Molecular Biology Reports (2021) 48:6131–6143
https://doi.org/10.1007/s11033-021-06585-6

REVIEW

Cytoplasmic FMR1 interacting protein (CYFIP) family members and their function in neural development and disorders

Ísis Venturi Biembengut[1] · Isabelle Leticia Zaboroski Silva[1] · Tatiana de Arruda Campos Brasil de Souza[1] · Patrícia Shigunov[1]

Received: 12 April 2021 / Accepted: 20 July 2021 / Published online: 29 July 2021

Abstract

In humans, the cytoplasmic FMR1 interacting protein (CYFIP) family is composed of CYFIP1 and CYFIP2. Despite their high similarity and shared interaction with many partners, CYFIP1 and CYFIP2 act at different points in cellular processes. CYFIP1 and CYFIP2 have different expression levels in human tissues, and knockout animals die at different time points of development. CYFIP1, similar to CYFIP2, acts in the WAVE regulatory complex (WRC) and plays a role in actin dynamics through the activation of the Arp2/3 complex and in a posttranscriptional regulatory complex with the fragile X mental retardation protein (FMRP). Previous reports have shown that CYFIP1 and CYFIP2 may play roles in posttranscriptional regulation in different ways. While CYFIP1 is involved in translation initiation via the 5′UTR, CYFIP2 may regulate mRNA expression via the 3′UTR. In addition, this CYFIP protein family is involved in neural development and maturation as well as in different neural disorders, such as intellectual disabilities, autistic spectrum disorders, and Alzheimer's disease. In this review, we map diverse studies regarding the functions, regulation, and implications of CYFIP proteins in a series of molecular pathways. We also highlight mutations and their structural effects both in functional studies and in neural diseases.

Keywords Cytoplasmic FMR1 interacting protein · WAVE regulatory complex · Neural development · Neural disorders

Introduction

Neurological development is highly regulated, and perturbations in this process can cause different types of neural disorders. These disorders may appear in childhood, such as autistic spectrum disorder (ASD) [1], infantile epileptic encephalopathy (IEE) [2], and attention deficit hyperactivity disorder (ADHD) [3], or they may appear later in life, such as schizophrenia [4] and Alzheimer's disease [5]. In addition to disorders, such as ASD and schizophrenia, which share some phenotypes, including the difficulty of social communication, they do not necessarily share the same genetic background [6]. However, the association of CYFIP family members with these disorders has been described by other studies [7–10]. For example, the same variant in *CYFIP1* has been described as a possible association in ASD and ADHD [11]. Other studies have pointed out the relationship between CYFIP1/CYFIP2 and other neural disorders, such as Alzheimer's disease [12], intellectual disabilities [13, 14], epileptic encephalopathy [15], and even compulsive behavior [16, 17]. Furthermore, CYFIP family members have important functions after neurodevelopment, and they have been implicated in adult synaptic plasticity and memory [12, 18].

The human CYFIP family consists of the following two homologous proteins: CYFIP1 and CYFIP2. CYFIP1 (cytoplasmic FMR1 interacting protein 1) is also known as Sra1 (specifically Rac1 associated protein 1) [19]. The gene (*CYFIP1* or *KIAA0068;* NG_054889.1) is located on chromosome 15q11.2 and encodes 7 isoforms (UniProtKB Database, Q7L576). The CYFIP1 structure inside the WAVE regulatory complex (WRC) has been resolved by crystallography (PDB 3P8C). The CYFIP2 (cytoplasmic

Ísis Venturi Biembengut and Isabelle Leticia Zaboroski Silva contributed equally to this work.

✉ Patrícia Shigunov
patricia.shigunov@fiocruz.br

Tatiana de Arruda Campos Brasil de Souza
tatiana.brasil@fiocruz.br

[1] Carlos Chagas Institute—FIOCRUZ-PR, Rua Prof. Algacyr Munhoz Mader, 3775, CIC, Curitiba, Paraná 81830-010, Brazil

FMR1 interacting protein 2), also known as PIR121 (p53 inducible protein), gene (*CYFIP2*, *KIAA1168* or *PIR121*; NC_000005.10) is located on chromosome 5q33.3 and encodes 4 isoforms (UniProtKB Database, Q96F07). The CYFIP1 and CYFIP2 proteins are approximately 145 kDa, and they share 88% identity and 95% similarity. These two proteins are highly conserved in several organisms [20], and they share 99% identity with their orthologs in mice [21].

Abekhoukh and Bardoni proposed that the function and role of these two proteins in neuronal maturation are similar [22]. Recently, Schaks and collaborators showed that mutations described for CYFIP2 can be transferred to CYFIP1 and impact the actin dynamics driven by WRC [23]. This result points to a conservative function of the CYFIP family concerning the regulatory complex. Moreover, other studies have suggested that they may perform some different biological functions [20]. CYFIP1 is expressed in most human tissues, and CYFIP2 is more abundant in the brain, kidney, and lymph nodes (Gene ID CYFIP1: 23191; Gene ID CYFIP2: 26999). CYFIP1-knockout mouse embryos die at approximately 9.5 days, and CYFIP2-knockout embryos die at approximately 18.5 days [24]. Zhang and collaborators also reported a difference in expression sites between the two proteins in the hippocampal cells of adult mice. In the brains of mice, CYFIP1 is more highly expressed in nonneuronal cells, whereas CYFIP2 is more highly expressed in neuronal cells [24]. Cioni and colleagues also highlighted the importance of both CYFIP proteins in neural development. Using retinal glial cells (RGCs) from zebrafish embryos and in vivo time-lapse imaging of the *Xenopus* brain, Cioni et al. observed how CYFIP1 and CYFIP2 knockdown affects axon sorting, and they found nonredundant functions of the proteins with CYFIP1 involved in axon extension and CYFIP2 involved in proper axon sorting [25]. Due to contradictory results, the functions of the CYFIP family at the molecular level need to be elucidated to identify the roles of CYFIP1/2 in neural development and the impact of CYFIP1/2 dysfunctions on neural disorders.

CYFIP1

CYFIP1 was initially described as a protein interacting with Rac family small GTPase 1 (RAC1), a member of the Rho small GTPases [19]. RAC1 was first described in HL-60 cells as a substrate for ADP-ribosylation by botulinum toxin C3 ADP-ribosyltransferase [26]. Subsequently, other studies have shown that RAC1 is involved in different cellular processes [27–30], including actin filament reorganization [31] and CYFIP1 signaling [19]. The WRC is a pentameric complex constitutively assembled (see "WAVE regulatory complex" section) [32]. The WRC is activated by Rac-GTP and recruited to the cell periphery, but it does not dissociate after activation [33]. This complex acts in the dynamics of actin cytoskeleton formation and is responsible for the activation of the Arp2/3 complex [34].

CYFIP1 also seems to be involved in the regulation of its WRC partners. Abekhoukh and collaborators suggested that CYFIP1 absence may affect the mRNA expression of WRC partners, including Nap1, Abi1, Wave1, and HSPC300, in mouse *Cyfip1*-depleted neurons and lymphoblastoid cell lines from patients with genomic deletion of *CYFIP1* [35]. Recent data have shown that CYFIP1 may act in proteins that traffic and recruit WRC proteins to the cytoskeleton, such as APC, SHANK1, SHROOM2, and TMSB10, which are downregulated in the amygdala of mice overexpressing *Cyfip1* [36]. These studies point to an important role for CYFIP1 inside the WRC and in its regulation.

CYFIP1 also interacts with fragile X mental retardation protein (FMRP) [21], a protein related to fragile X syndrome (FXS) [37]. FMRP absence is responsible for FXS, in which patients present intellectual disability and autistic spectrum behavior [38]. Although CYFIP1 interacts with FMRP, it does not interact with the FMRP-related proteins, FXR1P and FXR2P [21]. FMRP is also involved in the modulation of proteins that regulate cytoskeletal reorganization [39]. CYFIP1 has been described as an eIF4E-binding protein (4E-BP), forming a posttranscriptional regulatory complex with FMRP in synaptoneurosomes and decreasing the expression of FMRP target mRNAs involved in different neural processes, such as *MAP1B*, *ARC,* and *CaMKIIα*. Upon breve synaptic stimulation, CYFIP1 dissociates from eIF4E, releasing the FMRP target mRNAs for translation [40]. De Rubeis and colleagues showed that this free CYFIP1 binds to WRC, leading to actin cytoskeleton regulation at dendritic spines. This change in CYFIP1 between the two complexes is regulated by RAC1, promoting a conformational switch from a globular to a planar form of CYFIP1, which causes CYFIP1 to dissociate from eIF4E and promotes the binding of NCKAP1 and the subsequent formation of WRC [41]. Through molecular dynamics and docking simulations, Di Marino and collaborators corroborated these findings, showing that CYFIP1 has two conformations depending on its partner, changing with a butterfly-like motion in a RAC1-dependent way [42]. This switch between complexes, acting concomitantly, is important for proper dendritic spine formation and maturation [41].

Cyfip1 haploinsufficient mice present presynaptic dysfunction with lower presynaptic terminal size and abnormal actin polymerization at these sites, which influences synapse assembly and maturation [43]. Cell lineage SY5Y and mouse neurons overexpressing CYFIP1 present an increasing number of neurite branches, and pyramidal neurons from the mouse frontal cortex show an increase in abnormal dendritic spine formation [44]. Recently, Sahasrabudhe and collaborators showed that the absence of CYFIP1 increases RAC activation and mGluR levels on dendritic spines with

WRC being more activated and higher amounts of F-actin on these postsynaptic sites, impacting synaptic plasticity in the mouse hippocampus [45]. Another recent study has shown that treadmill exercise in an animal model of ischemic stroke increases CYFIP1 expression and that a reduction in CYFIP1 impairs proper dendritic spine density and synaptic plasticity recovery in these animals [46]. Kawano and collaborators showed that CYFIP1 is required for axon growth, suggesting that the CYFIP1/WAVE1 complex is carried out to the axon-growth cones by CRMP-2 interacting with kinesin-1, a motor protein responsible for intracellular transportation [47]. These studies point to an important role of CYFIP1 in the establishment of dendritic and axon connections as well as synaptic plasticity.

CYFIP1 has also been described as a target for the Notch signaling pathway. Dziunycz and collaborators showed that NOTCH1 binds to CSL motifs in the CYFIP1 gene promoter, and overexpression of NOTCH1 in squamous cell carcinoma (SCC) in vitro leads to an increase in CYFIP1 at both the mRNA and protein levels. This interaction may decrease the invasive phenotype of SCC [48]. Recently, Habela and collaborators showed that loss of CYFIP1 increases the proliferation of type B1 cells, a type of neural stem cell, in the subventricular zone in the brains of conditional and inducible knockout mice. These authors hypothesized that the absence of CYFIP1 may impair differentiation, leading Type B1 cells to symmetrically self-renew [49]. Because NOTCH is also an important factor in brain development, it may also regulate CYFIP1 expression during neural development by participating in the balance of symmetrical and asymmetrical division in neural stem cells [50, 51]. Further studies are needed to evaluate the impact of NOTCH on CYFIP1 in brain growth and its possible role in neural disorders.

CYFIP2

CYFIP2 interacts with FMRP, as well as other members of its family, namely, FXR1P and FXR2P [19]. The biological function of these interactions is still not well established. Napoli and collaborators showed a potential eIF4E-binding domain conserved at the C-terminus of CYFIP2, indicating a possible relationship of CYFIP2 regulating FMRP target mRNA as described for CYFIP1 [40]. In rat primary cortical neurons, CYFIP2 has been immunoprecipitated with eIF4E, suggesting their interaction [52]. At the same time, the levels of some FMRP targets, such as APP and CaMKIIα, are normal in the synaptosomes of *Cyfip2* heterozygous mice [14]. In contrast, Tiwari and collaborators showed an upregulation of APP and CaMKIIα proteins not accompanied by the increase of their mRNAs levels, indicating that this change occurred through post-transcriptional regulation, in hippocampal synaptosomes from *Cyfip2* $^{+/-}$ mice [12]. These differences could be due to the genetic background of the animals used in the studies; while the study of Han and collaborators used the C56BL/6 J lineage, the Tiwari's study used the C56BL/6 N lineage. This last lineage is known to have a point mutation in the Cyfip2 gene, and this mutation was already shown to reduce Cyfip2 function and was associated with behavior disorders [16] (see "CYFIP family and neural disorders" section). Once it is not well established how this mutation impacts the association of Cyfip2 with its partners, including FMRP, this difference may impact the results obtained from different animal lineages. *CYFIP2* mRNA also appears among the FMRP target mRNAs but not *CYFIP1* [53]. Recently, it has been described that CYFIP2 interacts with 25 other proteins related to RNA metabolism in mouse brains, including proteins involved in mRNA processing and the miRNA pathway, such as Pumilio1 (PUM1) and Argonaute2 (AGO2) [54]. Moreover, circ*CYFIP2*, a sense-overlapping circular RNA spliced from the CYFIP2 transcript, has been described as a sponge for miR-1205. This miRNA regulates the expression of E2F1, a protein previously described to be upregulated in tumors. This circ*CYFIP2*-miR-1205-E3F1 axis is involved in cell proliferation and migration in gastric cancer, suggesting that circ*CYFIP2* may be a biological marker for this disease [55]. *CYFIP2* is upregulated in basal cell carcinoma, suggesting that it may be a biological marker for this type of tumor, but its mechanism is still unclear [56]. Perhaps the *CYFIP2* RNA detected in this previous study was circ*CYFIP2,* which can be more stable than *CYFIP2* mRNA [55], acting in post-transcriptional regulation, such as in gastric cancer and basal cell carcinoma. These data suggest that while CYFIP1 may act in translation initiation, CYFIP2 may regulate translation via the 3′UTR. Additionally, CYFIP2 has been described as a p53-dependent apoptosis factor (hence, it is also known as PIR121). The p53 protein is considered a tumor suppressor, acting as an activating factor for apoptosis and inducing transcription of many genes [57]. Jackson and colleagues identified a p53-responsive element in the *CYFIP2* gene promoter, which leads to the activation of its transcription by the p53 factor [58]. Once p53 expression is lost in many types of cancer [59], CYFIP2 expression may be affected. Further studies are needed to establish the molecular role of CYFIP2 in posttranscriptional regulation and cancer development.

Additionally, researchers have suggested that CYFIP2 can be involved in T cell adhesion. Mayne et al. analyzed $CD4^+$ cells in patients with multiple sclerosis, a disease in which T cell adhesion plays an important role. In these cells, CYFIP2 expression is increased by approximately 4-fold. As the protein acts in the regulation of the WRC complex, high levels of CYFIP2 may facilitate the adhesion of T cells. The analysis of fibronectin-mediated binding in healthy T cells overexpressing CYFIP2 shows a significant increase in adhesion compared to the control. In addition, CYFIP2

knockdown in $CD4^+$ cells from multiple sclerosis patients decreases adhesion [60]. More studies are necessary to dissect the role of CYFIP2 in T cell adhesion and MS.

Levanon and collaborators reported that *CYFIP2* mRNA can be edited by adenosine deaminases acting on RNA (ADARs), enzymes responsible for the exchange of adenosine (A) for inosine (I) in mRNA posttranscriptional editing. This *CYFIP2* editing (resulting in a K320E substitution in the protein) occurs especially in the brain in different species, indicating that it is conserved [61]. Another study has shown that *CYFIP2* editing is related specifically to ADAR2 and that this process is more abundant in the cortex and cerebellar tissues [62]. Because I can be read as a G by the ribosome, A-to-I editing can change the amino acid during translation. This posttranscriptional alteration is important for the function of some proteins, such as GluR2, which is a subunit in the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor in neurons. Without editing by ADARs, the glutamine (Q) residue in the editing site is not substituted by the arginine (R) residue, increasing Ca^{2+} permeability by the AMPA receptor, which may trigger neuronal death [63]. A-to-I editing by ADARs also plays a role in embryogenesis and aging, especially in the brain, highlighting its importance throughout the lifetime. In mice, the editing of ADAR targets, including *CYFIP2*, increases through embryo development, and in some cases, it reaches almost 100% after 21 days postnatal [64]. Recently, Levitsky and collaborators showed that in addition to the high level of protein expression in murine brain cells, the levels of *CYFIP2* RNA editing are increased only in neurons [65]. In humans, embryonic stem cells and fetal brains show no indications of *CYFIP2* RNA editing, while it is present in the adult brain [66]. Interestingly, Nicholas and collaborators showed a decrease in *CYFIP2* editing levels in the human adult brain as the individual ages [67]. Additionally, Bonini and collaborators showed that rat cortical cells treated with glutamate, an important excitatory neurotransmitter, have decreased ADAR2 expression and self-editing, which affects *CYFIP2* RNA editing levels. It remains to be elucidated how this impacts neural functioning [68]. These reports show that *CYFIP2* RNA editing is important to neural function and maturation, and further studies are needed to understand this relationship.

CYFIP2 is also involved in WRC [69]. Derivery et al. showed that CYFIP2 immunoprecipitates with the complex and that WRC remained inactivated in the basal state of the cell [70]. Furthermore, it is known that Wiskott–Aldrich syndrome protein family verprolin-homologous (WAVE) proteins may need to interact with other proteins to form stable WRC [32]. Another study reported that CYFIP2 may stabilize WAVE protein in the cortex of mice. Also, it shows that *Cyfip2* haploinsufficiency allows the presence of an active WAVE for enough time to promote actin polymerization before WAVE's degradation by low stability [14]. Cioni et al. described how CYFIP2 interacts with xFXR (a RNP marker) or NCKAP1 (a component of WRC) in distinct subcellular compartments in *Xenopus laevis* RGC axons [25], and their data indicated that CYFIP2 is associated more with RNPs along the axon, thereby changing its association with the WRC in the growth cone periphery, corroborating De Rubeis's [41] study on CYFIP1.

WAVE regulatory complex

The WRC is a regulatory complex of approximately 400 kDa, and it is active in the regulation of actin filament polymerization. The WRC is a pentameric complex consisting of the following groups of proteins: (i) WAVE1 or WAVE2 or WAVE3; (ii) CYFIP1 or CYFIP2; (iii) NCKAP1 (Nck-associated protein 1) or NCKAP1L (Nck-associated protein 1 like); (iv) ABI1 (abl interactor 1) or ABI2 (abl interactor 2) or ABI3 (ABI family member 3); and (v) BRK1 (BRICK1 subunit of SCAR/WAVE actin nucleating complex) [33, 69, 71].

The WRC basal activity is in the inactive form [70]. The VCA domain (Verprolin-homology, Central, Acidic) of the WAVE protein is responsible for the activation of the Arp2/3 complex to initiate actin polymerization. The interaction between the complex proteins, mainly between CYFIP and the VCA domain, prevents their interaction with Arp2/3 [72]. The binding of Rac-GTP, the interaction with some phospholipid acids, and the phosphorylation state of some regions of the WAVE protein allow the exposure of the VCA domain of the WAVE protein, which links with Arp2/3 for its activation [73].[1]

To evaluate the interactions inside WRCs, several groups have generated *CYFIP1/2* point mutations to disrupt its binding with other components of the complex and evaluated the effects of these mutations by assays, such as immunoprecipitation and pull-down assays. Furthermore, the effects of these mutations have been observed in cell phenotypes (Table 1). Figure 1 shows the CYFIP1/2 structure, highlighting important sites inside the protein for its proper binding with WAVE, eIF4E, and RAC1.

Actin filaments and microtubules are structures of the cytoskeleton that are critical for cellular polarization and are particularly important in extremely polarized cells, such as neurons. Actin filaments are particularly important in controlling dendrite function and morphology [81]. Dendritic spines are small protrusions of dendrite membranes that help to pass signals to the neuron's body, and they are dynamic

[1] For an overview on the WRC and actin dynamics, we suggest the following reviews: [32, 34, 74–76].

Table 1 CYFIP1/2 mutations reported in the literature and their biological effects

Protein	Mutation	Organism (origin of protein sequence)	Molecular effect reported	Analysis method	Model	References
CYFIP1	C179R	Mouse	Inhibited Rac1 binding in the "A site"	Pull-down assay; Lamellipodia formation	Murine B16-F1 Melanome cells	[23, 77]
	C179R	Human	Impaired binding of WRC to Rac1 without altering the integrity of the recombinant complex	Pull down assay; Arp2/3-mediated pyrene–actin assembly assays	In vitro	[72, 78]
	R190D	Mouse	Inhibited Rac1 binding in the "A site"	Pull-down assay; Lamellipodia formation	Murine B16-F1 Melanome cells	[23, 77]
	R190D	Human	Impaired binding of WRC to Rac1 without altering the integrity of the recombinant complex	Fractional saturation of WRC versus free Rac1-GMPPNP; Pull down assay	In vitro	[72]
	M632D	Human	Impaired binding of WRC to Rac1 without altering the integrity of the recombinant complex	Pull down assay	In vitro	[72]
	E434K/F626A	Human	Impaired binding of WRC to Rac1 without altering the integrity of the recombinant complex	Fractional saturation of WRC versus free Rac1-GMPPNP; Pull down assay	In vitro	[72]
	Y967A	Mouse	Inhibited Rac1 binding in the "D site"	Pull-down assay	Murine B16-F1 Melanome cells	[77]
	Y967A	Human	Inhibited Rac1 binding in the "D site"	Pyrene-actin assembly assay; Pull down assay	In vitro	[78]
	P957A/K958D/I959A	Human	Inhibited Rac1 binding in the "D site"	Pull down assay	In vitro	[78]
	R961D/P963A/R964D	Human	Inhibited Rac1 binding in the "D site"	Pull down assay	In vitro	[78]
	G971W	Human	Inhibited Rac1 binding in the "D site"	Pull down assay	In vitro	[78]
	E974A/F975A/H978A/Q979A	Human	Inhibited Rac1 binding in the "D site"	Pull down assay	In vitro	[78]
	L697D/Y704D	human	Disrupted WAVE C helix contact sites with CYFIP1; Inhibited interaction between CYFIP1 and WAVE's VCA domain; Promoted WRC activation independent of Rac signal	Arp2/3-mediated pyrene–actin assembly assays	In vitro	[72]
	L841A/F844A/W845A	human	Disrupted WAVE V helix contact sites with CYFIP1; Inhibited interaction between CYFIP1 and WAVE's VCA domain; Promoted WRC activation independent of Rac signal	Arp2/3-mediated pyrene–actin assembly assays	In vitro	[72]
	L697D/Y704D/L841A/F844A/W845A (termed WCA*)	Mouse	Inhibited interaction between CYFIP1 and WAVE's VCA domain; Promoted WRC activation independent of Rac signal	Pull-down assay; Lamellipodia formation	B16 Melanome cells	[23, 77]
	F686E	human	Promoted WRC activation independent of Rac signal	Arp2/3-mediated pyrene–actin assembly assays	In vitro	[72]

Table 1 (continued)

Protein	Mutation	Organism (origin of protein sequence)	Molecular effect reported	Analysis method	Model	References
	R87C	Mouse	Restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation)	Lamellipodia formation	Murine B16-F1 Melanome cells	[23]
	I640M	Mouse	Restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation)	Lamellipodia formation	Murine B16-F1 Melanome cells	[23]
	E641K	Mouse	Restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation)	Lamellipodia formation	Murine B16-F1 Melanome cells	[23]
	D700H	Mouse	Restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation)	Lamellipodia formation	Murine B16-F1 Melanome cells	[23]
	Q701R	Mouse	Restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation)	Lamellipodia formation	Murine B16-F1 Melanome cells	[23]
	E1150Dfs*3	Mouse	Impaired lamellipodia formation; unable to associate with remaining WRC subunits; reduced half-life	Pull-down assay; Lamellipodia formation; CHX stability assay	Murine B16-F1 Melanome cells	[23]
	K725E (mutE)	Mouse; Human	Reduced the interaction with eIF4E, but not with the WRC; Inhibited CYFIP1's ability to regulate translation	Immunoprecipitation	Ex vivo HEK293T cells	[41]
	ΔCTD (deletion 1–921)	Mouse; Human	Disruptied regulation of actin dynamics; Partially reduced CYFIP1's regulation of mRNA translation; Abolished its interaction with the NCKAP1 on WRC	Immunoprecipitation	Ex vivo HEK293T cells	[41]
CYFIP2	R87C or R87P or R87L	Human	Weaker interaction with WAVE's VCA domain	Pull-down assay	HEK293T cells	[15]
	K727E (mutE)	*Xenopus*; Zebrafish	Normal axon sorting	Injection with DiI and DiO in the dorsal and ventral retina. Quantification of the Missorting Index	RGC retinal explants	[25]
	ΔCTD (deletion 968–1253)	*Xenopus*; Zebrafish	Increased axon missorting	Injection with DiI and DiO in the dorsal and ventral retina. Quantification of the Missorting Index	RGC retinal explants	[25]
	S968F	Mouse; Human	Protein destabilization	Protein stability assay	HEK293T cells	[16, 17]
	T1067A	Human	Decreased the density of stubby spines	Image analyses	Cultured hippocampal neurons	[79]

The table summarizes mutations induced by researchers to study in vitro protein interactions and functions. The mutations selected focus on understanding CYFIP interactions inside the WRC complex, between CYFIP and eIF4E or FMRP, or related to clinical neurological conditions

structures in which remodeling of the actin cytoskeleton is especially important [81, 82].

In dendritic spines, most of the actin cytoskeleton is in the form of branched structures because these structures have better tensile strength against the plasma membrane [83], which is necessary for the maturation and growth of dendritic spines. Interestingly, by using proteins coupled with GFP to evaluate proteins in structures, Pathania et al. observed how both the CYFIP1 and CYFIP2 proteins are located predominantly in dendritic spines in mouse brain cells. The overexpression of proteins has also resulted in increased complexity of dendrites and dendritic spines with structural changes [84]. This type of abnormality in the dendritic spines is also associated with different types of neural disorders [85]. Many studies have shown that alterations in the expression levels of CYFIP1 and CYFIP2 cause alterations in dendritic spine formation and maturation. For example, overexpression of CYFIP1 increases the proportion of mature spines and spine density [44], while its haploinsufficiency leads to an increase in immature spines and dysregulates the cytoskeleton in dendritic spines [84]. *Cyfip2* haploinsufficient mice also present differences in dendritic spine maturation in their cortex [14], and abnormalities in spines morphology are present in adult CA1 pyramidal neurons of Cyfip2 heterozygous mice [12]. These reports show that the CYFIP family is associated with different neural disorders.

CYFIP family and neural disorders

CYFIP family and its effects on behavior and cognitive parameters

CYFIP1 is associated with different types of behavior and cognitive abnormalities, including intellectual disabilities (ID) [10], autism spectrum disorders (ASD) [7], and schizophrenia [9]. For example, *CYFIP1* mRNA is downregulated

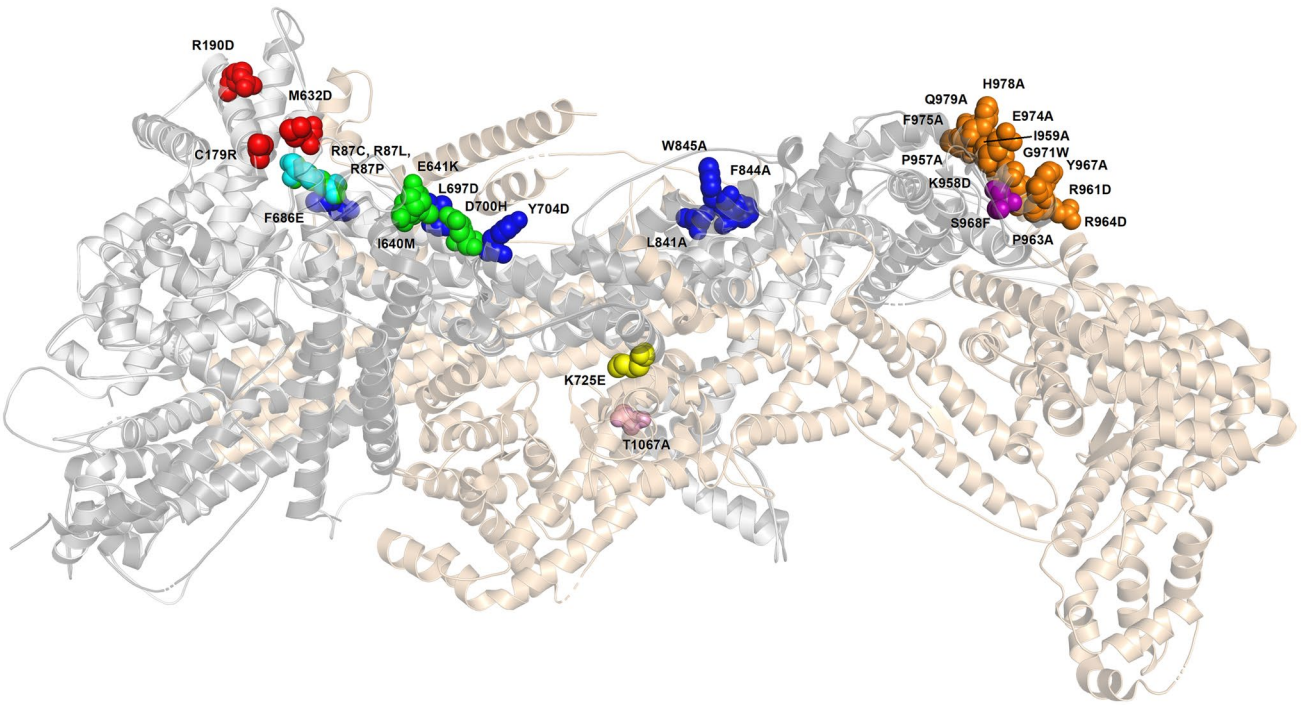

Fig. 1 Model of CYFIP1/2 protein (dark gray/light gray) interacting in the WRC complex (wheat). The model of CYFIP2 was constructed using Modeller and the CYFIP1 structure as a template (PDB: 3P8C, CYFIP1 shares 95% similarity with CYFIP2). Other WRC chains were obtained from PDB structure 3P8C. Spheres emphasize the regions where reported mutations in CYFIP proteins lead to biological effects. Mutations reported/analyzed in CYFIP1: The C179R, R190D, and M632D mutations inhibit RAC1 binding in the "A site" (red). The Y967A, P957A/K958D/I959A, R961D/P963A/R964D, G971W, and E974A/F975A/H978A/Q979A mutations eliminate RAC1 binding in the "D site" (orange). The L697D/Y704D, L841A/F844A/W845A, and F686E impair the interaction between CYFIP proteins and the VCA domain of WAVE (blue). The R87C, I640M, E641K, D700H, and Q701R restored lamellipodia formation even with impaired binding of WRC to Rac1 (C179R/R190D mutation) (green). The K725E mutation reduces the interaction between CYFIP proteins and eIF4E (yellow). Mutations reported/analyzed in CYFIP2: The R87C, R87P, and R87L promote weaker interaction between CYFIP and the VCA domain of WAVE (cyan). The S968F mutation is correlated to protein destabilization (purple). The T1067A mutation decreased the density of stubby spines in cultured hippocampal neurons (pink). More details are described in Table 1. Mutations linked by/were experimentally evaluated together. (Color figure online)

Table 2 CYFIP1/CYFIP2 mutations found in clinical/animal models studies

Protein	Mutation	Condition/pathology associated	Organism	References
CYFIP1	15q11-13	ASD, schizophrenia, intellectual disabilities	Human; Mouse	[9, 10]
CYFIP2	R87C or R87P or R87L or R87H or R87S	Epileptic encephalopathy	Human	[15, 98]
	Y108H	Epileptic encephalopathy	Human	[96]
	M311T	Profound ID	Human	[98]
	A455P	Epileptic encephalopathy	Human	[96]
	M456V	Mild ID	Human	[98]
	E468D	Epileptic encephalopathy	Human	[98]
	T490M	Severe ID, Epilepsy	Human	[98]
	I664M	Epileptic encephalopathy	Human	[96]
	E665K	Mild to moderate ID, epilepsy	Human	[96]
	Y690C	Moderate ID	Human	[98]
	D724H or D724Y or D724G	Epileptic encephalopathy	Human	[96, 98]
	Q725A	Epileptic encephalopathy	Human	[96]
	F888S	Epileptic encephalopathy	Human	[98]
	H1206Y	Moderate ID	Human	[98]
	E1174Aspfs*3	Profound ID, epilepsy, microcephaly	Human	[98]
	Premature stop codon after aminoacid 342	Reduced innate startle threshold	Zebrafish	[80]
	S968F	Lower acute and sensitized response to cocaine; Compulsive-like eating	Mouse	[16, 17]

The table summarizes mutations found in clinical studies associated with neurological diseases and mutations found in animal models affecting its behavior

in the peripheral blood of patients with schizophrenia and upregulated in patients with epilepsy [8]. Additionally, CYFIP1 is associated with Neuroligin3 for controlling hyperactivity, and its reduction affects motor learning, phenotypes associated with ASD, in mutant model animals [86]. These neural disorders are associated with deletions or gains in chromosome 15q11-13 where the *CYFIP1* gene is located (Table 2). The 15q11-13 region is associated with intellectual disabilities, behavior disturbances, and communication delays, such as Prader-Willi and Angelman syndromes [87], and individuals with these disorders usually present deletions or duplications in specific loci, known as breakpoints (BP1, BP2, and BP3). These breaks can occur between BP1-BP2 (Burnside-Butler locus), BP1-BP3 (type I), or BP2-BP3 (type II). Moreover, studies have suggested that alterations including deletions or duplications in the BP1-BP2 region present more severe effects in patients. The genes found in the BP1-BP2 region are *TUBGCP5*, *CYFIP1*, *NIPA1*, and *NIPA2* as well as the noncoding RNA, *WHAMML1* [22, 87]. These genes show potential relevance in neurodisorder development, but *CYFIP1* is considered to be a more significant effector [88]. Oguro-Ando et al. showed that ASD patients with duplication at 15q11-13 present overexpression of CYFIP1 in lymphoblastoid cell lines and the temporal cortex, and they also found an increase in *mTOR* levels and phosphorylation of S6 (p-S6, a downstream effector of mTOR) in the brains of these patients, indicating a role for CYFIP1 in the regulation of mammalian target of rapamycin (mTOR) signaling associated with ASD [44]. Corroborating this report, Abekhoukh and collaborators showed that cortical neurons of mice with *Cyfip1* knockdown have decreased mTOR protein levels and phosphorylation of S6. Interestingly, cortical neurons isolated from Fmr1 knockout mice have increased S6 phosphorylation levels [35]. Patients with FXS present a decrease in *CYFIP1* mRNA levels and an increase in the phosphorylation of two mTOR effectors, S6K1 and AKT, in lymphocytes and the brain [13]. These reports suggest that the effect of CYFIP1 on mTOR signaling may depend on the expression or absence of FMRP.

CYFIP1 is also involved in oligodendrocyte maturation. Silva and collaborators showed that *Cyfip1* haploinsufficient mice have altered brain white matter, decreased myelin thickness and decreased expression of oligodendrocyte maturation markers, such as Cc1 and Mbp. These authors also reported that the mutant mice show a decrease in behavior flexibility, consistent with the effects observed in the brain described by other studies [88]. Once the formation of the myelin sheath involves actin assembly and disassembly, in which Arp2/3 is required for actin assembly and MBP is required for disassembly [89], WRC can be involved in this process. Because proper myelination is important for behavioral flexibility and learning, CYFIP1 may be important in both neuronal and glial processes. Fricano-Kluger et al. showed that mice overexpressing CYFIP1 at the amygdala

have upregulated genes associated with myelination, and they reported that these animals also present learning deficits and increased fear conditioning, comorbidities associated with some cases of ASD [36]. Recent studies have reported that *Cyfip1* haploinsufficient mice also show compulsive-like eating behavior, which is associated with sex and genetic background [90].

CYFIP2 alterations are also associated with behavior and cognitive defects. Although there is some evidence that downregulation of Cyfip2 may be not associated with abnormal social and repetitive behaviour in C56BL/6N *Cyfip2* $^{+/-}$ mice [12], Han et al. showed that C56BL/6J mice heterozygous for *Cyfip2* present some fragile X-like behaviors, and they reported that these effects are enhanced in mice with FMRP knockout combined with *Cyfip2* haploinsufficiency. These behavior alterations are not associated with abnormal hippocampal synapse plasticity, as occurs in *Fmr-1* null mice, but instead impact spine elongation where mGluR agonist-induced *Cyfip2* translation and FMRP-mediated regulation are needed [14]. Patients with FXS show a decrease in CYFIP2 protein expression, while the expression of *CYFIP2* mRNA does not change, suggesting that the absence of FMRP may allow more *CYFIP2* mRNA to translate [13]. To evaluate this hypothesis, one alternative is to analyze the levels of *CYFIP2* mRNA associated with ribosomes through the polysome profiling technique [91], comparing samples with or without genetic alterations. If there is a shift in the amount of *CYFIP2* mRNA between free RNA and polysomal RNA, this could indicate a change in translation.

Kumar et al. showed that a single nucleotide polymorphism (SNP) is located in the *Cyfip2* gene, which causes a missense mutation in CYFIP2 (serine-to-phenylalanine at position 968, S968F) and can generate a minor acute response and sensitization in mice upon cocaine stimulation. These authors also showed a lower dendritic spine density in the brain and a decrease in the frequency of mini-excitatory postsynaptic signaling currents (mEPSCs), which can be associated with drug-induced structural plasticity and, consequently, addiction [16]. The CYFIP2 S968F mutation is also correlated with binge eating in mice, which is associated with obesity and other comorbidities related to eating disorders. Mice with this mutation also present compulsive-like eating, which may be associated with the downregulation of myelination genes in these animals, thereby correlating with other studies that show a decrease in the white matter of patients with eating disorders [17].

CYFIP2 has also been identified as a potential target for the treatment of Alzheimer's disease. In a recent study, Ghosh and collaborators showed that *Cyfip2* $^{+/-}$ aged mice present Aβ accumulation in the brain, gliosis, synapse loss, and memory deficits [52]. A reduction in CYFIP2 expression in neuronal cells initiates a cascade of modifications in the disease, such as hyperphosphorylation of the tau protein, the formation of amyloid plaques, and memory loss [12]. Kim and collaborators showed that neurons from layer 5 of the medial prefrontal cortex (mPFC L5) of *Cyfip2* $^{+/-}$ animals present fewer presynaptic boutons and axonal processes containing mitochondria, and they hypothesized that this alteration in mitochondrial amount may be related to trafficking disturbances [92]. Tau protein is a microtubule-stabilizing protein, and its hyperphosphorylation leads to mislocation to dendritic spines [93], which may compromise its role on microtubules and trafficking machinery. Perhaps the reduction in CYFIP2 levels increases tau phosphorylation, dislocating tau to dendritic spines and compromising the stability of microtubules and organelle trafficking, which may have caused the decrease in mitochondria in the presynaptic region. This has a direct effect on ATP levels needed for synapses and Ca^{2+} clearance, affecting short-term plasticity [92]. Additionally, the interactome of CYFIP2 contains 23 mitochondrial proteins [54], and Cyfip2 has been reported in an enriched mitochondrial fraction of mPFC L5 neurons [92]. It is also known that mitochondrial disturbances in the neural context may lead to neurodegenerative diseases, such as Alzheimer's disease [94]. Thus, CYFIP2 has an important role in mitochondrial pathways and in the development of neurodegenerative diseases that needs to be deepened.

CYFIP2 Arg87 variants and epileptic encephalopathy

Nakashima et al. reported, for the first time, an association of the CYFIP2 protein with early epileptic encephalopathy. These researchers studied 489 individuals with some type of epileptic encephalopathy. Four different and unrelated individuals showed the following variants in the Arg87 residue of the protein: Arg87Cys, Arg87Pro, and Arg87Leu (Fig. 1) [15].

Others have also reported at least six more patients with this syndrome with variants in the same protein residue [95–99]. Zweier and collaborators reported that other variants of the protein are also associated with the syndrome. Seven different "missense" variants have been found in the analyzed patients with the p. Arg87Cys and p.Ile664Met variants being the most recurrent [96]. Begeman and collaborators identified more patients diagnosed with epileptic encephalopathy who carry Arg87 variants, confirming previously described variants and identifying novel ones (p.Arg87His and p.Arg87Ser). Additionally, these authors described that patients with Arg87 substitutions present a profound developmental delay, intellectual disability, epilepsy, and muscle tonus anomalies [98], establishing this position as a hotspot for mutation and subsequently the development of a severe form of the neural disorder.

Although the correlation of early epileptic encephalopathy with the protein mutation at this specific site exists, it has

not yet been possible to prove the mechanisms with which it is associated. One hypothesis based on the structural analysis of the Arg87 site is that changes in that site would promote the continuous activation of the WRC. More precisely, the mutation would be at the interface of interaction with WAVE1, a protein that is part of the complex [15]. This interaction would cause the VCA domain to disconnect and become more exposed, creating a constant and aberrant activation of Arp2/3. Recently, Schaks and collaborators supported this hypothesis; once the induced expression of the R87C variant recovers lamellipodia formation in CYFIP1-2 knockout (KO) cells, the same phenotype is observed with induced expression of the VCA domain of WAVE. The recovery of lamellipodia formation is driven by the R87C variant, which occurs even in the absence of Rac activation, suggesting the constant activation of WRC in the presence of mutated CYFIP2 [21]. This would change the structures of the dendritic spines and affect the balance between excitation/inhibition of synapses [99].

Furthermore, Lee and collaborators showed that the R87 variants of CYFIP2 impact the formation of stress granules (SG). These authors showed that cells with R87 variants spontaneously form clusters containing CYFIP2 and that these clusters do not colocalize with G3BP, a SG marker. From 140 proteins reported as the CYFIP2 interactome, these authors identified 23 proteins as components of SG, and they hypothesized that R87 variants of CYFIP2 may impair the assembly of SG by clustering with the SG members, such as AGO2. In fact, under stress conditions, Ago2 remains in the CYFIP2 cluster and does not migrate to SG. Therefore, the R87 variants may maintain CYFIP2 clustering and impair its function as in WRC formation and actin polymerization [54]. Further studies are needed to understand the real impact of R87 variants on cell metabolism and perhaps establish a future treatment for epileptic encephalopathy.

Conclusions and future directions

Due to their similarity, many researchers have proposed a redundant function between CYFIP1 and CYFIP2, focusing on CYFIP1 function, regulation, and its role in neural diseases. Although CYFIP1 and CYFIP2 share some functions, studies have suggested that they also act in unique ways, considering that both proteins are essential during neuronal development. However, it is not yet precisely understood if this is because they have unique functions (besides their known shared functions) or if it is only related to their different regulatory mechanisms, developmental expression patterns, and cellular and subcellular locations. Thus, future studies are important for a better understanding of both proteins acting in diseases and their differences.

The role of CYFIP family variants in several neural diseases makes them major targets for drug development. Using a *Cyfip2* haploinsufficient mouse model, a recent study has shown that treatment with lithium decreases seizure scores and recovers hyperexcitability in layer 5 neurons of the prefrontal cortex [100]. The molecular mechanism of this process is still unclear, but understanding the structure, molecular dynamics, and the effect of mutations on the complex interactions between CYFIP proteins and their partners may be essential in the design of treatments for neural syndromes. There are future perspectives for treatments using mRNAs or gene therapy in the case of pathologies involving low expression or absence of CYFIPs genes. Even, there are expectations regarding the development of therapy for diseases that result in pathogenic variants, for example (1) use of genetic editing tools; (2) drugs that involve gene silencing by siRNA specific for the mRNA containing the genetic alteration, and (3) molecular docking for identification of drugs with blocking action only of CYFIPs containing the pathogenic variant. The translational knowledge about the structure of proteins, their genetic variants, their location, partners, expression site, affected tissues, and the clinical characteristics of patients affected with pathogenic variants of CYFIPs will allow in the future establishing the eligibility of each patient to different treatments strategies.

Author contributions ÍVB and ILZS contributed to the study conception and design, performed the literature search, and wrote the original manuscript. PS and TACBS critically revised the work. All authors read and approved the final manuscript.

Funding CNPq/Instituto Carlos Chagas Nº 15/2019—PROEP/ICC—442324/2019-7.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any results with human participants or animals performed by any of the authors.

References

1. Courchesne E, Pramparo T, Gazestani VH et al (2019) The ASD living biology: from cell proliferation to clinical phenotype. Mol Psychiatry 24:88–107. https://doi.org/10.1038/s41380-018-0056-y
2. Gataullina S, Bienvenu T, Nabbout R et al (2019) Gene mutations in pediatric epilepsies cause NMDA-pathy, and phasic and tonic GABA-pathy. Dev Med Child Neurol 61:891–898. https://doi.org/10.1111/dmcn.14152
3. Dark C, Homman-Ludiye J, Bryson-Richardson RJ (2018) The role of ADHD associated genes in neurodevelopment. Dev Biol 438:69–83. https://doi.org/10.1016/j.ydbio.2018.03.023

4. Zhuo C, Hou W, Li G et al (2019) The genomics of schizophrenia: shortcomings and solutions. Prog Neuropsychopharmacol Biol Psychiatry 93:71–76. https://doi.org/10.1016/j.pnpbp.2019.03.009
5. Gulisano W, Maugeri D, Baltrons MA et al (2018) Role of amyloid-β and tau proteins in Alzheimer's disease: confuting the amyloid cascade. J Alzheimers Dis 64:S611–S631. https://doi.org/10.3233/JAD-179935
6. Pourcain BS, Robinson EB, Antilla V et al (2018) ASD and schizophrenia show distinct developmental profiles in common genetic overlap with population-based social communication difficulties. Mol Psychiatry 23:263–270. https://doi.org/10.1038/mp.2016.198
7. Noroozi R, Omrani MD, Sayad A et al (2018) Cytoplasmic FMRP interacting protein 1/2 (CYFIP1/2) expression analysis in autism. Metab Brain Dis 33:1353–1358. https://doi.org/10.1007/s11011-018-0249-8
8. Sayad A, Ranjbaran F, Ghafouri-Fard S et al (2018) Expression analysis of CYFIP1 and CAMKK2 genes in the blood of epileptic and schizophrenic patients. J Mol Neurosci 65:336–342. https://doi.org/10.1007/s12031-018-1106-2
9. Domínguez-Iturza N, Lo AC, Shah D et al (2019) The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour. Nat Commun 10:3454. https://doi.org/10.1038/s41467-019-11203-y
10. Clifton NE, Thomas KL, Wilkinson LS et al (2020) FMRP and CYFIP1 at the synapse and their role in psychiatric vulnerability. Complex Psychiatry. https://doi.org/10.1159/000506858
11. Waltes R, Freitag CM, Herlt T et al (2019) Impact of autism-associated genetic variants in interaction with environmental factors on ADHD comorbidities: an exploratory pilot study. J Neural Transm 126:1679–1693. https://doi.org/10.1007/s00702-019-02101-0
12. Tiwari SS, Mizuno K, Ghosh A et al (2016) Alzheimer-related decrease in CYFIP2 links amyloid production to tau hyperphosphorylation and memory loss. Brain 139:2751–2765. https://doi.org/10.1093/brain/aww205
13. Hoeffer CA, Sanchez E, Hagerman RJ et al (2012) Altered mTOR signaling and enhanced CYFIP2 expression levels in subjects with fragile X syndrome. Genes Brain Behav 11:332–341. https://doi.org/10.1111/j.1601-183X.2012.00768.x
14. Han K, Chen H, Gennarino VA et al (2015) Fragile X-like behaviors and abnormal cortical dendritic spines in cytoplasmic FMR1-interacting protein 2-mutant mice. Hum Mol Genet 24:1813–1823. https://doi.org/10.1093/hmg/ddu595
15. Nakashima M, Kato M, Aoto K et al (2018) De novo hotspot variants in *CYFIP2* cause early-onset epileptic encephalopathy. Ann Neurol 83:794–806. https://doi.org/10.1002/ana.25208
16. Kumar V, Kim K, Joseph C et al (2013) C57BL/6N mutation in cytoplasmic FMRP interacting protein 2 regulates cocaine response. Science 342:1508–1512. https://doi.org/10.1126/science.1245503
17. Kirkpatrick SL, Goldberg LR, Yazdani N et al (2017) Cytoplasmic FMR1-interacting protein 2 is a major genetic factor underlying binge eating. Biol Psychiatry 81:757–769. https://doi.org/10.1016/j.biopsych.2016.10.021
18. Zhang Y, Kang Hyae R, Lee SH et al (2020) Enhanced prefrontal neuronal activity and social dominance behavior in postnatal forebrain excitatory neuron-specific Cyfip2 knock-out mice. Front Mol Neurosci 13:1–10. https://doi.org/10.3389/fnmol.2020.574947
19. Kobayashi K, Kuroda S, Fukata M et al (1998) p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. J Biol Chem 273:291–295. https://doi.org/10.1074/jbc.273.1.291
20. Zhang Y, Kang H, Lee Y et al (2019) Smaller body size, early postnatal lethality, and cortical extracellular matrix-related gene expression changes of Cyfip2-null embryonic mice. Front Mol Neurosci 11:482. https://doi.org/10.3389/fnmol.2018.00482
21. Schenck A, Bardoni B, Moro A et al (2001) A highly conserved protein family interacting with the fragile X mental retardation protein (FMRP) and displaying selective interactions with FMRP-related proteins FXR1P and FXR2P. Proc Natl Acad Sci USA 98:8844–8849. https://doi.org/10.1073/pnas.151231598
22. Abekhoukh S, Bardoni B (2014) CYFIP family proteins between autism and intellectual disability: links with Fragile X syndrome. Front Cell Neurosci. https://doi.org/10.3389/fncel.2014.00081
23. Schaks M, Reinke M, Witke W, Rottner K (2020) Molecular dissection of neurodevelopmental disorder-causing mutations in CYFIP2. Cells 9:1355. https://doi.org/10.3390/cells9061355
24. Zhang Y, Kang HR, Han K (2019) Differential cell-type-expression of CYFIP1 and CYFIP2 in the adult mouse hippocampus. Anim Cells Syst 23:380–383. https://doi.org/10.1080/19768354.2019.1696406
25. Cioni J-M, Wong HH-W, Bressan D et al (2018) Axon–axon interactions regulate topographic optic tract sorting via CYFIP2-dependent WAVE complex function. Neuron 97:1078-1093.e6. https://doi.org/10.1016/j.neuron.2018.01.027
26 Didsbury J, Evans T, Snyderman R (1989) Rac, a novel ras-related family of proteins that are botulinum toxin substrates. J Biol Chem 264:5
27. Abo A, Pick E, Hall A et al (1991) Activation of the NADPH oxidase involves the small GTP-binding protein p21rac1. Nature 353:668–670
28. Kim SK (2000) Cell polarity: new PARtners for Cdc42 and Rac. Nat Cell Biol 2:E143–E145. https://doi.org/10.1038/35019623
29. Castilho RM, Squarize CH, Patel V et al (2007) Requirement of Rac1 distinguishes follicular from interfollicular epithelial stem cells. Oncogene 26:5078–5085. https://doi.org/10.1038/sj.onc.1210322
30. Guo F, Cancelas JA, Hildeman D et al (2008) Rac GTPase isoforms Rac1 and Rac2 play a redundant and crucial role in T-cell development. Blood 112:1767–1775. https://doi.org/10.1182/blood-2008-01-132068
31. Ridley AJ, Paterson HF, Johnston CL et al (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70:401–410
32. Takenawa T, Suetsugu S (2007) The WASP–WAVE protein network: connecting the membrane to the cytoskeleton. Nat Rev Mol Cell Biol 8:37–48. https://doi.org/10.1038/nrm2069
33. Innocenti M, Zucconi A, Disanza A et al (2004) Abi1 is essential for the formation and activation of a WAVE2 signalling complex. Nat Cell Biol 6:319–327. https://doi.org/10.1038/ncb1105
34. Campellone KG, Welch MD (2010) A nucleator arms race: cellular control of actin assembly. Nat Rev Mol Cell Biol 11:237–251. https://doi.org/10.1038/nrm2867
35. Abekhoukh S, Sahin HB, Grossi M et al (2017) New insights into the regulatory function of CYFIP1 in the context of WAVE- and FMRP-containing complexes. Dis Model Mech 10:463–474. https://doi.org/10.1242/dmm.025809
36. Fricano-Kugler C, Gordon A, Shin G et al (2019) CYFIP1 overexpression increases fear response in mice but does not affect social or repetitive behavioral phenotypes. Mol Autism 10:25. https://doi.org/10.1186/s13229-019-0278-0
37. Verkerk AJMH, Pieretti M, Sutcliffe JS et al (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914. https://doi.org/10.1016/0092-8674(91)90397-H

38. Bagni C, Oostra BA (2013) Fragile X syndrome: from protein function to therapy. Am J Med Genet A 161A:2809–2821. https://doi.org/10.1002/ajmg.a.36241
39. Michaelsen-Preusse K, Feuge J, Korte M (2018) Imbalance of synaptic actin dynamics as a key to fragile X syndrome? J Physiol 596:2773–2782. https://doi.org/10.1113/JP275571
40. Napoli I, Mercaldo V, Boyl PP et al (2008) The fragile X syndrome protein Represses Activity-Dependent Translation through CYFIP1, a New 4E-BP. Cell 134:1042–1054. https://doi.org/10.1016/j.cell.2008.07.031
41. De Rubeis S, Pasciuto E, Li KW et al (2013) CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron 79:1169–1182. https://doi.org/10.1016/j.neuron.2013.06.039
42. Di Marino D, D'Annessa I, Tancredi H et al (2015) A unique binding mode of the eukaryotic translation initiation factor 4E for guiding the design of novel peptide inhibitors: Cyfip1 and eIF4E Inhibitor Peptides. Protein Sci 24:1370–1382. https://doi.org/10.1002/pro.2708
43. Hsiao K, Harony-Nicolas H, Buxbaum JD et al (2016) Cyfip1 regulates presynaptic activity during development. J Neurosci 36:1564–1576. https://doi.org/10.1523/JNEUROSCI.0511-15.2016
44. Oguro-Ando A, Rosensweig C, Herman E et al (2015) Increased CYFIP1 dosage alters cellular and dendritic morphology and dysregulates mTOR. Mol Psychiatry 20:1069–1078. https://doi.org/10.1038/mp.2014.124
45. Sahasrabudhe A, Begum F, Guevara C et al (2021) Cyfip1 regulates SynGAP1 at hippocampal synapses. Front Synaptic Neurosci 12:581714. https://doi.org/10.3389/fnsyn.2020.581714
46. Shen W, Jin L, Zhu A et al (2021) Treadmill exercise enhances synaptic plasticity in the ischemic penumbra of MCAO mice by inducing the expression of Camk2a via CYFIP1 upregulation. Life Sci 270:119033. https://doi.org/10.1016/j.lfs.2021.119033
47. Kawano Y, Yoshimura T, Tsuboi D et al (2005) CRMP-2 is involved in kinesin-1-dependent transport of the sra-1/WAVE1 complex and axon formation. Mol Cell Biol 25:9920–9935. https://doi.org/10.1128/MCB.25.22.9920-9935.2005
48. Dziunycz PJ, Neu J, Lefort K et al (2017) CYFIP1 is directly controlled by NOTCH1 and down-regulated in cutaneous squamous cell carcinoma. PLoS ONE 12:e0173000. https://doi.org/10.1371/journal.pone.0173000
49. Habela CW, Yoon K-J, Kim N-S et al (2020) Persistent Cyfip1 expression is required to maintain the adult subventricular zone neurogenic niche. J Neurosci 40:2015–2024. https://doi.org/10.1523/JNEUROSCI.2249-19.2020
50. Egger B, Gold KS, Brand AH (2010) Notch regulates the switch from symmetric to asymmetric neural stem cell division in the Drosophila optic lobe. Development 137:2981–2987. https://doi.org/10.1242/dev.051250
51. Contreras EG, Egger B, Gold KS, Brand AH (2018) Dynamic Notch signalling regulates neural stem cell state progression in the Drosophila optic lobe. Neural Dev 13:25. https://doi.org/10.1186/s13064-018-0123-8
52. Ghosh A, Mizuno K, Tiwari SS et al (2020) Alzheimer's disease-related dysregulation of mRNA translation causes key pathological features with ageing. Transl Psychiatry 10:192. https://doi.org/10.1038/s41398-020-00882-7
53. Darnell JC, Van Driesche SJ, Zhang C et al (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146:247–261. https://doi.org/10.1016/j.cell.2011.06.013
54. Lee Y, Zhang Y, Kang H et al (2020) Epilepsy- and intellectual disability-associated CYFIP2 interacts with both actin regulators and RNA-binding proteins in the neonatal mouse forebrain. Biochem Biophys Res Commun 529:1–6. https://doi.org/10.1016/j.bbrc.2020.05.221
55. Lin J, Liao S, Li E et al (2020) circCYFIP2 acts as a sponge of miR-1205 and affects the expression of its target gene E2F1 to regulate gastric cancer metastasis. Mol Ther Nucleic Acids 21:121–132. https://doi.org/10.1016/j.omtn.2020.05.007
56. Liu Y, Liu H, Bian Q (2020) Identification of potential biomarkers associated with basal cell carcinoma. BioMed Res Int 2020:1–10. https://doi.org/10.1155/2020/2073690
57. May P, May E (1999) Twenty years of p53 research: structural and functional aspects of the p53 protein. Oncogene 18:7621–7636. https://doi.org/10.1038/sj.onc.1203285
58. Jackson RS, Cho Y-J, Stein S, Liang P (2007) CYFIP2, a direct p53 target, is leptomycin-B sensitive. Cell Cycle 6:95–103. https://doi.org/10.4161/cc.6.1.3665
59. Ozaki T, Nakagawara A (2011) Role of p53 in cell death and human cancers. Cancers 3:994–1013. https://doi.org/10.3390/cancers3010994
60. Mayne M, Moffatt T, Kong H et al (2004) CYFIP2 is highly abundant in CD4+ cells from multiple sclerosis patients and is involved in T cell adhesion. Eur J Immunol 34:1217–1227. https://doi.org/10.1002/eji.200324726
61. Levanon EY, Halleger M, Kinar Y et al (2005) Evolutionarily conserved human targets of adenosine to inosine RNA editing. Nucleic Acids Res 33:1162–1168. https://doi.org/10.1093/nar/gki239
62. Nishimoto Y, Yamashita T, Hideyama T et al (2008) Determination of editors at the novel A-to-I editing positions. Neurosci Res 61:201–206. https://doi.org/10.1016/j.neures.2008.02.009
63. Kwak S, Nishimoto Y, Yamashita T (2008) Newly identified ADAR-mediated A-to-I editing positions as a tool for ALS research. RNA Biol 5:193–197. https://doi.org/10.4161/rna.6925
64. Wahlstedt H, Daniel C, Enstero M, Ohman M (2009) Large-scale mRNA sequencing determines global regulation of RNA editing during brain development. Genome Res 19:978–986. https://doi.org/10.1101/gr.089409.108
65. Levitsky LI, Kliuchnikova AA, Kuznetsova KG et al (2019) Adenosine-to-inosine RNA editing in mouse and human brain proteomes. Proteomics 19:1900195. https://doi.org/10.1002/pmic.201900195
66. Shtrichman R, Germanguz I, Mandel R et al (2012) Altered A-to-I RNA editing in human embryogenesis. PLoS ONE 7:e41576. https://doi.org/10.1371/journal.pone.0041576
67. Nicholas A, de Magalhaes JP, Kraytsberg Y et al (2010) Age-related gene-specific changes of A-to-I mRNA editing in the human brain. Mech Ageing Dev 131:445–447. https://doi.org/10.1016/j.mad.2010.06.001
68. Bonini D, Filippini A, La Via L et al (2015) Chronic glutamate treatment selectively modulates AMPA RNA editing and ADAR expression and activity in primary cortical neurons. RNA Biol 12:43–53. https://doi.org/10.1080/15476286.2015.1008365
69. Eden S, Rohatgi R, Podtelejnikov AV et al (2002) Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. Nature 418:790–793. https://doi.org/10.1038/nature00859
70. Derivery E, Lombard B, Loew D, Gautreau A (2009) The Wave complex is intrinsically inactive. Cell Motil Cytoskelet 66:777–790. https://doi.org/10.1002/cm.20342
71 Gautreau A, Ho H, Li J et al (2004) Purification and architecture of the ubiquitous Wave complex. PNAS 101:4379–4383
72. Chen Z, Borek D, Padrick SB et al (2010) Structure and control of the actin regulatory WAVE complex. Nature 468:533–538. https://doi.org/10.1038/nature09623
73. Lebensohn AM, Kirshner MW (2009) Activation of the WAVE complex by coincident signals controls actin assembly. Mol Cell 36:512–524. https://doi.org/10.1016/j.molcel.2009.10.024

74. Takahashi K (2012) WAVE2 protein complex coupled to membrane and microtubules. J Oncol. https://doi.org/10.1155/2012/590531
75. Svitkina T (2018) The actin cytoskeleton and actin-based motility. Cold Spring Harb Perspect Biol 10:a018267. https://doi.org/10.1101/cshperspect.a018267
76. Rottner K, Schaks M (2019) Assembling actin filaments for protrusion. Curr Opin Cell Biol 56:53–63. https://doi.org/10.1016/j.ceb.2018.09.004
77. Schaks M, Singh SP, Kage F et al (2018) Distinct interaction sites of Rac GTPase with WAVE regulatory complex have non-redundant functions in vivo. Curr Biol 28:3674-3684.e6. https://doi.org/10.1016/j.cub.2018.10.002
78. Chen B, Chou H-T, Brautigam CA et al (2017) Rac1 GTPase activates the WAVE regulatory complex through two distinct binding sites. Elife 6:1–22. https://doi.org/10.7554/eLife.29795
79. Lee Y, Kim D, Ryu JR et al (2017) Phosphorylation of CYFIP2, a component of the WAVE-regulatory complex, regulates dendritic spine density and neurite outgrowth in cultured hippocampal neurons potentially by affecting the complex assembly. NeuroReport 28:749–754
80. Marsden KC, Jain RA, Wolman MA et al (2018) A Cyfip2-dependent excitatory interneuron pathway establishes the innate startle threshold. Cell Rep 23:878–887. https://doi.org/10.1016/j.celrep.2018.03.095
81. Konietzny A, Bär J, Mikhaylova M (2017) Dendritic actin cytoskeleton: structure, functions, and regulations. Front Cell Neurosci 11:147. https://doi.org/10.3389/fncel.2017.00147
82. Alvarez VA, Sabatini BL (2007) Anatomical and physiological plasticity of dendritic spines. Annu Rev Neurosci 30:79–97. https://doi.org/10.1146/annurev.neuro.30.051606.094222
83. Koseki K, Taniguchi D, Yamashiro S et al (2019) Lamellipodium tip actin barbed ends serve as a force sensor. Genes Cells 24:705–718. https://doi.org/10.1111/gtc.12720
84. Pathania M, Davenport EC, Muir J et al (2014) The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stabilization of mature spines. Transl Psychiatry 4:e374–e374. https://doi.org/10.1038/tp.2014.16
85. Kulkarni VA, Firestein BL (2012) The dendritic tree and brain disorders. Mol Cell Neurosci 50:10–20. https://doi.org/10.1016/j.mcn.2012.03.005
86. Sledziowska M, Kalbassi S, Baudouin SJ (2020) Complex interactions between genes and social environment cause phenotypes associated with autism spectrum disorders in mice. eNeuro 7:1–15. https://doi.org/10.1523/ENEURO.0124-20.2020
87. Butler MG (2017) Clinical and genetic aspects of the 15q11.2 BP1-BP2 microdeletion disorder: 15q11.2 BP1-BP2 microdeletion. J Intellect Disabil Res 61:568–579. https://doi.org/10.1111/jir.12382
88. Silva AI, Haddon JE, Ahmed Syed Y et al (2019) Cyfip1 haploinsufficient rats show white matter changes, myelin thinning, abnormal oligodendrocytes and behavioural inflexibility. Nat Commun 10:3455. https://doi.org/10.1038/s41467-019-11119-7
89. Zuchero JB, Fu M, Sloan SA et al (2015) CNS myelin wrapping is driven by actin disassembly. Dev Cell 34:152–167. https://doi.org/10.1016/j.devcel.2015.06.011
90. Babbs RK, Beierle JA, Ruan QT et al (2019) Cyfip1 haploinsufficiency increases compulsive-like behavior and modulates palatable food intake in mice: dependence on *Cyfip2* genetic background, parent-of origin, and sex. Genes Genet Genome 9:3009–3022. https://doi.org/10.1534/g3.119.400470
91. Faye MD, Graber TE, Holcik M (2014) Assessment of selective mRNA translation in mammalian cells by polysome profiling. J Vis Exp 92:e52295. https://doi.org/10.3791/52295
92. Kim GH, Zhang Y, Kang HR (2020) Altered presynaptic function and number of mitochondria in the medial prefrontal cortex of adult Cyfip2 heterozygous mice. Mol Brain 13:123. https://doi.org/10.1186/s13041-020-00668-4
93. Hoover BR, Reed MN, Su J et al (2010) Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neuron 68:1067–1081. https://doi.org/10.1016/j.neuron.2010.11.030
94. Islam T (2016) Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol Res 39:73–82. https://doi.org/10.1080/01616412.2016.1251711
95. Peng J, Wang Y, He F et al (2018) Novel West syndrome candidate genes in a Chinese cohort. CNS Neurosci Ther 24:1196–1206. https://doi.org/10.1111/cns.12860
96. Zweier M, Begemann A, McWalter K et al (2019) Spatially clustering de novo variants in CYFIP2, encoding the cytoplasmic FMRP interacting protein 2, cause intellectual disability and seizures. Eur J Hum Genet 27:747–759. https://doi.org/10.1038/s41431-018-0331-z
97. Zhong M, Liao S, Li T et al (2019) Early diagnosis improving the outcome of an infant with epileptic encephalopathy with cytoplasmic FMRP interacting protein 2 mutation: case report and literature review. Medicine (Baltimore) 98:e17749. https://doi.org/10.1097/MD.0000000000017749
98. Begeman A, Sticht H, Begtrup A et al (2020) New insights into the clinical and molecular spectrum of the novel CYFIP2-related neurodevelopmental disorder and impairment of the WRC-mediated actin dynamics. Genet Med 23:543–554. https://doi.org/10.1038/s41436-020-01011-x
99. Zhang Y, Lee Y, Han K (2019) Neuronal function and dysfunction of CYFIP2: from actin dynamics to early infantile epileptic encephalopathy. BMB Rep 52:304–311. https://doi.org/10.5483/BMBRep.2019.52.5.097
100. Lee SH, Zhang Y, Park J et al (2020) Haploinsufficiency of Cyfip2 causes lithium-responsive prefrontal dysfunction. Ann Neurol 00:1–18. https://doi.org/10.1002/ana.25827