoms show different numbers, plastidic locations, and structures of the pyrenoids (Bedoshvili *et al.* 2009). In *P. tricornutum* a single pyrenoid is visible, which occupies the centre of the plastid and is crossed by special thylakoids called 'pyrenoid-penetrating thylakoids' (Kikutani *et al.* 2016), (Fig. 3a, brown) swelling in the centre of the Rubisco rich compartment (Fig. 3a, yellow).

Besides revealing the topology of photosynthetic membranes, the 3D reconstruction of a *P. tricornutum* cell has also revealed interesting aspects of organelles (Fig. 1b). The chloroplast and nucleus appear to have physical contacts at specific locations (Fig. 1c) (Flori *et al.* 2017). This is understandable, because the outermost membrane of the plastid envelope forms a continuum with the outer nuclear membrane in these organisms. Moreover, the inner nuclear membrane and the periplastid membrane (PPM), but also the outer and inner envelope membrane (oEM and iEM) are facing each other (Flori *et al.* 2016). It has been proposed (Flori *et al.* 2017) that these contacts between the chloroplast and nucleus could facilitate exchanges (e.g. of signalling molecules) between the two organelles. The plastid-nucleus contacts of diatoms are somehow reminiscent of chloroplast evaginations in plants (stromules) that transiently connect to the nucleus for metabolite exchange and retrograde signalling (Dietz *et al.* 2016). This process, largely studied in plants, has recently

received some experimental support also in *P. tricornutum* (Lepetit *et al.* 2013). Signalling between the two compartments could be mediated by the presence of a vesicular network (VN) that is seen close to the nucleus and in connection with both the oEM and the PPM. The proximity between the nucleus and the plastid, and the vesicular system resembling the one seen in *P. tricornutum* (see also (Tanaka *et al.* 2015)) have been reported in other diatom species (Bedoshvili and Likhoshway 2012; Bedoshvili *et al.* 2009), suggesting that nucleus-plastid interactions may be conserved in both pennate and centric diatoms.

Plastid-mitochondria contacts are also seen in *P. tricornutum*. Mitochondria-plastid juxtaposition results in numerous physical contacts between the two organelles, which are visible in chemically fixed samples (Bailleul *et al.* 2015). These interactions could facilitate energy exchange to optimise CO₂ assimilation. This might be achieved by establishing an ATP flux commensurate to CO₂ assimilation in the light (Bailleul *et al.* 2015; Kim *et al.* 2016). This process likely requires the presence of transporters facilitating the energetic interactions possible between the two organelles. Because diatom plastids are surrounded by four membranes rather than two as in plants, the molecular nature of these transporters could be different from those of plants, reflecting the different phylogenetic origin of these organisms and their complex evolutionary history.

Endosymbiotic Diatoms. Although most diatoms are free-living individuals, there are a few examples of endosymbiotic diatoms, e.g. in dinoflagellates (Chesnick *et al.* 1997), in foraminifera (Bernhard and Bowser 1999; Lee *et al.* 1980; Lee *et al.* 1989), and in flatworms (Van Steenkiste *et al.* 2019). Heterotrophic dinoflagellates of different groups may take up the cytoplasm of algal cells, including diatoms, and store them in a single membrane vacuole, the symbiosome (Tomas *et al.* 1973). Such a kleptoplasty process may either involve selectively the ingested plastids only, or in other cases can also include some of the other ingested prey organelles, especially mitochondria and nuclei (Johnson *et al.* 2007). Most of these associations are temporary, but in a specific group of dinoflagellates, the dinotoms, some endosymbiotic diatoms have evolved into permanent stages (Tippit and Pickett-Heaps 1976; Yamada *et al.* 2019). Dinotoms are widely distributed in both freshwater and marine environments. They may possess at least two types of plastids of different origins (Dodge 1971; Hehenberger *et al.* 2014). Furthermore, there are different stages of integration of the diatom endosymbionts into

the host cells, ranging from permanent endosymbionts to temporary endosymbionts as kleptoplastids (Yamada *et al.* 2019). In the latter case, diatoms have to be taken up by the dinoflagellate regularly to keep the dinoflagellate alive. Interestingly, there is a high specificity regarding the diatom species that can be taken up and utilised as photosynthetic endosymbiont by the host cell. A comparison of two closely related diatoms, *Durinskia capensis* and *D. kwazulunatalensis*, recently revealed two different evolutionary stages. While *D. capensis* is a kleptoplastic protist, keeping its diatoms temporarily for up to 2 months, *D. kwazulunatalensis* is able to keep several diatoms species permanently (Yamada *et al.* 2019).

Conclusions. Diatoms have been studied for centuries, mostly regarding their taxonomy. Only in the past 25 years, their molecular cell biology and genetics came into focus, revealing a number of unexpected findings. Modern tools in cell biology now allow a better understanding of peculiar processes and structures, like the formation of a silica cell wall, the complex cellular structures like the chloroplast ER, and their unusual photophysiology. Because of the recent progress in developing molecular tools, like for instance genome editing and genetic transformation via bacterial conjugation, diatoms will have an increasing importance as model organisms to study specific functions of organelles in stramenopile algae.

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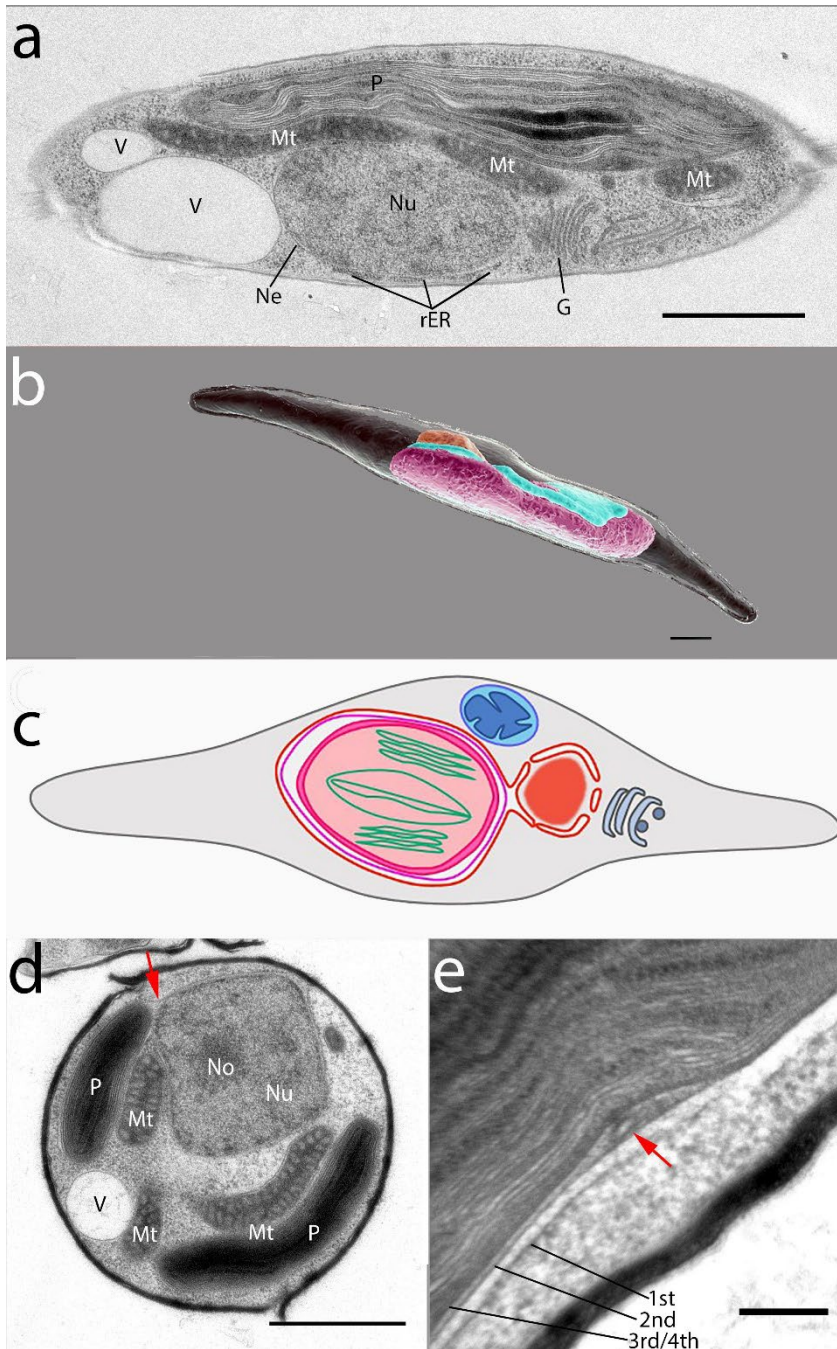


Fig. 1 (a) Ultrastructure of the diatom *P. tricornutum* (after high-pressure freezing, freeze substitution, and Epon embedding) as seen on 50 nm ultrathin sections by Transmission Electron Microscopy (TEM). P, Plastid; Mt Mitochondrion; Nu, Nucleus; V, Vacuoles; G, Golgi apparatus; rER, rough Endoplasmic Reticulum. Bar: 1 μ m. (b) 3D topology of a *P. tricornutum* cell, obtained with Focused Ion Beam-Scanning Electron Microscopy (FIB-SEM). The compact organisation of the main organelles (nucleus: orange; plastid: magenta; and mitochondria: cyan) is shown. Note that one single mitochondrion per cell is winding along the plastid. Bar: 1 μ m. (c) Schematic organisation of a fusiform *P. tricornutum* cell. The plastid is limited by four membranes, the outermost of them being continuous with the outer nuclear envelope membrane. Nucleus: orange; plastid: magenta; mitochondria: cyan. (d) Ultrathin section of *P. tricornutum* highlighting that the nuclear envelope and the outermost membrane surrounding the complex plastid are a continuous membrane (red arrow); P, Plastid; Mt, Mitochondrion; V, Vacuole; Nu, Nucleus; No, Nucleolus; bar 1 μ m. (e) Detail of the *P. tricornutum* plastid showing its membranes and a putative membranous structure (red arrow) inside the periplastid compartment. Bar: 200 nm

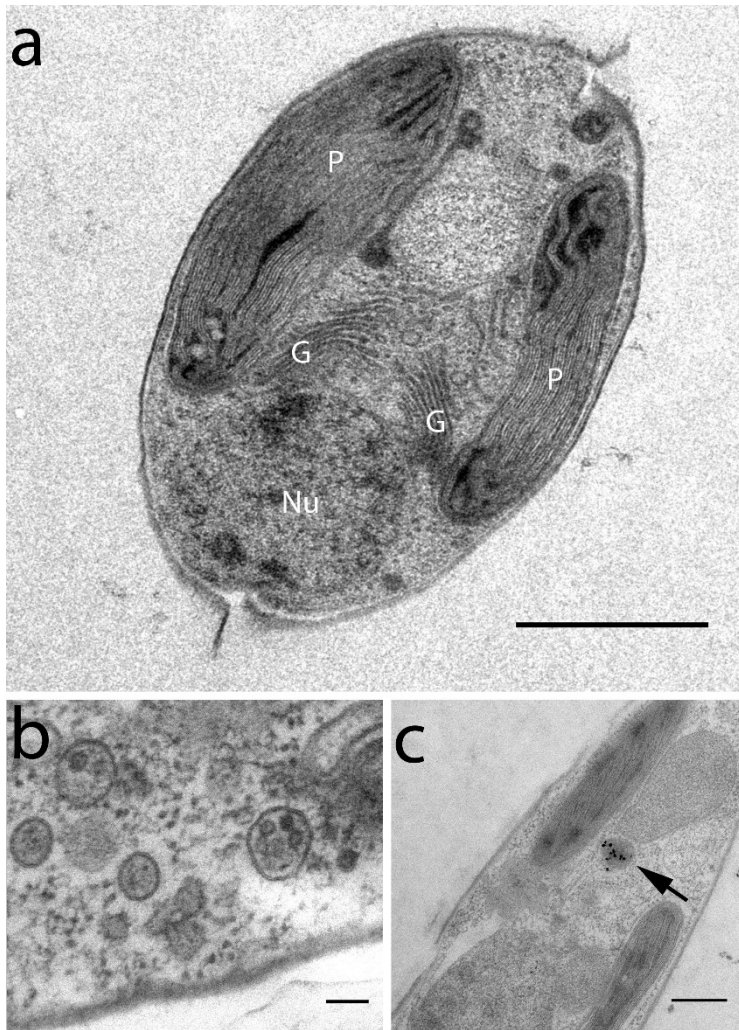


Fig. 2 (a) Ultrastructure of *P. tricornutum* (after high pressure freezing, freeze substitution, and Epon embedding) as seen on 50 nm ultrathin sections by transmission electron microscopy, showing that (at least) two Golgi stacks per cell can occur; P, Plastid; Nu, Nucleus; G, Golgi apparatus; bar 1 μm . (b) Detail of putative multivesicular bodies (MVBs). Bar: 100 nm. (c) Immunogold labeling of (heterologously) overexpressed GFP-tagged Pex16 protein, showing peroxisomal localisation (arrow) in *P. tricornutum* (primary antibody α -GFP 1: 1000; secondary antibody (rabbit anti goat) coupled to ultrasmall gold 1:100; silver enhancement 15 min). Bar: 500 nm

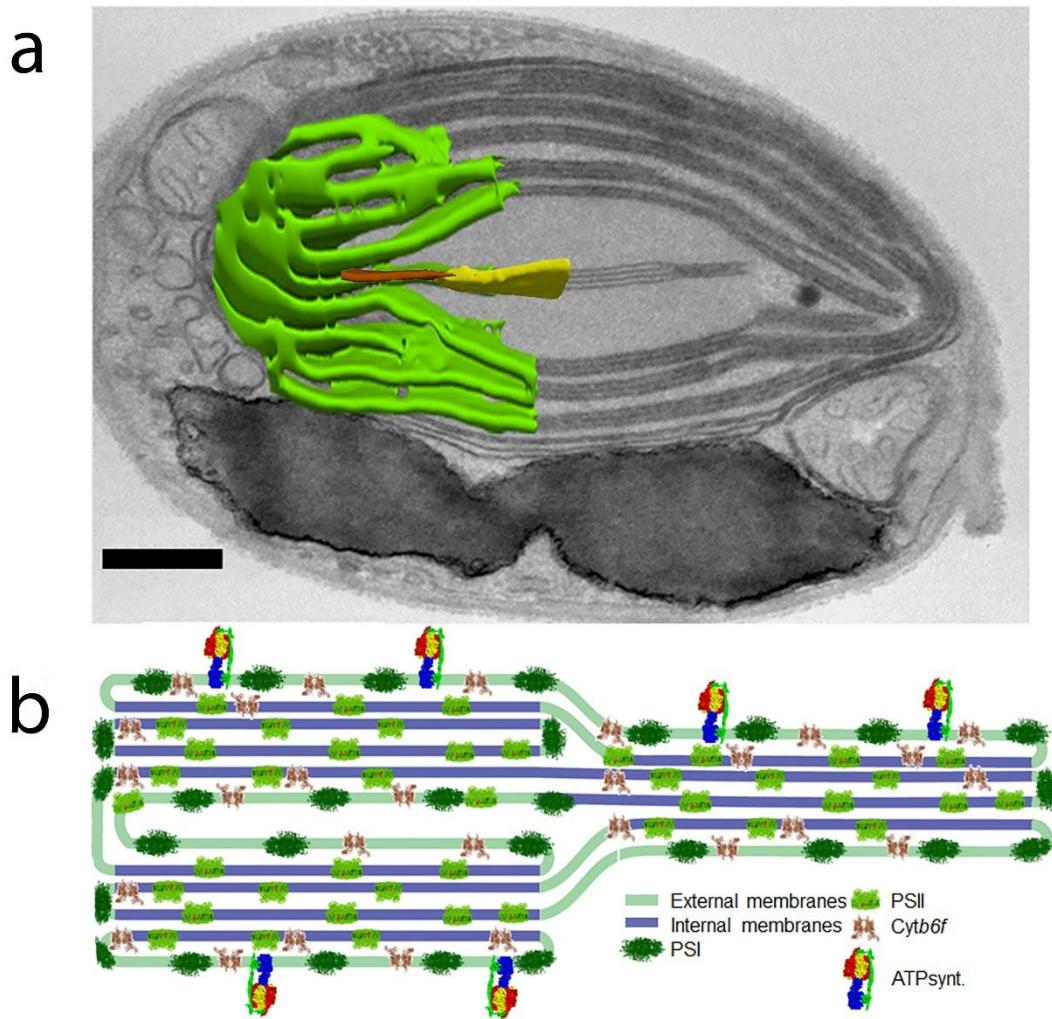


Fig. 3 (a) EM picture of a *P. tricornutum* cell, superimposed to a partial 3D reconstruction of the thylakoid membranes network (green) from FIB-SEM imaging. Single thylakoids connecting the three layers are visible. Pyrenoid-penetrating thylakoids are shown in brown. They become swollen in their centre (yellow). Bar: 1 μm . (b) Cartoon of the thylakoid membranes in *P. tricornutum*. Complexes are localised either in the external (green) or in the internal membranes (violet). PSI: photosystem I; PSII: photosystem II, cytb6f: cytochrome b6f complex. ATP synth. ATP synthase