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Hypothesis

TPPP orthologs are ciliary proteins

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Abstract Tubulin polymerization promoting protein, (TPPP/p25), was identified as a brain-specific protein. The potential function of this protein resembled that of MAPs. It is mainly expressed in oligodendrocytes; however, immunopositivity was also detected in glial and neuronal inclusions in synucleinopathies. Here, we show that TPPP gene(s) are conserved in the genomes of ciliated organisms, but are lacking from the nonciliated ones. This recognition is based upon homologous gene sequence analysis, in silico comparative genomic studies, bioinformatic search and experimental evidence. Cilia (flagella) are microtubule-based cellular extensions of sensory and/or motile function. TPPP orthologs are among the only 16 genes that can be found in all ciliated organisms, suggesting that TPPP orthologs may be associated with a basic function of cilia.

Keywords: TPPP/p25; TPPP ortholog; Cilia; Flagella; Microtubule; Synucleinopathy

1. Introduction

The research on eukaryotic cilium/flagellum has come to a renaissance of interest, which was triggered by the recognition that defects of this organelle are at the centre of several human disorders, including most importantly polycystic kidney disease, the most common inherited disease in the US [1,2]. The cilium evolved very early in the development of eukaryotes. This organelle, virtually in its present form, had been present in the last common ancestor of eukaryotes [3,4]. However, it has been lost secondarily in multiple lineages as red algae, cellular slime molds and in the majority of land plants or fungi [5,6]. However, gametes and sperms of several fungi and ancient plants such as mosses, ferns or Ginkgo possess flagella/cilia [6,7]. There are two main types of cilia, motile and non-motile or sensory. Their axonemes consist of nine doublet microtubules; motile cilia (named also flagella) contain an additional central pair of microtubules. The axonemal microtubules of all cilia nucleate and extend from a basal body, a centriolar structure. Most cilia are assembled in a compartment separate from the cytoplasm via a process called intraflagellar transport (IFT), mediated by the IFT multiprotein complex (IFT particle) and IFT motors [8]. However, *Plasmodium* cilia and *Drosophila* sperm flagella are assembled in the cytoplasm by a process that does not appear to require IFT [4,5]. The molecular makeup of motile and non-motile cilia is likely to overlap substantially, although each type will boast components required accommodating their specific functions. For example, non-motile cilia lack motility-associated proteins (e.g. outer and inner dynein arm and radial spoke proteins, microtubule central pair-associated proteins) but can be enriched for proteins related to sensory perception [9].

Recently, we isolated a partially unfolded protein from bovine brain [10] and named it tubulin polymerization promoting protein/p25, (TPPP/p25), which has no well-defined structure [11,12]. It was first identified by co-purification together with the tau protein kinase, Cdk5, in the bovine brain [13], which probably causes its phosphorylation, together with other kinases, ERK2 and PKA [14]. We have shown that it promotes tubulin polymerization into normal and double walled tubules and polymorph aggregates; its binding to paclitaxel-stabilized microtubules induces their bundling [14,15]. It exhibits microtubule associated proteins-like functions stabilizing the microtubular network both in vitro and in transfected HeLa and rat kidney cells [14,16]. The physiological role of TPPP/p25 is unknown, although our data strongly suggest that TPPP/p25 may take part in the stabilization of the microtubular network. Under pathological conditions, TPPP/p25 is enriched in filamentous α-synuclein bearing Lewy bodies of Parkinson's and diffuse Lewy body diseases, as well as in glial inclusions of multiple system atrophy, as demonstrated by immunohistochemistry and confocal microscopy [11,12,17,18]. TPPP/p25 promotes the fibrillization of α-synuclein in vitro [19].

2. Phyletic distribution of TPPP proteins

TPPP/p25 is the first member of a new protein family, the primary sequence of which differs from that of other known proteins, but shows homology with TPPP-like hypothetical proteins [15] sought via BLAST. There are two homologous gene sequences in the human genome encoding two shorter proteins, TPPP2/p18 and TPPP3/p20. Only the latter one was isolated from bovine brain which displays similar microtubule bundling activity as TPPP/p25 [20]. The paralogous genes can be found only in vertebrates but not in other

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Abbreviations: TPPP, tubulin polymerization promoting protein; IFT, intraflagellar transport; PSC, photoreceptor sensory cilium; CCV, clathrin-coated vesicle; SV, synaptic vesicle

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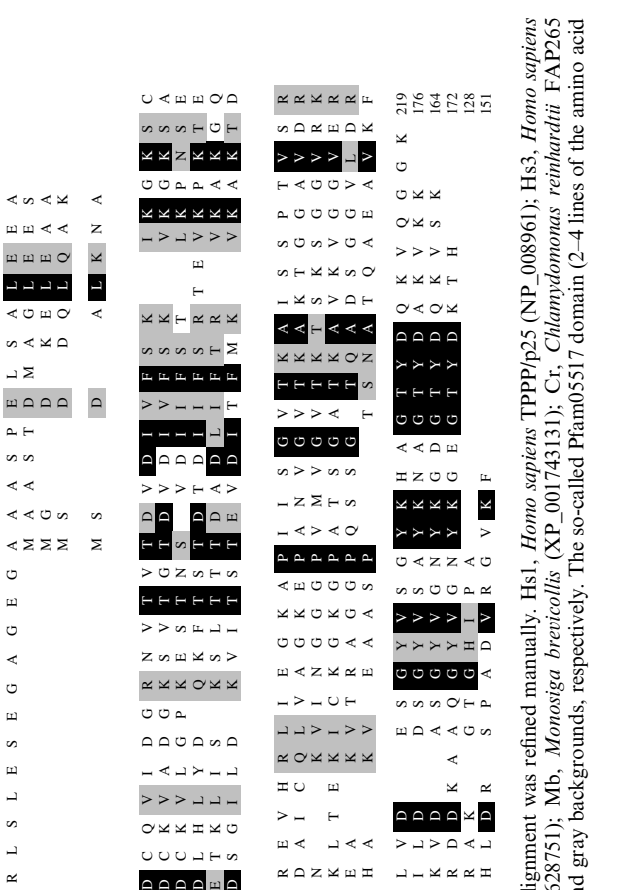

Fig. 1. Multiple sequence alignment of several members of the TPPP family by ClustalW [51]. The alignment was refined manually. Hs1, *Homo sapiens* TPPP/p25 (NP_008961); Hs3, *Homo sapiens* TPPP3/p20 (NP_057048); Ci, *Ciona intestinalis* (BW358083); Nv, *Nematostella vectensis* (XP_001628751); Mb, *Monosiga brevicollis* (XP_001743131); Cr, *Chlamydomonas reinhardtii* FAP265 (XP_001695016). Residues identical and similar in at least all but one species are indicated by black and gray backgrounds, respectively. The so-called Pfam05517 domain (2–4 lines of the amino acid sequences) involves the major part of the proteins except the N-terminal tail specific for TPPP/p25s.

organisms. BLASTP or TBLASTN analysis [21] was performed on complete genome sequences and EST collections available at the NCBI website using the sequences of human TPPP proteins. Fig. 1 shows the sequence homology of several TPPP proteins from unicellular organism to vertebrates. Similarity can be seen in all parts of the proteins except the N-terminal tail of TPPP/p25s, which is missing in other homologs. A standard and simple method for determining orthology is the reciprocal best hit approach. It helped to reveal 1:1 orthology also in cases when the BLAST E score was higher than 1e−10. Our search has shown that such proteins/genes can be found throughout the animal kingdom from protists to vertebrates but not in prokaryotes, land plants and fungi (Table 1). Thus they cannot be found in the completed genomes of *Arabidopsis thaliana*, *Oryza sativa* or *Saccharomyces cerevisiae* etc. No evidence for TPPP orthologs was found either in bacteria or archaea. The species in Table 1 are only representative examples. (The only possible exception was the moss, *Physcomitrella patens*, which has a best-reciprocal-hit protein, although the similarity is very low, and probably it cannot be considered as a TPPP ortholog. The very recent establishment of the draft genome sequence of this model species and the comparison of its features with those of flowering plants and the unicellular green algae revealed genomic changes concomitant with the evolutionary movement from aquatic environment to land, including loss of some but not all flagellar components for gametic motility as proteins of the outer dynein arms of flagella [22]. However, we guess that it would be premature to make a final conclusion concerning the occurrence of TPPP gene/protein in land plants with ciliated sperm and we should wait until more genomes will be available.) TPPP is also absent in the slime mold, *Dictyostelium discoideum*. Interestingly, it occurs in the biflagellated green alga, *Chlamydomonas reinhardtii* but not in the red alga, *Cyanidioschyzon merolae*, which does not have cilia/flagella. A detailed investigation of protists revealed that a TPPP ortholog occurs in many species, however it is absent from some of them. It can be found in Alveolates including Apicomplexa (e.g. *Plasmodium falciparum*) and Ciliophora (e.g. *Tetrahymena thermophila*), in Euglenozoa (e.g. *Trypanosoma brucei*) and in Diplomonadida (*Giardia lamblia*) but not in *Entamoeba histolytica* (Entamoebidae) and *Acanthamoeba castellanii* (Acanthamoebidae).

The data listed in Table 1 show a tight parallel between the phyletic distribution of cilia and the TPPP proteins, which suggests that the *TPPP* gene is conserved in the genomes of ciliated organisms but is absent from those that are non-ciliated. *TPPP* genes can be found in all organisms possessing these organelles, independently of the type of the cilium: (i) in organisms with motile cilia (most of the ciliated organisms); (ii) in organisms with non-motile cilia only (e.g. *C. elegans*); (iii) in organisms with compartmentalized (but not cytosol-assembled) cilia (most of the ciliated organisms); (iv) and in organisms with cytosol-assembled cilia (e.g. *P. falciparum*).

There are only two unicellular green algae, *Ostreococcus lucimarinus* and *Ostreococcus tauri,* whose genomes contain genes that can be considered as TPPP orthologs but these species do not have cilia (cf. Table 1). *Ostreococcus* belonging to the Prasinophyceae, an early-diverging class within the green plant lineage, are the smallest known eukaryotes. They have a very simple cellular organization, with no cell wall or flagella, and with a single chloroplast and mitochondrion. In