Genome Biology

PROTEIN FAMILY REVIEW

# The tubby family proteins

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**Abstract**

The *tubby* mouse shows a tripartite syndrome characterized by maturity-onset obesity, blindness and deafness. The causative gene *Tub* is the founding member of a family of related proteins present throughout the animal and plant kingdoms, each characterized by a signature carboxy-terminal tubby domain. This domain consists of a β barrel enclosing a central α helix and binds selectively to specific membrane phosphoinositides. The vertebrate family of tubby-like proteins (TULPs) includes the founding member TUB and the related TULPs, TULP1 to TULP4. *Tulp1* is expressed in the retina and mutations in *TULP1* cause retinitis pigmentosa in humans; *Tulp3* is expressed ubiquitously in the mouse embryo and is important in sonic hedgehog (Shh)-mediated dorso-ventral patterning of the spinal cord. The amino terminus of these proteins is diverse and directs distinct functions. In the best-characterized example, the TULP3 amino terminus binds to the IFT-A complex, a complex important in intraflagellar transport in the primary cilia, through a short conserved domain. Thus, the tubby family proteins seem to serve as bipartite bridges through their phosphoinositide-binding tubby and unique amino-terminal functional domains, coordinating multiple signaling pathways, including ciliary G-protein-coupled receptor trafficking and Shh signaling. Molecular studies on this functionally diverse protein family are beginning to provide us with remarkable insights into the *tubby*-mouse syndrome and other related diseases.

## Gene organization and evolutionary history

Monogenic diabetes-obesity syndromes in mice have been historically important in the discovery of genes important in their pathogenesis. The *tubby* mouse was initially identified as a spontaneous maturity-onset obesity syndrome in inbred backgrounds maintained at the Jackson Laboratory [1]. The mice were later found to be deficient in hearing and vision [2]. Positional cloning strategies by two groups mapped the causative mutation to a novel gene of unknown function called *Tub* [3,4]. Subsequent studies identified a family of related proteins, present throughout the animal and plant kingdoms, each possessing a signature carboxy-terminal tubby domain capable of highly selective binding to specific phosphoinositides [5,6]. The amino terminus of these proteins is varied and imparts diverse functions to them.

The vertebrate family of tubby-like proteins (TULPs) encompasses the founding member TUB and the related TULPs, TULP1 to TULP4. TUB and TULP1-3 are closely related, but TULP4 is more distant [7,8] (Figure 1a). Human TUB and TULP1-3 are 442 to 561 amino acids long and are encoded by 12 to 15 exons spanning 12 to 15 kb. Human TULP4 is longer; its 1,543 amino acids are encoded by 14 exons, spanning around 200 kb. The expression of these genes is also varied and tightly regulated. *Tub* is expressed broadly in the brain and retina [3,9]. *Tulp1* is selectively expressed in the retina [8], whereas *Tulp3* is expressed ubiquitously in the mouse embryo [10,11] (Figure 1b). The importance of these genes in mammalian development and physiology is evident from the diseases resulting from their disruption. Mutations in *TULP1* cause retinitis pigmentosa in humans [12-14] and retinal degeneration in mice [15], whereas *Tulp3* mutants show embryonic lethality with defects in dorso-ventral patterning of the spinal cord [10,16,17].

Phylogenetic analysis of the tubby family proteins suggests that the ecdysozoan TUB homologs, such as *Drosophila* TULP and *Caenorhabditis elegans* TUB-1, although related to the mammalian group of TULPs, might not be orthologs of a specific mammalian TULP [7] (Figure 1a). However, the *C. elegans tub-1* regulates fat storage similarly to the mouse *Tub*, suggesting remarkable conservation in the fat storage pathways between invertebrates and vertebrates [18,19]. TULP4, the distantly related TULP family member, is characterized by a large amino terminus containing WD repeats and E3 ubiquitin ligase binding motifs [7]. TULP4 is also conserved in evolution, the *Drosophila* TUSP (tubby domain superfamily protein) and *C. elegans* TUB-2 being related homologs [7] (Figure 1a). The mammalian TULP4 is also distantly related to the intraflagellar transport complex A (IFT-A) protein subunit WDR35 (Figure 1a).

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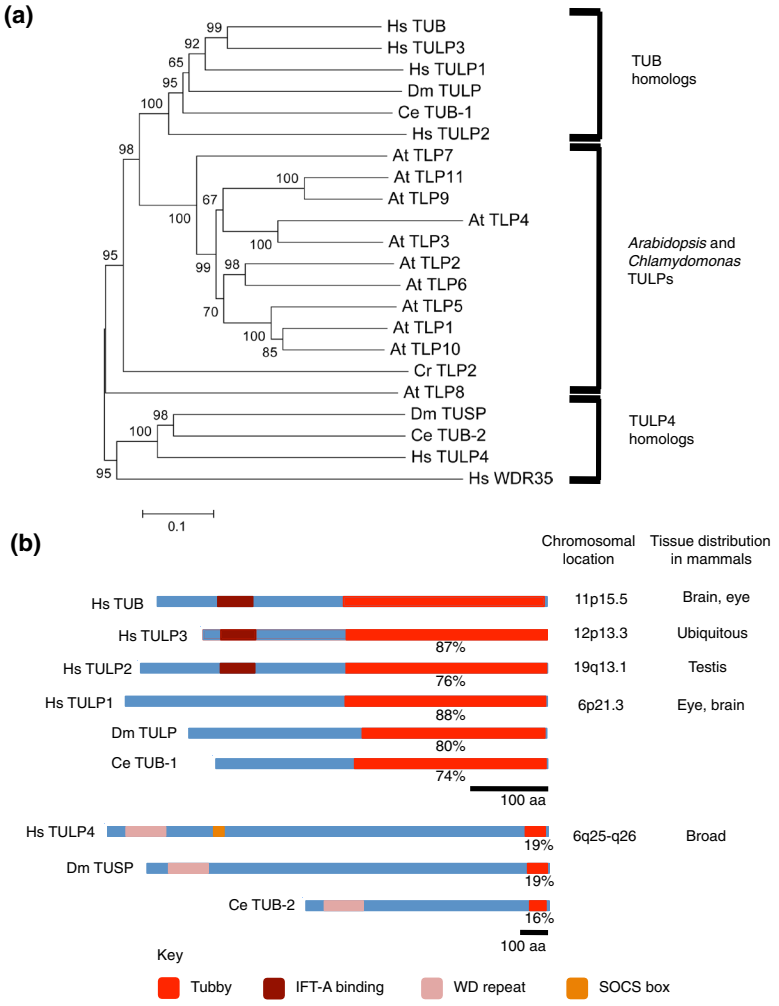


**Figure 1. Evolutionary relationships and domain architecture of the tubby family proteins. (a)** Evolutionary relationships between the tubby family proteins from different species. Sequences were aligned using the neighbor-joining method in ClustalW2 and the phylogenetic tree was drawn using Mega 5 [67]. Bootstrap values were calculated over 1,000 iterations and values greater than 50% are shown as percentages next to branches. The proportion of amino acid differences is indicated by the bar below. Note that the IFT-A complex protein WDR35 is related to TULP4. The NCBI accession numbers of the various members are as follows: Hs TUB, NP_813977.1; Hs TULP3, NP_003315.2; Hs TULP2, NP_003314.2; Hs TULP1, NP_003313.3; Hs TULP4, NP_064630.2; Dm TULP, NP_995911.1; Ce TUB-1, NP_495710.1; Cr TLP2, XP_001692116.1; Ce TUB-2, NP_490913.2; Dm TUSP, NP_651573.4; AtTLP7, NP_564627.1; AtTLP10, NP_173899.1; AtTLP5, NP_564485.1; AtTLP1, NP_849894.1; AtTLP6, NP_175160.2; AtTLP2, NP_849975.1; AtTLP3, NP_850481.1; AtTLP9, NP_187289.1; AtTLP11, NP_197369.2; AtTLP4, NP_176385.1; AtTLP8, NP_173059.1; Hs WDR35, NP_001006658.1. For a more detailed phylogenetic tree see http://www.treefam.org (accession TF314076). **(b)** Comparison of the domain structures of the tubby family proteins from different species. The percentage amino acid similarity of the tubby domain with respect to the canonical human TUB tubby domain is shown below each protein. Hs, *Homo sapiens*; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Cr, *Chlamydomonas reinhardtii*; At, *Arabidopsis thaliana*; IFT-A, Intraflagellar transport complex A; SOCS, suppressor of cytokine signaling.

The tubby-like family of proteins is more extensive in plants, arising predominantly from segmental duplication events. This family is distinct from the mammalian cluster of tubby-like proteins and comprises 11 and 14 members in *Arabidopsis* and rice, respectively [20,21] (Figure 1a). Most of these proteins have an amino-terminal F-box domain, which may function as a binding motif for specific E3 ubiquitin cullin family ligases. The *Chlamydomonas* tubby homolog TLP2 is also not closely related to any of the mammalian tubby-family proteins and was found to be highly upregulated in a genome-wide transcriptional analysis during flagellar regeneration in the green algae [22].

The tubby family proteins are related to the phospholipid scramblase family (PLSCRs). This was deduced from crystallography studies on the *Arabidopsis* protein At5g01750, a member of the DUF567 family [23]. This *Arabidopsis* protein is related to PLSCRs and bears

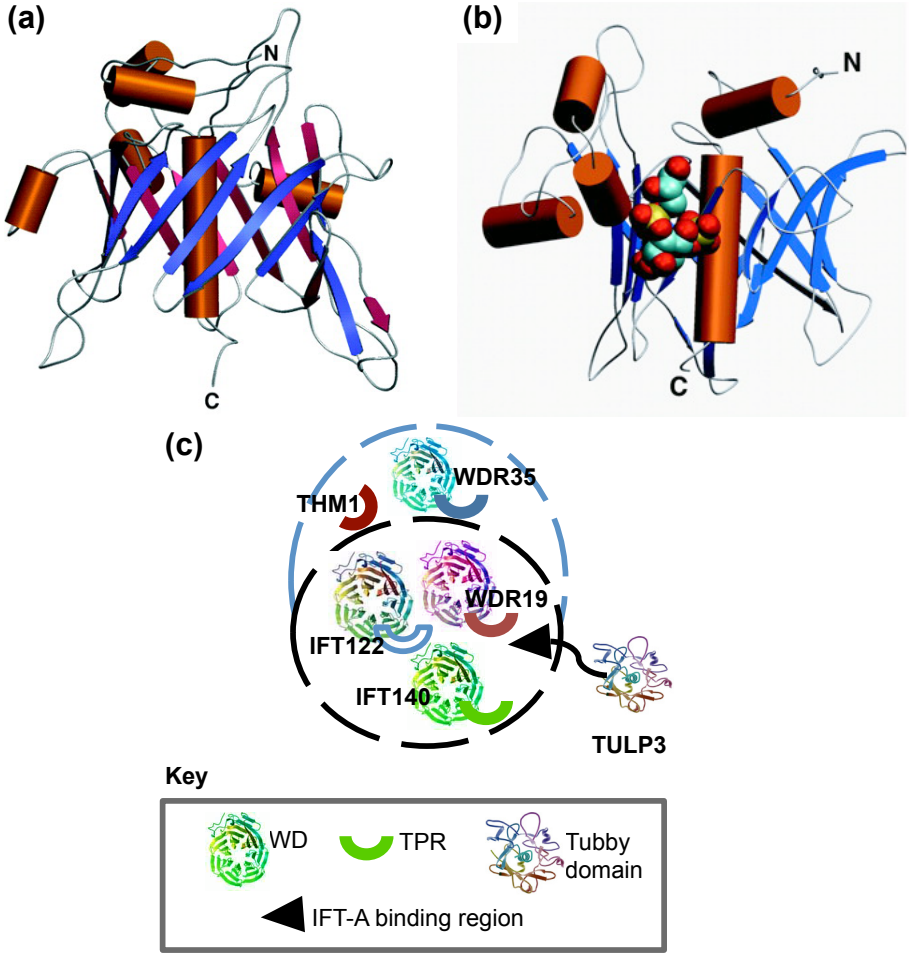


**Figure 2. Functional regions in the tubby family proteins. (a)** Ribbon diagram of the tubby domain from Tub [5]. Helices and β sheets are depicted as cylinders and arrows, respectively. The structure consists of a 12-stranded β barrel filled by a central hydrophobic helix. **(b)** Co-crystal structure of the tubby domain from Tub bound to GPMI-P2, a soluble analog of the head group from PIP2 [6]. The PIP2 analog is shown in a van der Waals sphere representation. The PIP2 analog binds at a site on the tubby β-barrel adjacent to helix 6A. Panels (a) and (b) reproduced with permission from [5] and [6], respectively. **(c)** TULP3 binds to the IFT-A complex. A summary cartoon of the IFT-A complex subunits and their association with TULP3 is shown. The black dotted line encircles the 'core' complex subunits, and the blue dashed line contains the accessory subunits. TULP3 associates with the 'core' IFT-A complex, and this interaction is dependent on an amino-terminal region conserved between TULP3, TUB and TULP2. TPR, tetratricopeptide repeat.

strong structural similarity to the prototypical tubby domain. PLSCRs are a family of cytoplasmic membrane-associated proteins linked by palmitoylation (as opposed to phosphoinositide binding in the case of the tubby family) and mediate flippase activity, the trans-bilayer exchange of membrane phospholipids [23]. The tubby family is found only in eukaryotes, whereas the scramblase/DUF567 family is found in eukaryotes and eubacteria, suggesting that the tubby family of proteins evolved from an ancestral scramblase-like protein.

## Characteristic structural features

The tubby family proteins share a common domain, the signature carboxy-terminal tubby domain, but the amino terminus is much more varied among the members (Figure 1b). The crystal structure of the tubby domain of Tub was solved by the Shapiro lab in 1999 and provides us with remarkable insights into its function [5]. The tubby domain comprises a closed β barrel consisting of 12 anti-parallel strands, surrounding a central hydrophobic α helix (Figure 2a). The hydrophobic helix is located at the carboxyl terminus of the protein. This core central helix is completely disrupted by the spontaneous G to T transversion at a splice donor site in the *Tub* genomic locus in the *tubby* mouse. Subsequent experiments by the same group suggested that the tubby domain interacts with certain membrane phosphoinositides, predominantly phosphatidylinositol 4,5-bisphosphate

($PIP_2$) [6]. Co-crystal structures of the tubby domain and $PIP_2$ analogs show that the lipid-head group interaction occurs in a positively charged cavity in the domain (Figure 2b). The interaction with the phosphate groups is mediated by conserved amino acids K330 (coordinates interaction between the 4- and 5-phosphates) and R332 (stabilizes the 4-phosphate). In addition, the R363 residue coordinates with the inositol ring at the 3-position, whereas the side-chain $NH_2$ group of N310 hydrogen bonds to the oxygen atoms at the 4- and 5-phosphoester positions [6]. The central K330 and R332 residues are most widely conserved among the members of this family of proteins, and R363 and N310 are conserved among the mammalian TULPs. The selective and highly specific binding of the Tub tubby domain to $PIP_2$ has been exploited to create a $PIP_2$ biosensor and allows live tracking of the spatial distribution of this phosphoinositide in cells [24,25].

The amino termini of these proteins are diverse and direct distinct functions. For example, a conserved domain in the amino terminus enables some members of the tubby family (TULP3, TULP2 and TUB but not TULP1 and TULP4) to bind to ciliary IFT-A [26] (Figures 1b and 2c). This unexpected insight came from tandem affinity purification and mass spectrometry analysis of tubby family interacting proteins. Primary cilia are microtubule-based cellular antennae acting as sensory signaling compartments in processes ranging from mammalian sonic hedgehog (Shh) signaling to neuronal control of obesity. Intraflagellar transport is an ancient, conserved mechanism required to assemble cilia and for trafficking within primary cilia [27]. IFT-A has historically been believed to mediate retrograde intraflagellar transport inside the cilia. However, the binding of the IFT-A complex to TULP3 imparts IFT-A with a novel function of directing TULP3's entry into the cilia [26]. Deletion analysis of the TULP3 amino terminus narrowed this binding region to a conserved helix in the amino terminus of the IFT-A-binding TULP members [26,28] (Figure 1b). Furthermore, small interfering RNA (siRNA) depletion of individual subunits of the IFT-A complex showed that three subunits (WDR19, IFT122 and IFT140) form a 'core' IFT-A sub-complex (important in maintaining the stability of the holo-IFT-A complex) and that this core is important for binding to TULP3 [26] (Figure 2c). It is interesting that this IFT-A binding region overlaps with a nuclear localization sequence [6,26,29]. Nuclear localization sequences and ciliary localization motifs often share similarities [29], suggesting evolutionary parallels in mechanisms for localization to these different cellular domains.

Other regions in the amino terminus of tubby family proteins have been proposed to have distinct functional motifs. For example, Tulp1 and Tub have been identified as phagocytosis-stimulating molecules in retinal pigment epithelium (RPE) cells and macrophages, using a phage display strategy [30,31]. These proteins act as extracellular ligands of members of the TAM receptor tyrosine kinase subfamily, including MerTK [31]. The minimal phagocytosis determinant has been mapped to five $K/R(X)_{1\text{-}2}KKK$ motifs in the mouse Tulp1 amino terminus, and combined mutagenesis of all five motifs abrogates its effect on RPE phagocytosis and MerTK binding [31]. However, most of these sites in the mouse Tulp1 are poorly conserved even with other mammalian Tulp1 homologs, and the physiological relevance of MerTK binding remains unclear.

TULP4 and the plant tubby-like proteins also have distinct domains in their amino termini. TULP4 has a WD40 repeat region at positions 78 to 218 and a suppressor of cytokine signaling (SOCS) domain at positions 82 to 208 [7] (Figure 1b). All of the *Arabidopsis* AtTLPs except AtTLP8 contain highly conserved F-box domains in their amino terminus [20,32]. The F-box or SOCS box domain containing proteins act as bridges between specific substrates and generic components of the SCF-type (Skp1-Cullin-F-box) or ECS-type (ElonginC-cullin-SOCS-box) E3 ubiquitin ligase complexes, respectively [33,34]. The F-box domain in AtTLP9 interacts with the *Arabidopsis* Skp1-like 1 or ASK1 protein [20], whereas human TULP4 interacts with cullin 5 and elongins B and C, generic components of E3 ubiquitin ligases (SM and PKJ, unpublished). However, the specific substrates for these E3 ubiquitin ligases are unknown.

In addition to the canonical function in $PIP_2$ binding, the tubby domain has been proposed to have other functions. The tubby domain of Tub has been suggested to function in double-stranded DNA binding, an interaction depending largely on the positively charged surface of this domain [5]. Fusing the amino-terminal half of the Tub or Tulp1 protein to the DNA-binding domain of GAL4 activated transcription of a reporter gene downstream of the GAL4 DNA-binding site [5]. Another study using a protein microarray strategy detected DNA-binding motifs for TULP1 [35]. However, the role of these proteins in transcriptional regulation is not clear, because clear downstream Tub/Tulp1-regulated genes have not been identified, and acidic-domain-GAL4 fusions can activate transcription nonspecifically. The tubby domain of Tub and Tulp1 is thought to function in binding to phagocytic debris [31]. Purified Tulp1 and Tub were observed to bind the surface of apoptotic cells, as assayed by flow cytometry. This binding was especially dependent on the carboxy-terminal 54 amino acids of Tub/Tulp1 and did not depend on the conserved $PIP_2$ binding central residues. However, the identity of the anchoring molecules for Tulp1/Tub on phagocytic cells

and the physiological relevance of these proteins as phagocytic ligands for maintaining retinal homeostasis are unclear.

## Localization and function

Studies on the tissue distribution and subcellular localization of the diverse members of the tubby family help clarify their functions. *Tub* is expressed in the retina and the brain, including the hippocampus, and the paraventricular, ventromedial and arcuate nuclei of the hypothalamus [3,9]. *Tulp1* and *Tulp2* are mainly expressed in the retina and the testis, respectively [8]. However, *Tulp3* is broadly expressed during mouse development and retains a widespread expression pattern in the adult, including in the central nervous system [10,11]. The related invertebrate homologs are also expressed in the nervous system. *C. elegans tub-1* is expressed in ciliated neurons [18,19] and fly *TULP* is expressed in a subset of neuroblasts in early stage embryos and more broadly in the nervous system in late-stage embryos [36]. The distant family member *Tulp4* is broadly expressed, including in the mouse brain and testis [7]. In humans, the full-length transcript is detected in the brain, skeletal muscle and kidney and a smaller transcript is strongly expressed in the heart and kidney [7]. Fly *TUSP* is detected in bilateral groups of brain cells and in the antennal-maxillary sensory neurons in the embryos [36]. Most of the plant *TULP*s are expressed ubiquitously except *AtTLP5* and *AtTLP8*, which have a more restricted expression [20].

The tubby family proteins have been reported to localize to cytoplasmic, plasma membrane and nuclear fractions in transiently transfected cells and in stable cultured cell lines [5,16,26,37]. *TUB* has at least two alternative splice forms, differing in their amino termini, encoding predicted proteins of 561 and 506 amino acids [26,37]. Immunohistochemical analyses using an antibody capable of detecting both the isoforms show Tub to be localized notably in high concentrations in the nucleoli of brain neurons, with lower protein levels in the cytoplasm [37]. In addition, in transfected cells, mutations in the conserved $PIP_2$-binding residues of Tub result in its translocation to the nucleus [6], suggesting that membrane association anchors Tub to sequester it from transport to the nucleus. *Tulp1* is expressed exclusively in the photoreceptors, localizing to the inner segment, connecting cilia, perikarya and synaptic terminals [38,39]. Clonal stable lines of tubby family proteins in cultured cells suggest that TULP2 and TULP4 are exclusively cytoplasmic [7,26]. Tulp3 is detected in both the cytoplasmic and nuclear fractions of mouse embryo extracts [16]. In TULP3-expressing stable cell lines, inhibition of nuclear export using leptomycin-B or mutations in its conserved $PIP_2$ binding residues results in its translocation to the nucleus [26]. In the cytoplasm, Tulp3/TULP3 is localized to the primary ciliary base and also to punctate spots throughout the cilia, a localization observed in a wide range of cultured ciliated cells, including mouse embryonic fibroblasts [16,26]. The localization of TULP3/Tulp3 to the cilia is strongly dependent on its binding to the core-IFT-A proteins, as siRNA-mediated depletion (WDR19, IFT122 and IFT140) or knockouts (Ift122) prevent its ciliary localization [26,40] (Figure 2b). However, the holo-IFT-A complex also regulates retrograde intraflagellar transport of TULP3 inside the cilia, as depletion of other accessory components of the IFT-A complex (THM1 and WDR35, which are not important in maintaining core IFT-A complex architecture) results in its being accumulated at ciliary tips. Therefore, the IFT-A complex not only provides ciliary access to TULP3, but also regulates its retrograde transport inside the primary cilia [26].

Defects in the tubby-family proteins result in characteristic phenotypes and severe disease syndromes. The *tubby* mouse shows maturity-onset obesity, blindness and deafness. Obesity in *tubby* mice is slowly progressive, with weights beginning to diverge at about 12 to 16 weeks and subsequently reaching twice that of wild-type controls [1,41]. Along with the weight gain, the *tubby* mice show increased insulin levels (insulin resistance) but normal glucose levels (normoglycemic) [1,41]. Even before the onset of obesity, the earliest defects in metabolism in the *tubby* mice seem to be a paradoxical failure to use carbohydrates as an energy source and an increased reliance on fat metabolism and β-oxidation (used normally during starvation for energy needs) [41]. Although *tubby* mice increase food intake as they age, their food intake surpasses that of wild-type controls only after they weigh significantly more, reflecting their need for higher energy to maintain the increased body mass [41]. Hypothalamic mediators important in the central control of obesity, including neuropeptide Y (NPY), Agouti-related peptide (Agrp) and Orexin, are upregulated in the hypothalamus by 7 to 8 weeks, before the onset of obesity [41]. After the onset of obesity, the *tubby* mice show altered levels of NPY and proopiomelanocortin (POMC) in the hypothalamus [42]; however, it is not clear whether these observed neurochemical changes are causative or arise as a consequence of the obesity syndrome. Aside from the central effects, Tub might also be a mediator of insulin signaling and energy metabolism in the adipose tissue [43,44]. *Tub* is expressed in the adipose tissue, is upregulated in 3T3-L1 pre-adipocytes during adipocyte differentiation and is upregulated in insulin-resistant 3T3-L1 adipocytes [44]. It is also tyrosine phosphorylated following insulin treatment in both neuronal PC12 and 3T3-L1 cells [43,44]. In humans, *TUB* has been identified as a candidate gene influencing body weight [45] and

polymorphisms of this gene are associated with body composition and eating behavior in middle-aged women [46]. In addition to obesity, *tubby* mice also develop progressive neurosensory deficits, including retinal and cochlear degeneration [2]. The cochlear degeneration is dependent on the presence of polymorphisms in the microtubule-associated protein gene *Map1a* in the C57BL/6J background; however, the biochemical mechanism of this genetic interaction is unclear [47]. A null mutant of *Tub* is phenotypically indistinguishable from *tubby* mice with regard to weight gain and retinal degeneration [48].

*TULP1* mutations in humans result in retinitis pigmentosa type 14, which is inherited in an autosomal recessive manner [12-14]. Null mutations of *Tulp1* in mice result in early-onset (abnormal outer and inner segments by 2 weeks of age) and progressive photoreceptor degeneration [15,39,49]. The retinal degeneration in these knockout mice is earlier than in the *tubby* mice, and the visual deficits are finally associated with apoptosis of the retinal photoreceptors in both *Tub* and *Tulp1* knockout mice. *Tulp1* knockout retinal photoreceptors show mislocalization of rod and cone opsins in the inner segments even before the photoreceptor degeneration starts, suggesting that Tulp1 is important in intracellular vesicular trafficking [39]. In addition, *Tulp1* knockout mice show early defects in photoreceptor synapses and stunting of bipolar dendrites at stages before retinal degeneration, suggesting that Tulp1 might be critical for normal development of the photoreceptor synapse [38]. Double knockouts of *Tulp1* and *tubby* show more rapid retinal degeneration than either single knockout [39].

*Tulp3* mutant mice show embryonic lethality on or before embryonic day 14.5 and have defects, including exencephaly, spina bifida, micropthalmia and polydactyly [10,16,17,50]. On closer inspection, the lumbar neural tube shows increased Shh signaling apparent from the ventralization of the neuronal subtypes [16,17]. Similar phenotypes are present in mutants of IFT-A complex subunits Ift122 and Thm1 [40,51,52].

Currently, there are no knockouts for either mouse *Tulp2* or *Tulp4*. However, the *C. elegans tub-2* was identified in an RNA interference (RNAi) screen for altered innate immune responsiveness, and siRNA-mediated depletion of Tulp4 decreased production of the cytokine interleukin-6 in murine macrophages in response to bacterial lipopolysaccharides [53]. Of the plant tubby family members, mutants of *AtTLP9* are abscisic-acid-insensitive, suggesting that *AtTLP9* is important in the abscisic acid signaling pathway [20]. Expression of the members of the rice tubby family is induced on infection with microorganisms that cause bacterial blight, suggesting a role in host-pathogen interactions [54].

## Frontiers

The *tubby*-mouse syndrome belongs to a growing class of monogenic obesity syndromes that includes congenital leptin deficiency and leptin receptor deficiency [55]. Recent studies suggest that monogenic obesity syndromes can also be caused by defects in neuronal cilia or defects in trafficking to this compartment. For example, disruption of intraflagellar transport in adult mice in the POMC-expressing hypothalamic axis results in hyperphagia-induced obesity [56]. In addition, primary cilia in the central nervous system neurons are rich in G protein-coupled receptors (GPCRs) such as melanin concentrating hormone receptor (Mchr1 [57]) and downstream effectors such as adenylyl cyclase type 3 (ACIII [58]). Mchr1 is involved in the regulation of feeding and energy balance [59,60], and ACIII-deficient mice become obese with age [61]. Patients with complex developmental disorders such as Bardet-Biedl syndrome (BBS) also develop obesity, and a complex of proteins involved in BBS, known as the BBSome, has been shown to transport specific ciliary proteins [62-64]. The cause of obesity in the *tubby* mice has been attributed to defects in hypothalamic signaling, as *Tub* is highly expressed in the hypothalamus. However, the mechanism by which Tub regulates the neuroendocrine axis in regulating obesity is not clear and is one of the pressing questions that need to be addressed to understand the pathogenesis of obesity.

Recent insights into the functions of tubby family proteins are beginning to provide us with intriguing possibilities into how this could be achieved. The bipartite structural organization of tubby family proteins helps to bridge the membrane compartment with additional components, and disruption of domains important in either function result in severe functional consequences. This is most clear in the case of TULP3, which localizes to the cilia and regulates GPCR trafficking to this compartment, in both cultured cell lines and in primary hippocampal neurons [26]. Both the IFT-A-binding domain and the phosphoinositide-binding tubby domain are important in GPCR trafficking, because disruption of either motif in full-length TULP3 results in similar defects. In addition, the amino-terminal IFT-A-binding fragment also acts as a dominant negative in these processes. Thus, TULP3 functionally links the membrane phosphoinositides to the IFT-A complex to gate GPCR traffic to the cilia [26] (Figure 3a). Similar bridging functions are postulated for Tulp1, in which the MerTK-binding amino-terminal motif bridges the phagocytic debris with its carboxy-terminal tubby domain, thereby regulating phagocytosis in cultured RPEs [31]. In the case of Tub, the functional consequences of its disruption in hypothalamic neurons are not clear, but it is possible that it regulates GPCR trafficking and cellular signaling in these neurons. Although TUB is

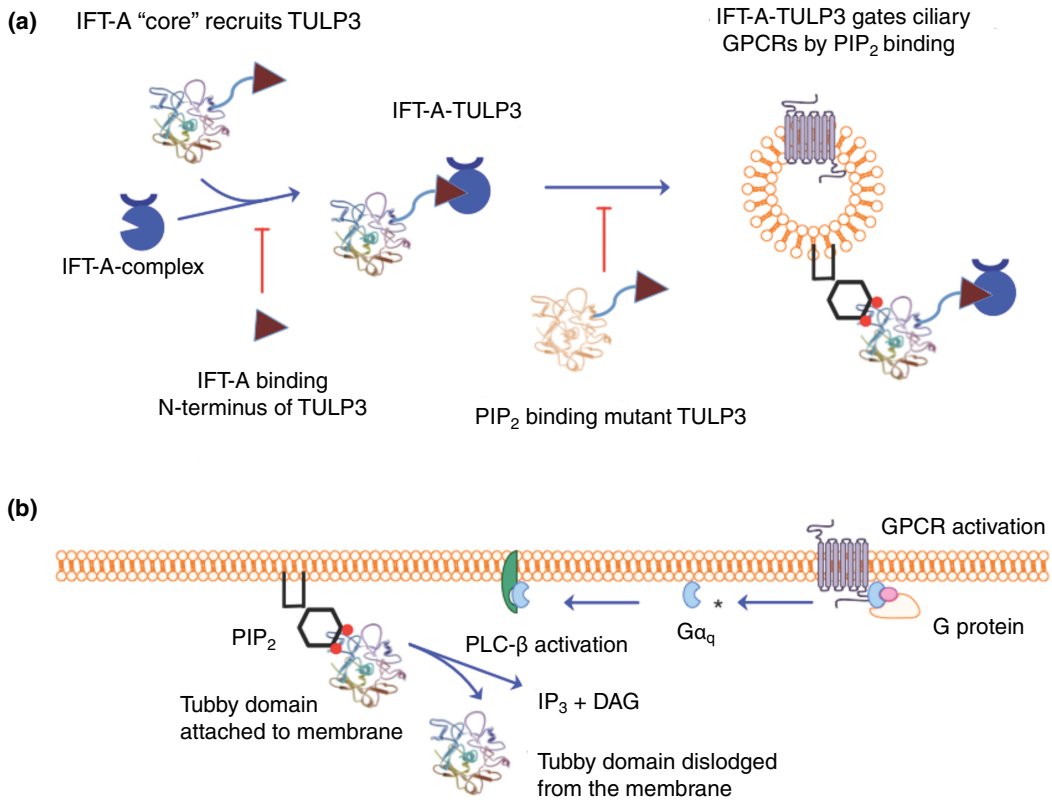


**Figure 3. Functional regulation of the tubby family proteins. (a)** The IFT-A core recruits TULP3 by binding to its IFT-A binding region in the amino terminus. The TULP3 amino-terminal fragment inhibits TULP3 loading to the IFT-A complex. The IFT-A-TULP3 interaction gates ciliary GPCRs by $PIP_2$ binding through TULP3's tubby domain. A full-length TULP3 with defective $PIP_2$ binding is thought to inhibit loading of TULP3-IFT-A to the $PIP_2$ vesicles [26]. Thus, TULP3 bridges the membrane phosphoinositides and IFT-A complex to gate ciliary GPCR trafficking. **(b)** The tubby domain is attached to membrane $PIP_2$. Following activation of $G\alpha_q$ to $G\alpha_q$* by GPCR activation, $PIP_2$ hydrolysis causes the tubby domain to be dislodged from the membrane [6]. $IP_3$, inositol triphosphate; DAG, diacylglycerol; PLC, phospholipase C.

associated with the IFT-A complex [26], the localization of TUB to the neuronal cilia has not yet been detected. Given the fact that neuronal ciliary function contributes to the development of obesity, more detailed analyses of endogenous TUB in these cilia are thus necessary.

Other studies also suggest the role of these proteins in related vesicular trafficking processes. For example, the *C. elegans* TUB-1 interacts with a Rab GTPase-activating protein, RBG-3, in a yeast two-hybrid screen, and RNAi of *rbg-3* reduces fat deposition in the *tub-1* mutant [19], suggesting an evolutionarily conserved role of these proteins. Tulp1 is important in rhodopsin trafficking to the outer photoreceptor segment [39], and its association with dynamin-1, a protein implicated in endocytic vesicle trafficking, may be important in this process [65]. Cellular signaling might in turn affect tubby domain containing proteins, and this adds another level of functional complexity in their regulation. This is exemplified by the regulated dislodgement of this domain from the plasma membrane on activation of $G\alpha_q$-coupled GPCRs. Activation of these GPCRs result in changes in membrane $PIP_2$ levels and subsequent translocation of transfected Tub to the nucleus [6] (Figure 3b). Thus, the tubby family proteins promote vesicular trafficking in a highly regulated manner, and future research should focus on the role of these processes in the *tubby*-mouse syndrome.

A recurring functional feature of this class of proteins is their involvement in diverse signaling processes. For example, *Tulp3* is expressed early during mouse development and, notably, mutations in *Tulp3* result in developmental defects in Shh-dependent dorso-ventral patterning of the neural tube [16,17]. Similar phenotypes are observed in the IFT-A mutants [40,51,52]. IFT-A associates with Tulp3 and has both an early role in delivering Tulp3 to the cilia and another role in retrograde ciliary traffic of Tulp3, compromising Tulp3 function in either case. This explains why the Tulp3 and IFT-A complex act coordinately as negative regulators of the Shh signaling pathway. However, it is not clear how

Tulp3-IFT-A regulates Shh signaling. The frizzled family GPCR Smoothened (Smo) is trafficked to the cilia in a Shh-dependent manner [66]. However, Tulp3 does not regulate Smo trafficking to the cilia [16,26], and genetic epistasis experiments suggest that, similar to the IFT-A subunit mutants, Tulp3 restricts activity of the transcription factor Gli2 in an intraflagellar transport-dependent manner downstream of Shh and Smo [16]. Besides, Gli3 processing is not impaired [16,17,26]. As TULP3 regulates GPCR trafficking in a wide variety of ciliated cells, defects in the developmental patterning of the neural tube could be regulated by the trafficking of a GPCR other than Smo. In addition, the Tulp3-IFT-A interactome would shed new light on the mechanisms by which the negative regulation on the Shh pathway is achieved. Future research should be directed towards the discovery of the key players important in Tulp3-mediated developmental patterning.

Since the positional cloning of *Tub* in 1996, studies on Tub and related family members are starting to provide us with remarkable insights into the *tubby*-mouse syndrome and related diseases. These molecules have now been shown to have major roles in coordinating multiple signaling pathways, including ciliary GPCR trafficking and Shh signaling during development. The emerging paradigm of these proteins serving as bipartite bridges, through their phosphoinositide-binding tubby and unique amino-terminal functional domains, simplifies the inherent complexity of their diverse functional attributes. Better understanding of the mechanisms of action of this protein family promises novel therapeutic targets for treating obesity.

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