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Euglenoid pellicle morphogenesis and evolution in light of comparative ultrastructure and trypanosomatid biology: Semi-conservative microtubule/strip duplication, strip shaping and transformation

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Abstract

Uniquely in eukaryotes, euglenoid pellicles comprise longitudinal proteinaceous, epiplasmic strips underlain by microtubules. Contradictory interpretations of pellicle microtubule duplication and segregation assumed opposite microtubule polarity from kinetoplastid Euglenozoa and conservative microtubule segregation. *Distigma* shows new pellicle microtubules nucleating posteriorly as in trypanosomatids, unifying euglenoid and kinetoplastid pellicle morphogenesis, but strip-growth is unpolarised. Epiplasmic insertion and cutting make new strip junctions between alternating wide and narrow daughter strips that grow intussusceptively. Nanotubules, overlooked epiplasm-associated components, define strip edges. At strip heel/toe junctions all euglenoids have a morphogenetic centre microtubule mt2/3 pair. Arguably, proteolysis, epiplasmic growth, and toe-nanotubule-associated epiplasmic scission initiate daughter strips, separating old mts2/3; new mt2/3/bridge-B assembly, sub-heel scission, nanotubule-bridge-A assembly complete duplication. Only mt2/3 pair fully enters the canal, one master microtubule also the reservoir, other pellicle microtubules terminating near canal rims. A related cytokinesis model involving ciliary attachment zone duplication explains near-universally even spirocute strip number. I consider *Serpenomonas* and *Entosiphon* alternating heteromorphic strips developmental stages of 'strip transformation'; explain intergroup diversity of strip morphology and dorsoventral strip differentiation causally by specific pellicle-complex components; propose centrin-based mechanisms for strip shaping and euglenoid movement; unify pellicle cytokinetic microtubule segregation across Euglenozoa; and discuss origin and diversification of pellicle complexes.

Keywords: Euglenoid strip junctions; *Serpenomonas*; Postgaardea homologies; Trypanosomatid-euglenoid pellicle unity; Microtubule polarity; Epiplasmic strip transformation

Abbreviations: AA, articulin anchor; CAZ, ciliary attachment zone; CL, contractile lattice of ciliates; BB, bypassing mt band of chromists; DB, dorsal band of microtubules in reservoir; DF, dorsal fan of mts; DR, dorsal root; EFZ, epiplasm-free zone; FA, feeding apparatus of Euglenozoa; IR, intermediate root; JZ, junction zone between euglenoid pellicle strips; LS, longitudinal section; MAB, microtubule-associated bridge; MDM, microgroove-defining material; MIB, microtubule-independent bridge; MC, morphogenetic centre; mt, microtubule; MTR, 'reinforced microtubules' of euglenozoan cytopharnyx; nt, nanotubule; PC, pellicle complex of euglenoids; PM, plasma membrane; R1, centriolar mt root R1; R2, centriolar mt root R2; R3, centriolar mt root R3; R4, centriolar mt root R4; TF, traversing fibre; TS, transverse section; VR, ventral root.

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Introduction

Protozoan phylum Euglenozoa was established to embrace euglenoid and kinetoplastid flagellates (Cavalier-Smith 1981). Later expansion added Diplonemea and Postgaardea (Cavalier-Smith 1993, 2003) also ancestrally with two cilia with unique dissimilar latticed paraxonemal rods (Cavalier-Smith 1981; Simpson 1997) extending from parallel centrioles at the base of a deep ciliary pocket. Euglenozoa have three distinctive microtubular centriolar roots attached to a microtubule-rich pellicle (Cavalier-Smith 2013) differently from other eukaryotes, and a unique cemented feeding apparatus (FA) and cytopharnyx radically different from those of all other protists (Belhadri and Brugerolle 1992; Breglia et al. 2010; Burzell 1973, 1975; Elbrächter et al. 1996; Frolov and Karpov 1995; Mignot 1963, 1966; Triemer and Farmer 1991a,b; Simpson 1997). Many also have unique cylindrical extrusomes (Brugerolle 1985). Their latest classification (Cavalier-Smith 2016) has three subphyla: Glycomonada (classes Diplonemea, Kinetoplastea) with peroxisomes modified to glycosomes and mitochondrial genomes of multiple heterogeneous circles; anaerobic/microaerophilic Postgaardia (class Postgaardea only) with bacterial epibionts and highly aberrant FA; and Euglenoida characterised by unique longitudinal proteinaceous pellicular strips (five classes, each with distinctive FA and pellicle).

Euglenoid pellicle strips consist of an even-thickness epiplasmic layer of euglenoid-specific articulins, two or three related hetero-oligomeric hydrophobic β-sheet phosphoproteins (Bricheux and Brugerolle 1986, 1987; Dubreuil and Bouck 1985; Marrs and Bouck 1992), and a junction zone (JZ) involving cross bridges between their overlapping edges (Dubreuil and Bouck 1988). Strips are underlain by characteristic arrays of microtubules (mts) (Leedale 1967; Sommer 1965) some of which continue into and partially line the ciliary pocket [in the reservoir forming the dorsal row (Mignot et al. 1987) or dorsal band (DB) (Willey and Wibel 1985)] and are distinct from centriolar dorsal root (DR) mts (Shin et al. 2002; Shin and Boo 2001; Solomon et al. 1987; Surek and Melkonian 1986). More primitive euglenoids (class Entosiphonea and superclass Rigimonada, comprising classes Stavomonadea and Ploeotarea: Cavalier-Smith 2016) have rigid pellicles with 12 or fewer strips. More advanced euglenoids (i.e. clade and superclass Spirocuta, comprising heterotrophic Peranemea and ancestrally photosynthetic Euglenophyceae: Cavalier-Smith 2016) have more numerous strips (14–80) typically differentiated into longitudinal ridges and grooves with mts in fixed positions (Fig. 1), many species exhibiting squirming 'euglenoid movement', sometimes called 'metaboly', absent in *Entosiphon* and rigimonads. Euglenoid movement (Arroyo et al. 2012) of these plastic pellicles may involve mutual sliding of strips held together laterally by interlocking complementary hooked edges (Leedale 1967; Suzaki and Williamson 1985, 1986a,b) or changes in width/shape of strips (Angeler et al. 1999), being driven by an unknown calcium-dependent motor that is neither dynein nor myosin (Murray 1981). Squashing *Euglena* separates its strips (Leedale 1963, 1966); each appears as a narrow lamina with a thickened edge, the heel (Mignot et al. 1987), which forms the pellicular groove into which fits the hooked thinner edge of the lamina of the adjacent strip, now called the toe (Cavalier-Smith 2016). Some species, notably petalomonads like *Scytomonas* and some *Distigma*, have unhooked strips with no ridge/groove structure whose JZ organisation is less clear (Cavalier-Smith et al. 2016a; Kim et al. 2010). As I shall show, both strip patterns share an underlying unity: Fig. 1A, M.

Strip number varies greatly among species but is generally conserved within them, being lower in lineages branching early on sequence trees (Cavalier-Smith 2016); conservation depends on morphogenetic intercalation of new strips between old ones once every cell cycle prior to cell division (Pochman 1953; Leedale 1967). Strip morphogenesis is complex, carefully studied ultrastructurally only in one of the 12 euglenoid orders now recognised (Cavalier-Smith 2016) — Euglenida. Hofmann and Bouck (1976) studied *Euglena gracilis* with contractile pellicle, whereas Mignot et al. (1987) used the phylogenetically distant non-contractile osmotroph *Cyclidiopsis acus*, now called *Lepocinclis cyclidiopsis* (Bennett and Triemer 2014). Despite different views (arguably both incorrect) on detailed mt segregation pattern, both studies unambiguously showed standard euglenoid strip development to be spread across two cell cycles (Hofmann and Bouck 1976; Mignot et al. 1987), analogously to ciliary and centriolar transformation over two cell cycles (or more: Nohýnková et al. 2006) that evolved in the ancestral eukaryote that was biciliate like ancestral Euglenozoa (Cavalier-Smith 2014; the uniciliate *Scytomonas* is derived — Cavalier-Smith et al. 2016a). These contradictions were overlooked in previous discussions of euglenoid pellicle evolution (e.g. Leander and Farmer 2000, 2001; Leander et al. 2007) that focused largely on Spirocuta not early diverging euglenoids. This paper resolves them by critically reevaluating euglenoid pellicle morphogenesis and evolution, and pellicle homologies across Euglenozoa, and proposing a new synthesis more harmonious with trypanosomatid cell biology.

Euglenozoan pellicle morphogenesis is best understood in kinetoplastid trypanosomes, whose cytoskeleton is highly simplified compared with other Euglenozoa through parasitism and associated loss of one cilium and most centriolar roots, because their medical importance provides far more funding for their molecular cell biology. Their small size has facilitated complete ultra-high-resolution tomographic reconstruction of every mt in the ciliary pocket area (Lacomble et al. 2009), including importantly their polarity and roles in cell morphogenesis prior to and during division (Lacomble et al. 2010; Alcantara et al. 2014). A key finding is that in *Trypanosoma* the growing plus end of pellicle mts is at the ciliary pocket end of the cell; pellicle mts grow towards not away from centrioles, and have opposite polarity