Apicortin and Its Tentative Role

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Apicortin was identified in silico, in 2009, as a characteristic protein of apicomplexans. It combines a partial p25alpha domain with a DCX (doublecortin) one. Based on its occurrence and one of its characteristic domains, it was termed apicortin. Apicortin, when identified, was shown to occur in apicomplexan parasites and in the placozoan animal, Trichoplax adhaerens. The apicomplexan genomes known then contained it without exception. This situation practically has not changed since then; this statement is valid for the newly sequenced genomes and transcriptomes of apicomplexans as well, almost without exception.

Keywords: apicortin ; Plasmodium ; Toxoplasma ; p25alpha domain ; DCX domain ; apicomplexa ; conoid

1. Overview

In 2009, apicortin was identified in silico as a characteristic protein of apicomplexans that also occurs in the placozoa, *Trichoplax adhaerens*. Since then, it has been found that apicortin also occurs in free-living cousins of apicomplexans (chromerids) and in flagellated fungi. It contains a partial p25-α domain and a doublecortin (DCX) domain, both of which have tubulin/microtubule binding properties. Apicortin has been studied experimentally in two very important apicomplexan pathogens, *Toxoplasma gondii* and *Plasmodium falciparum*. It is localized in the apical complex in both parasites. In *T. gondii*, apicortin plays a key role in shaping the structure of a special tubulin polymer, conoid. In both parasites, its absence or downregulation has been shown to impair pathogen–host interactions. Based on these facts, it has been suggested as a therapeutic target for treatment of malaria and toxoplasmosis.

2. Description

2.1. Name

Apicortin was identified in silico, in 2009, as a characteristic protein of apicomplexans[1]. It combines a partial p25alpha domain with a DCX (doublecortin) one. Based on its occurrence and one of its characteristic domains, it was termed apicortin. In *T. gondii* it is also known as TgDCX [2].

2.2. History and occurrence

Apicortin, when identified, was shown to occur in apicomplexan parasites and in the placozoan animal, *Trichoplax adhaerens*[1]. The apicomplexan genomes known then contained it without exception. This situation practically has not changed since then; this statement is valid for the newly sequenced genomes and transcriptomes of apicomplexans as well. The only exception is the Apicomplexa with the smallest genome, *Babesia microti*[3].

Later it has been found that apicortin also occurs in chromerids[4], the recently discovered[5][6], free-living cousins of apicomplexans. This is not surprising, given the phylogenetic proximity and the structural similarity of these phyla. Unlike apicomplexan species, both *Chromera velia* and *Vitrella brassicaformis* have three apicortin paralogs. Apicortin has not been found in other related phyla of the Alveolata superphylum, although its remnant is present in the genome in the case of Perkinsozoa[7]. However, the very recently published draft genome of *Perkinsus olseni* contains hypothetical protein(s) possessing both p25alpha and DCX domains (KAF4710163, KAF4750811)[8].

It has also been revealed that some primitive fungi also possess this protein; first it was shown in the cases of *Spizellomyces punctatus*[7] and *Rozella allomycis*[4]. Later, a systematic examination of fungal genomes showed that the flagellated fungi contain apicortin almost without exception; and it is present even in a non-flagellated but also deeper branching clade (phylum Mucoromycota, class Endogonomycetes)[9].

Apicortin is one of the most abundant proteins of *T. adhaerens*[10]. This is the only animal that possesses apicortin[11]. Animal draft genomes and transcriptomes contain sometimes nucleotide sequences, contigs and TSAs (transcriptome

shot-gun assemblies), homologous to apicortin, but they have been shown to be contaminations from parasitizing apicomplexans, based on sequence similarities and GC ratios[12][13]. In the case of *T. adhaerens*, this option was excluded by phylogenetic analysis. The origin of a few plant and algal apicortin-like nucleotide sequences needs further investigations.

Strong correlation between the presence of the p25alpha domain and that of the eukaryotic flagellum was suggested before the identification of apicortin[14]. With very few exceptions, each flagellated organism contains p25alpha domain-containing proteins; on the other side, in non-flagellated species, the p25alpha domain generally does not occur[11]. The protein that contains the p25alpha domain varies depending on the phylum; e.g., it is the so called “long-type” TPPP in animals (except *T. adhaerens*)[11][15], a fungal-type TPPP and apicortin in flagellated fungi, while the “short-type” TPPP and apicortin are found in apicomplexan species[11]. With the exceptions of two non-flagellated fungi and some apicomplexans, apicortin can be found only in species which are flagellated, at least in some life stage.

2.3. Domains

Apicortin belongs to a eukaryotic protein superfamily, the TPPP-like proteins, characterized by the presence of the p25alpha domain (Pfam05517, IPR008907), and named after the first identified member, TPPP/p25, which exhibits microtubule stabilizing function[11]. TPPP stands for tubulin polymerization promoting protein[16][17] and was first identified by Takahashi et al. as p25 protein[18]. Full-length p25alpha domain consists of about 160 amino acids; however, that of the apicortin is incomplete, containing only the last 30–40 amino acids (a “partial p25alpha domain”). Importantly, the tubulin/microtubule binding amino acid sequence is located in this part of the domain[19][20][21][22]. This is the most conserved part of the domain, which contains a characteristic “Rossmann-like motif”, GXGXGXXGR[11][15].

However, apicortins possess another characteristic domain, the DCX one (Pfam03607, IPR003533)[1]. The DCX (doublecortin) domain is named after the brain-specific X-linked gene doublecortin[23]. It is a structural domain, which generally appears in duplicate as two tandemly repeated 80 amino acid regions (N- and C-terminal type DCX domains), but proteins containing only one DCX-repeat have also been identified[24][25]. This domain is also known to play a role in the stabilization of microtubules[23][24].

2.4. Structure

Apicortins consist of four structural units: (i) a long, disordered N-terminal domain; (ii) a partial p25alpha domain; (iii) a disordered linker region; (iv) and a DCX domain[7].

The N-terminal part consists of about 70–90 amino acids in apicomplexan apicortins, similarly to the apicortins of the few non-flagellated fungi; this part is 40 amino acid long in one of the *C. velia* apicortins (Cvel_6797) while in other species (placozoa, chromerids, flagellated fungi) it is absent. The N-terminus of the proteins is rather different among the various apicomplexan species. It is not conserved among the species belonging to different classes of Apicomplexa, or the various genera of the same class, or even among the various *Plasmodium* subgenera. The extra N-terminal regions are significantly similar in the two fungal apicortins possessing it. Concerning the secondary structure, the N-terminal region of apicortins was shown to be highly disordered by predictor programs based on different principles[4][7][9].

The partial p25alpha domain, in general, is the most conservative part of the protein, however, there are two exceptions. *R. allomycis* and *Plasmodium* orthologs present in species infecting mammals (but not birds) lack the final part of this domain, which includes the Rossmann-like motif. Otherwise, the sequences are very similar, independently of whether the protein can be found in an apicomplexan species or not. It was shown by Leung et al.[2] that this region of the protein has an outstanding role in tubulin binding.

In the linker (interdomain) region, which has also been predicted to be unstructured, similarity is much lower between protist and non-protist apicortins. Moreover, this part of *Plasmodium* proteins also differs somewhat from those of the other apicomplexan orthologs.

In the DCX domain, the overall similarity is somewhat lower between the two groups than in the case of the partial p25alpha domain; however, there is no exception: the similarity occurs through the whole domain in all orthologs. Phylogenetic analysis showed that DCX domains of apicortins are clustered neither with C- nor N-terminal type domains but form a separate group[4].

2.5. Phylogenetics

Phylogenetic analysis clearly showed that apicomplexan apicortins form a monophyletic group and are well separated from opisthokont (fungal and placozoan) and chromerid apicortins[4][9]. One pair of the chromerid apicortins is a sister group to them, the other 2-2 chromerid homologs are significantly different.

2.6. Apicomplexan apicortins

Apicortin is a characteristic protein of Apicomplexa. It is present in both classes, Aconoidasida and Conoidasida, and in each order and family whose species are fully sequenced. According to the NCBI website, 66 genomes of apicomplexan species have been fully sequenced, 65 of which contain apicortin. The only exception, *B. microti* has a significantly decreased genome which is the smallest one in the phylum[3]. However, it contains a p25alpha domain containing protein, a short-type TPPP (XP_012649535).

The genomes of most *Plasmodium* species have been sequenced (21), due to their epidemiological significance, followed by 15 sequenced *Cryptosporidium* species genomes. Apicomplexans are obligate parasites causing serious illnesses in humans and domestic animals[26]. Species in the genus *Plasmodium* cause malaria, from which over 200 million people suffer each year. The official deaths according to WHO were about 400,000 both in 2018 and 2019[27]. Other members of the phylum Apicomplexa are responsible for animal sicknesses, such as coccidiosis and babesiosis, resulting in significant economic burden for animal husbandry. *Cryptosporidium* causes cryptosporidiosis in humans and animals, *Theileria* causes tropical theileriosis and East Coast fever in cattle, and *Toxoplasma* causes toxoplasmosis in immunocompromised patients and congenitally infected fetuses. The disease-causing parasites belong to the orders Haemosporida, Piroplasmida and Eucoccidiorida, which explains the bias in genome sequencing.

2.7. Function in apicomplexans

Apicomplexans are an ancient and diverse phylum with peculiar cell biological properties. Many of the distinct traits are related to the unique cytoskeletal elements of these parasites[28]. In addition to microtubules, the main cytoskeletal constituents, several apicomplexans possess another polymer form of tubulin, the conoid[29] that has an important role in host cell invasion. The conoid fibers resemble 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[30]. In *T. gondii*, apicortin is only localized at the conoid as shown by immunofluorescence staining and the labeling suggested that it is distributed all along the conoid fibers[2][31]; moreover, apicortin is essential for providing the correct structure and function of conoid[2]. In *P. falciparum,* which has no conoid, apicortin was observed at the apical end of the parasite suggesting its role in apical complex formation. It was co-localized with both α- and β-tubulin[32][33]. In both species, downregulation of apicortin leads to impaired host cell invasion[2][33]. Moreover, apicortin knockout *T. gondii* grew about four times slower compared with the wild-type ones[31].

3. Conclusions and Prospects

When apicortin was identified in silico, it was proposed that its function may be to stabilize specific cytoskeletal structures that are unique to apicomplexans[1]. It has also been hypothesized that it might have a role in parasite–host interactions[1][7]. Recent experimental results support both assumptions[2][31][32][33][34]. Apicortin has been shown to be localized in the apical complex; more precisely, in *T. gondii*, it is localized exclusively at the conoid, a tubulin-based structure that has an important role in host cell invasion. Apicortin is essential for providing the correct structure and function of conoid. In *P. falciparum*, which has no conoid, apicortin is localized at the apical end; it has been suggested that it is involved in the formation of the apical complex. In both species, downregulation of apicortin leads to impaired host cell invasion .

Apicomplexan species possess several hundred genes, which are specific for the phylum, and even more genes, which are absent in mammals/vertebrates but are present in these parasites. For example, apicomplexans have evolved hundreds of specialized invasion proteins, and contain lineage- and species-specific gene families, which are specialized for modulating host-specific adaptation[35]. Targeting parasite proteins that have crucial roles in these interactions is a key focus in the development of therapeutic agents against diseases caused by apicomplexan infection. Thus, apicortin, which seems to influence parasite–host interactions, is also a potential drug target. Since some mechanistic details of its function have been known, this protein has become more than a desirable target in drug development. However, the role of apicortin in phyla other than Apicomplexa has remained enigmatic.

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