# TPPP-like Proteins in Myzozoa

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TPPP-like proteins contain one or more p25alpha domains. They obtained their name after tubulin polymerization promoting protein (TPPP1), the first identified member of this protein family. Originally, it was named p25alpha protein, which became the eponym of the domain. Myzozoans are a monophyletic clade, and a sister clade to the Ciliata, within Alveolata.

apicortin     TPPP     Myzozoa     chrompodellids     dinoflagellates

## 1. Introduction

TPPP-like proteins contain one or more p25alpha domains [1]. They obtained their name after tubulin polymerization promoting protein (TPPP1), the first identified member of this protein family [2][3]. Originally, it was named p25alpha protein, which became the eponym of the domain [4]. P25alpha (Pfam05517; IPR008907) is not a structural domain but is derived from a sequence alignment (https://bioinf.umbc.edu/DMDM/generatelogo.php?accession=pfam05517 (accessed on 5 March 2023)). The p25alpha domain is exclusive to eukaryotes and there is a strong correlation between its presence and the presence of the eukaryotic flagellum/cilium [5][6]. TPPP-like proteins occur in different types, such as long-, short- and truncated (the C-terminal third is completely missing) TPPPs, depending on the length of the p25alpha domain (about 160, 140, and 120 amino acids, respectively) (**Figure 1**). The main difference between long- and short-type TPPPs is that the C-terminal end of short-type TPPPs is incomplete; long-type TPPPs but not short-type ones contain a very conservative sequence of 31–32 amino acids, the most typical part of which is the GXGXGXXGR 'Rossmann-like' motif (**Figure 1**). Some TPPP-like proteins also possess another domain/region such as EF-hand (CDD:428504) or doublecortin (DCX; Pfam 03607, IPR003533) in addition to the p25alpha [1]. The latter one is named apicortin, which unifies partial p25alpha and DCX domains [7] (**Figure 1**).
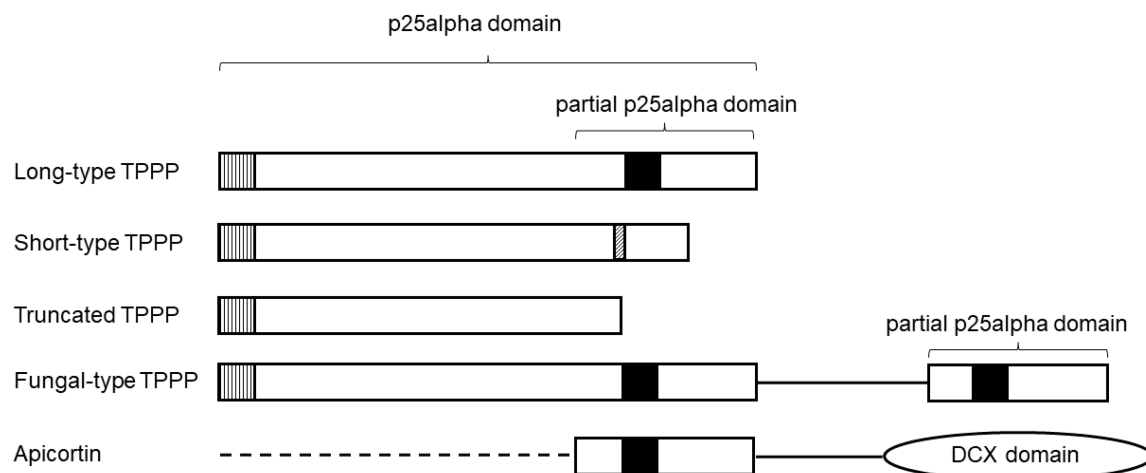
**Figure 1.** Schematic structure of some TPPP-like proteins. Highly conservative sequence motives are denoted with black boxes (GxGxGxxGR), vertical-striped boxes (L(V)xxxF(Y)xxF), and diagonal-striped boxes (GGP). The dashed line represents a disordered region unique to some apicortins.

The first TPPP-like protein, TPPP/p25 or TPPP1, was identified in mammalian brain [2][3][4] and its physiological significance is connected to the nervous system [8][9] as well as having a role in neurodegenerative disorders, as Parkinson's disease and multiple system atrophy [10][11][12]. Obviously, the role of TPPP-like proteins will be different in eukaryotic microbes without a nervous system. However, their interaction with tubulin and the microtubular system is a conserved property [13][14][15].

The various members of the TPPP-like protein family are characteristic for the phylogenomic supergroups [1]. For example, animals contain only long-type TPPPs, except for the placozoan *Trichoplax adhaerens*, which contains apicortin instead [7]. Truncated- and fungal-type TPPPs occur only in Endopterygota (Holometabola) [16] and in fungi [6], respectively. In this research, the occurrence, and the possible role of TPPP-like proteins in Myzozoa, which include apicomplexans and related taxa, chrompodellids (chromerids plus colpodellids), dinoflagellates, and perkinsids, is reviewed.

# 2. TPPP-like Proteins in Myzozoa

## 2.1. Apicortin

Apicortins unite two conserved domains, a DCX motif and a partial p25alpha sequence, that are separately found in other proteins, in doublecortins and TPPPs, respectively [7] (**Figure 1**). The DCX domain is named after the brain-specific X-linked gene doublecortin [17]. Both the p25alpha and the DCX domains play an important role in the stabilization of microtubules [2][3][17][18]; thus, a similar role was suggested for apicortin [7]. Apicortin was originally thought to occur only in apicomplexans and in the placozoan animal *T. adhaerens* [7]. Later, it was found that its occurrence is broader than thought earlier: it is present in chromerids (*Chromera velia* and *Vitrella brassicaformis*) [19] and in flagellated fungi [6][20][21]. The presence of apicortin in chromerids was not surprising, given the phylogenetic proximity and the structural similarity of chromerids and apicomplexans.

BLASTP analyses [22] were performed on myzozoan protein and nucleotide sequences available at the NCBI webpage. Since apicortins contain two different domains, various domain databases were also checked for proteins with both the DCX and the partial p25alpha domains that BLAST may not have been able to detect. The results of the search, i.e., the new apicortins not known before, are listed in **Table 1**. They were found in all myzozoan phyla, in Apicomplexa, chrompodellids, dinoflagellates, and Perkinsozoa. The newly re-interpreted [23][24] squirmids [25] (*Digyalum oweni* [26]) also possess it (the list of apicortins identified earlier is published in the references [6][7][20]).

Table 1. Newly identified apicortins.

| Species | Accesion Number (TSA) | Class | Order | Identity with *T. adhaerens* Apicortin [3], % |
|---|---|---|---|---|
| **Apicomplexans** | | | | |
| *Haemoproteus columbae* | GGWD01002623 | Aconoidasida | Haemosporida | 41.21 |
| *Cardiosporidium cionae* | KAF8822549 [1] | Aconoidasida | Nephromycida | 36.67 |
| *Nephromyces* sp. ex *Molgula occidentalis* | GHIL01104982 | Aconoidasida | Nephromycida | 40.35 |
| *Eleutheroschizon duboscqi* | GHVT01063535 | Conoidasida | Protococcidiorida | 40.57 |
| *Ancora sagittata* | GHVO01023203 [2] | Conoidasida | Eugregarinorida | 36.25 |
| *Cephaloidophora* cf. *communis* | GHVH01004777 | Conoidasida | Eugregarinorida | 40.00 |
| *Lankesteria abbotti* | HBHB01002866 [2] | Conoidasida | Eugregarinorida | 33.82 |
| *Porospora* cf. *gigantea* | KAH0481109 [1] | Conoidasida | Eugregarinorida | 39.52 |
| *Siedleckia nematoides* | GHVV01274235 [2] | Conoidasida | Eugregarinorida (Blastogregarinorida) | 51.35 |
| *Selenidium pygospionis* | GHVN01000425 [2] | Conoidasida | Archigregarinorida | 35.66 |
| *Rhytidocystis* sp. ex *Travisia forbesii* | GHVS01047420 [2] GHVS01057697 [2] | Marosporida | Agamococcidiorida | 37.65 37.11 |
| **Chrompodellids** | | | | |
| *Alphamonas edax* | GDKI01002741 | | | 36.26 |
| *Colpodella angusta* | GDKK01042800 | | | 40.88 |

| Species | Accesion Number (TSA) | Class | Order | Identity with *T. adhaerens* Apicortin [3], % |
|---|---|---|---|---|
| **Squirmids.** | | | | |
| *Digyalum oweni* | GHRU01063100 | | | 48.52 |
| **Dinoflagellates** | | | | |
| *Karenia papilionacea* | GJRB01069842 [2]<br>GFLM01035203 [2] | Dinophyceae | Gymnodiniales | 21.86<br>24.40 |
| *Karlodinium armiger* | GJRA01036780<br>GJQZ01074857 | Dinophyceae | Gymnodiniales | 30.17<br>29.48 |
| *Oxyrrhis marina* | HBQX01034077<br>HBIT01006257 | Dinophyceae | Oxyrrhinales | 33.33<br>33.33 |
| *Symbiodinium* sp. clade D | HBTB01100466 | Dinophyceae | Suessiales | 38.31 |
| **Perkinsids** | | | | |
| *Perkinsus chesapeaki* | KAF4672084 [1] | | Perkinsida | 23.90 |
| *Perkinsus olseni* | KAF4750811 [1] | | Perkinsida | 23.57 |

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*Polyrhabdina* sp., *Siedleckia nematoides*, *Selenidium pygospionis* [28], and *Porospora gigantea* [29], as well as coccidiomorphs as *Cardiosporidium cionae* [30], *Eleutheroschizon duboscqi*, *Rhytidocystis* sp. [28], and *Nephromyces* ex *Molgula occidentalis* [31]. All species listed here contain apicortin.

Protein. [2] Incomplete sequence. [3] XP_002111209.

The photosynthetic chromerids, *C. velia* and *V. brassicaformis*, belong to a monophyletic group, called the chrompodellids, with heterotrophic colpodellids, namely, *Alphamonas edax*, *Voromonas pontica*, and *Colpodella angusta* [32][33]. It is clear that although apicortin has not been known in them until now, it was worth examining them in this regard (cf. **Table 1**). There are another two myzozoan phyla, Dinoflagellata and Perkinsozoa, which are sisters to each other, and together, are sisters to Apicomplexa and chrompodellids. So far, apicortin has not been detected in dinoflagellates, while there were traces of its presence in perkinsids [34][35]. For evolutionary reasons, it is obvious that if, on the one hand, apicomplexans and chromerids contain apicortins, and on the other hand, their presence in perkinsids can be counted on, then their occurrence in dinoflagellates is not surprising (**Table 1**).

A few sequences are incomplete; this may be due to the fact that in the case of some species, a significant percentage of the genome/transcriptome data are missing, e.g., 48% of *Lankesteria abbotti* data are missing [28], and the N-terminus of the HBHB01002866 (**Table 1**) is not present in the available TSA sequence. The missing transcriptome data may also explain that while apicortin was identifiable in *C. angusta*, it was not found in the closely related (same family) *V. pontica*, in which 22% of the data are missing [28].

## 2.2. Short-Type TPPP

Short-type TPPPs have not been previously systematically investigated. They occur in various protists, mostly in Ciliata, Euglenozoa, and Chlorophyta [1], and they are present in Apicomlexa and Perkinsozoa [7]. Based on BLAST search, new short-type TPPPs were found in many myzozoans, not only in Apicomplexa and Perkinsozoa, but also in chrompodellids and dinoflagellates (**Table 2**). Within the apicomplexans, short-type TPPPs were found in Haemosporida, Piroplasmida, Eimeriidae, and Sarcocystidae, but they were absent in Cryptosporidiidae [7]. This analysis showed that they also occur in Nephromycida, gregarines (Eu-, Blasto-, and Archigregarinorida) and the recently defined class, Marosporida [23]. There are some characteristic sequences in short-type TPPPs. For example, one of the sequences, which is common with long-type TPPPs, is the L(V)xxxF(Y)xxF at the very beginning of the p25alpha domain. Another one is a GGP sequence in the C-terminal half of the protein (**Figure 1**). The length of the proteins varies between 120 and 170 amino acids.

**Table 2.** Newly identified short-type TPPPs.

| Species | Accesion Number (TSA) | Class | Order | Identity with *T. thermophila* TPPP [5], % |
|---|---|---|---|---|
| **Apicomplexans** | | | | |
| *Haemoproteus columbae* | GGWD01012446+ GGWD01012443 | Aconoidasida | Haemosporida | 48.61 |
| *Hepatocystis* sp. ex *Piliocolobus tephrosceles* | VWU48670 [1] | Aconoidasida | Haemosporida | 42.07 |
| *Plasmodium gallinaceum* | XP_028529806 [1] | Aconoidasida | Haemosporida | 44.83 |
| *Cardiosporidium cionae* | KAF8819752 [1] | Aconoidasida | Nephromycida | 50.68 |
| *Nephromyces* sp. ex *Molgula occidentalis* | GHIL01028850 | Aconoidasida | Nephromycida | 49.32 |
| *Babesia microti* | XP_012649535 [1] | Aconoidasida | Piroplasmida | 27.05 |
| *Cyclospora cayetanensis* | XP_022592352 [1] | Conoidasida | Eucoccidiorida | 56.55 |
| *Neospora caninum* | XP_003880535 [1] XP_003883867 [1] | Conoidasida | Eucoccidiorida | 56.94 50.34 |
| *Eleutheroschizon duboscqi* | GHVT01063834 | Conoidasida | Protococcidiorida; | 55.56 |
| *Ancora sagittata* | GHVO01049457 | Conoidasida | Eugregarinorida | 3730 |

| Species | Accesion Number (TSA) | Class | Order | Identity with *T. thermophila* TPPP [5], % |
|---|---|---|---|---|
| *Cephaloidophora* cf. *communis* | GHVH01010051 | Conoidasida | Eugregarinorida | 44.59 |
| *Gregarina niphandrodes* | GNI_040770 [2] | Conoidasida | Eugregarinorida | 37.04 |
| *Polyrhabdina* sp. | GHVP01031609 [3] | Conoidasida | Eugregarinorida | 35.64 |
| *Porospora* cf. *gigantea* | KAH0473834 KAH0475585 | Conoidasida | Eugregarinorida | 34.01 36.05 |
| *Siedleckia nematoides* | GHVU01040477 GHVV01097121 | Conoidasida | Eugregarinorida (Blastogregarinorida) | 52.03 48.85 |
| *Selenidium pygospionis* | GHVN01019431 [4] | Conoidasida | Archigregarinorida | 47.30, 45.64 |
| *Rhytidocystis* sp. ex *Travisia forbesii* | GHVS01062019 GHVS01062016 | Marosporida | Agamococcidiorida | 41.22 43.24 |
| **Chrompodellids** | | | | |
| *Chromera velia* | HBKZ01015069 HBKZ01021816 | | | 61.38 40.69 |
| *Vitrella brassicaformis* | CEM02660 [1] | | | 50.99 |
| *Alphamonas edax* | GDKI01002338 | | | 56.16 |
| *Colpodella angusta* | GDKK01046869 | | | 45.77 |
| *Voromonas pontica* | GDKH01013421 [3] | | | 57.69 |
| **Squirmids** | | | | |
| *Digyalum oweni* | GHRU01021184 | | | 45.27 |
| **Dinoflagellates** | | | | |
| *Karenia mikimotoi* | GHKS01124154 | Dinophyceae | Gymnodiniales | 45.39 |
| *Gambierdiscus australes* | HBLT01006012 | Dinophyceae | Gonyaulacales | 53.24 |
| *Dinophysis acuminata* | GKBP01057714 | Dinophyceae | Dinophysiales | 30.07 |
| *Symbiodinium* sp. CCMP2456 | CAE7788281 [1] | Dinophyceae | Suessiales | 27.69 |
| *Symbiodinium natans* | CAE7214312 [1] | Dinophyceae | Suessiales | 28.69 |

## 2.3. Multidomain Proteins Containing Short p25alpha Domains

| Species | Accesion Number (TSA) | Class | Order | Identity with T. thermophila TPPP [5], % |
|---|---|---|---|---|
| *Symbiodinium microadriaticum* | CAE7469644 [1] | Dinophyceae | Suessiales | 27.69 |
| **Perkinsids** | | | | |

| Species | Accession Number (Protein) | p25alpha Domains | Other Domains | Length (aa) |
|---|---|---|---|---|
| **Chrompodellids** | | | | |
| *Chromera velia* | Cvel_4181 [1,12] | 2 | | 232 |
| | Cvel_31116 [1] | 2 | EF-hand | 499 |
| *Vitrella brassicaformis* | CEL98751 | 2 | EF-hand | 433 |
| **Squirmids** | | | | |
| *Digyalum oweni* | GHRU01002363 [2] | 2 | | 399 |
| **Dinoflagellates** | | | | |
| *Polarella glacialis* | CAE8635994 | 2 | EF-hand | 344 |
| | CAE8640696 | 2 | EF-hand | 347 |
| | CAE8623567 | 2 | EF-hand | 637 |
| *Cladocopium goreaui* | CAI4012958 | 2 | Nc [3] and others [4] | 1808 |
| | CAI4010985 | 2 | PRK08691 [5] | 1704 |
| | CAI3986388 | 2 | several [6] | 1281 |
| *Symbiodinium* sp. CCMP2592 | CAE7826117 | 2 | sec 7 [7]; Ank_2 [8], Nc and others [9] | 2611 |
| | CAE7226582 | 2 | | 333 |
| *Symbiodinium* sp. CCMP2456 | CAE7634140 | 2 | sec 7; Nc | 1368 |
| | CAE7264691 | 2 | | 333 |
| *Symbiodinium* sp. *KB8* | CAE7914847 | | sec 7; Ank_2, Nc, and others [10] | 2627 |

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| Species | Accession Number (Protein) | p25alpha Domains | Other Domains | Length (aa) |
|---|---|---|---|---|
| | CAE7947347 | 2 | PTZ00121 [11] | 2004 |
| *Symbiodinium natans* | CAE7514938 | 2 | | 338 |
| | CAE7230103 | 2 | Nc | 735 |
| *Symbiodinium microadriaticum* | CAE7819146 | | Nc | 1367 |
| | OLP89249 | 2 | Nc | 1405 |
| | OLQ07037 | 2 | PTZ00121 | 2338 |
| | CAE7562082 | 2 | PTZ00121 | 2161 |
| *Symbiodinium necroappetens* | CAE7814517 | 2 | sec 7; Nc | 1356 |
| | CAE7554473 | 2 | PTZ00121 | 1082 |
| *Symbiodinium pilosum* | CAE7666671 | 2 | Nc | 1354 |
| **Perkinsids** | | | | |
| *Perkinsus chesapeaki* | KAF4658947 | 2 | | 349 |
| *Perkinsus olseni* | KAF4688416 | 2 | | 358 |
| | KAF4711908 | 2 | Ank_2 | 445 |
| | KAF4678978 | 2 | Ank_2 | 489 |

e found in lpodellids duplicate er domain (*C. velia*, *D. oweni*, *Symbiodinium* genus; *Perkinsus* genus). Strictly speaking, only these proteins can be considered to be TPPP-like. In other cases, an EF-hand or ankyrin repeats are present. In the third case, the duplicated p25alpha domains are associated with various catalytic domains, most often with a Nucleotidyl_ cyc_III one, which is typical of adenylate and guanylate cyclases; these are mostly proteins containing more than a thousand amino acids.

<sup>1</sup> From https://cryptodb.org [36]. <sup>2</sup> TSA. <sup>3</sup> Nc—Nucleotidyl_cyc_III .

# 3. Possible Function of TPPP-like Proteins in Myzozoa

## 3.1. Tubulin-Based Structural Elements of Apicomplexa and Other Myzozoa

It is known that TPPP binds to tubulin, promotes its polymerization into microtubules, and stabilizes them [2][3]. This function is conserved in animals from sponges to mammals [12]. Tubulin-based cytoskeletal elements of apicomplexans have distinct traits, which may be related to the unique biology of these parasites [37]. In principle, TPPP-like proteins can interact with and stabilize any of these microtubular elements, which has been confirmed experimentally.

Apicomplexans and their relatives possess discrete populations of microtubules and other tubulin polymers. Subpellicular microtubules and spindle microtubules are associated with the apical polar ring and with centrioles as the microtubule-organizing center, respectively. Axonemal microtubules are present in species possessing flagella in any of their life stages. Beside microtubules, some myzozoan species possess another polymer form of tubulin, the conoid fibers. They are similar to microtubules but their subunits are curled into an extremely tight coil, where tubulin is arranged into a polymer form that is different from typical microtubules [38]. Both the apical polar ring and the conoid are components of the namesake of apicomplexans, the apical complex, which was originally used for feeding in the common ancestor of apicomplexans and other myzozoans but was transformed for infection when the parasitism evolved [38][39].

It should be noted that the apical complex is complete only in some apicomplexans (e.g., Eimeriidae, Sarcocystidae, Cryptosporidiidae, and gregarines) [38]; other species of this phylum (in Piroplasmida and Haemosporida) lost the conoid, but they retained an apical polar ring; moreover, a conoid-like structure was found in the ookinete stage of *Plasmodium gallinaceum* [40] and in another haemosporidian, *Leucocytozoon simondi* [41]. Chrompodellids, but not *V. brassicaformis* [42], and perkinsids have an uncomplete 'pseudoconoid', while dinoflagellates lack it but instead, they have another specialized tubular structure called peduncle [43].

## 3.2. Connection between Apical Complex/Conoid and Apicortin

The apical complex can be divided into three components, namely, the apical cap, the conoid, and the secretory organelles, micronemes, and rhoptries [38]. The conoid, which plays an important role in the host cell invasion, is best described from the apicomplexan families Eimeriidae and Sarcocystidae (*Sarcocystis*, *Toxoplasma*, *Neospora*, etc.). Apicortin seems to be in strict relation with this organelle. In *T. gondii*, apicortin was shown to be localized exclusively at the conoid and is essential for providing its correct structure and function [14][44]. Tubulin disappeared or was present in a reduced amount in the apical complex of an apicortin-null mutant, while microtubules were normal [44]. The deletion of apicortin resulted in shorter and misshaped conoids [44]. These defects were reversed by the expression of the apicortin coding sequence. Apicortin possesses two microtubule-binding domains (a partial p25alpha and a DCX), which stabilize and bundle microtubules [2][3][17][18]. Thus, apicortin is an ideal protein for stabilizing the tubulin-based conoid fibers. This stabilizing effect of apicortin was shown in a reconstituted in vitro system as well [14].

In *P. falciparum*, which has no conoid, apicortin is localized at the apical end and may be involved in the formation of the apical complex [45]. In both *T. gondii* and *P. falciparum*, the downregulation of apicortin leads to impaired host cell invasion [14][15]. Interestingly, in mammalian, but not in avian, *Plasmodium* parasites, the p25alpha domain is degenerated, i.e., some otherwise conservative amino acids are missing. At this moment, it is not clear yet whether the presence of a conoid-like structure in the ookinete stage of the avian parasite *P. gallinaceum* [40] is in connection with these sequence differences, or whether it happened accidentally, and a similar structure will also be found in mammalian parasites in the future. However, this altered sequence does not affect the ability of *P. falciparum* apicortin to bind to tubulin; in silico docking showed that the amino acids of the p25alpha domain are involved in the binding [45].

## 3.3. Flagella and Short-Type TPPP

TPPP-like proteins occur predominantly in species that are flagellated. The eukaryotic flagellum is a microtubule-based organelle; thus, the connection is not surprising. Although the correlation between the incidence of flagellum and the p25alpha domain is strong [5][6], there is only some evidence for the functional relationship between TPPP-like proteins and the flagellum. They are related to short-type TPPPs. The connection was first shown in *Chlamydomonas reinhardtii*, a biflagellate green alga [46]. Its TPPP ortholog, FAP265 protein, can be found in the flagella, and is indispensable in their formation, as proven by using FAP265 null mutants [46]. Myzozoan species are flagellated; however, apicomplexans are in a special position in this respect [47]. The flagella present in the common ancestor of the Myzozoa (and Alveolata) were lost during evolution. In many parasitic apicomplexans, flagella are only found in male microgametes with exceptions, such as Piroplasmida and the *Cryptosporidium* genus, where no flagellum occurs. Flagellum formation in apicomplexans does not require the so-called intraflagellar transport proteins, but all elements of the flagellar apparatus assemble directly within the cytoplasm in a process termed exflagellation [48].

There are examples for the flagellum-connected role of short-type TPPPs in Apicomplexa. In *Plasmodium* genus, *P. falciparum* short-type TPPP (PFL1770c; XP_001350760) was shown to be essential for gametocytogenesis, using piggyBac transposon-mediated insertional mutagenesis, but the mutant failed to form mature gametocytes [49]. Recently, it has also been found that the TPPP (*Py*05543; EAA17578) is necessary for male gametocyte exflagellation in *Plasmodium yoelli* [50]. *Py*05543⁻ KO parasites, obtained by CRISPR/Cas9-mediated genome editing, were deficient in the exflagellation of male gametes by observing deficient exflagellation center formation [50].

In this respect, it is noteworthy that *Cryptosporidium* species, which lack flagella [51][52][53], do not have short-type TPPPs. This can be interpreted as meaning that the parasite does not need this protein. A similar loss was described in *Cryptosporidium* for δ- and ε-tubulin genes/proteins, which are marker proteins for the flagellum and basal bodies [50]. Piroplasmids also have no flagella, not even in microgametes [54]. Their short-type TPPPs are the shortest among all short-type TPPPs. The lengths of TPPP of *Theileria annulata*, *B. microti,* and *B. bovis* are 121, 123, and 124 amino acids, respectively. The length of most short-type TPPPs is approximately 140–150 amino acids. Furthermore, among all apicomplexan TPPPs, the piroplasmid TPPPs are the most divergent. With the loss of the flagellum, their function may have been lost, so they evolved faster, and perhaps acquired a new function.

## 3.4. Function of the p25alpha Domain-Containing Multidomain Proteins

Researchers find very different proteins in this group. Their length varies between 232 and 2627 amino acids. Interestingly, there are two short-type p25alpha domains in all of them. Sometimes, they do not have another domain; this type of protein is not found in apicomplexans and colpodellids, but is present in chromerids, perkinsids, and dinoflagellates. There is such a protein also in *D. oweni*; this species was previously classified as an apicomplexan, but according to the latest phylogenetic classification, it belongs to a separate group, the

squirmids, which are sisters to (apicomplexans and chrompodellids) [23][24]. The fact that *D. oweni* has this type of protein (two p25alpha domains and no other domains) fits into this picture; i.e., *D. oweni* is not an apicomplexan. The function of these proteins is unknown; it would be expected that, similar to short-type TPPPs and apicortins, they exert their effect by binding to a tubulin polymer.

Only in dinoflagellates can find larger proteins consisting of 1–2 thousand amino acids that contain more than three domains, including catalytic ones. For these proteins, the additional domains determine the function, e.g., adenylate cyclase or methyltransferase activity. The role of the p25alpha domains is unknown; however, there are cases when the importance of the tubulin-binding ability is likely, e.g., in the alpha-tubulin suppressor domain-containing protein CAI3986388 (*Cladocopium goreaui*). In the narrow sense, I do not consider these proteins to be TPPP-like ones.

# 4. Evolutionary Considerations

Among the TPPP-like proteins, short-type TPPPs and apicortins occur in all myzozoan phyla. Both experimental results and phylogenetic considerations indicate that these tubulin-binding proteins play an important role in the structure and/or function of the flagellum and the apical complex/conoid, respectively. Moreover, an evolutionary connection between the flagellum and apical complex was proposed [38][55]. Recent results suggest that the conoid complex evolved from flagellar components [56][57] or the flagellar root apparatus [38][58]; that is, 'the apical complex is the most conspicuously retained element of the associated flagellar root structures' [38].

In light of this, it is not surprising that short TPPPs are also common in Ciliata, the sister phylum of Myzozoa, since their surface is covered by hundreds of cilia; they usually contain several paralogs of short-type TPPPs. However, they do not have apicortin, and they do not possess conoid or any similar structural elements. The short-type TPPP is also present in other protists (Euglenozoa and Chlorophyta), which are also flagellated. Myzozoans are flagellated, but in apicomplexans, only the male gametes have flagellum/flagella in some species. If not, then the loss of the flagellum resulted in either the loss of TPPPs (*Cryptosporidium*) or a degenerate protein significantly diverging from other TPPPs (Piroplasmida). The conoid is also absent from some apicomplexans (Piroplasmida and Haemosporida), but despite this, apicortin is present in almost all of them. There can be several reasons for this. On the one hand, conoid-like structures are also found in some of these species [40][41], and on the other hand, the apical polar ring is also present in these cases; furthermore, since these species once possessed a conoid, the apicortin remained as a relic. The only exception is *B. microti*, which has an extremely small genome [27] and lacks the 'redundant' protein.

It cannot be ignored the fact that apicortin is also found in some Opisthokonta (flagellated fungi, the placozoan *T. adhaerens*) [6][59], which do not have a conoid or similar structure. These apicortins differ from apicomplexan orthologs in that, although they have a partial p25alpha domain and the entire DCX domain, the opisthokont apicortin lacks the long, disordered N-terminal region [6][34], which appears to be necessary for the formation of the proper conoid structure [14]. Their function is probably to stabilize tubulin polymers/microtubules in these species as well; this is also indicated by the fact that apicortin is the only TPPP-like protein in *T. adhaerens* and some

flagellated fungi [6][7]. Since apicortin is present only in these few primitive opisthokonts apart from Myzozoa, it is possible that the opisthokonts acquired this protein from myzozoans by horizontal gene transfer. This is supported by the high degree of sequence identity and similarity between apicomplexan and chromerid apicortins on the one hand, and *Trichoplax* and fungal apicortins on the other, which can reach 53% and 67%, respectively, which is roughly the same value as the similarity of apicomplexan apicortins to each other. The similarity exists not only between the domain sequences, but also in the interdomain linker. Since the last common ancestor of myzozoans and opisthokonts could be close to the first eukaryote, it is extremely unlikely that the gene remained so conserved for that much time, and horizontal gene transfer is the best explanation.

# 5. Conclusions

A common feature of TPPP-like proteins is that they contain one or more p25alpha domains [1]. They occur only in eukaryotes, and like their name indicates, they are tubulin-binding proteins [2][3]. This trait is conserved in animals, as it is present in everything from sponges to humans [13]. The phylogenetic distribution of TPPP-like proteins is widespread in protists as well, but not in plants [1]. The role of TPPP-like proteins may be quite different in protists than in humans. Short-type TPPPs and apicortins are found in Myzozoa, a major monophyletic group of Alveolata. Although there are few experimental examples so far, short-type TPPPs have a role in the flagellar structures, interacting with tubulin polymers, and perhaps play a role in the exflagellation of microgametes [49][50]. Apicortin may play a crucial role in the formation of the conoid/apical complex [14][15][44][45]. Thus, TPPP-like proteins may be potential therapeutic targets for the treatment of diseases such as malaria and toxoplasmosis [55].

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