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Longin domain GAP complexes in nutrient signaling, membrane traffic, and neurodegeneration

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Abstract

Small GTPases act as molecular switches and control numerous cellular processes by virtue of their binding and hydrolysis of guanosine triphosphate (GTP). The activity of small GTPases is coordinated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Recent structural and functional studies have characterized a subset of GAPs whose catalytic units consist of longin domains. Longin domain containing GAPs regulate small GTPases that facilitate nutrient signaling, autophagy, vesicular trafficking, and lysosome homeostasis. All known examples in this GAP family function as part of larger multiprotein complexes. The three characterized mammalian protein complexes in this class are FLCN:FNIP, GATOR1, and C9orf72:SMCR8. Each complex carries out a unique cellular function by regulating distinct small GTPases. In this article, we explore the roles of longin domain GAPs in nutrient sensing, membrane dynamic, vesicular trafficking, and disease. Through a structural lens, we examine the mechanism of each longin domain GAP and highlight potential therapeutic applications.

Keywords

small GTPases; GTPase activating proteins; GAPs; FLCN; GATOR1; autophagy; nutrient signaling; C9orf72; membrane trafficking

Introduction:

Small GTPases function as molecular switches in cell growth, death, migration, and membrane trafficking thanks to their ability to bind and hydrolyze GTP in a regulated manner. The conversion between small GTPases in their GTP-bound and GDP-bound states is facilitated by GTPase activating proteins (GAPs) and guanine exchange factors (GEFs). GAPs stimulate the hydrolysis of GTP, so generating the GDP-bound state, while GEFs promote the exchange back to the GTP-bound state (1).

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Longin domains are a structural motif that serves multiple structural and regulatory functions in cells, including functions related to small GTPase regulation (GEFs, GAPs, and GTPase effectors), trafficking, and vesicle formation. The longin domain was first identified and named for its presence in a family of extended (or “long”) SNARE proteins (2). Longin domains have since been annotated in numerous proteins across eukaryotes (2). Longin domains are composed of a five-stranded antiparallel β-sheet nestled between an α-helix on one side and two α-helices on the other (3). This structural motif is nearly identical to another regulatory motif, the roadblock domain. Roadblock domains, first identified for their role in modulating dynein function, contain five β-sheets and three helices and are distinct from longin domains only in the order of the helices (4). Structural insights into a bacterial Ras-like G protein, MglA and its roadblock domain containing binding partner, MglB, first showed that roadblock domains could function as GAP proteins (5).

Longin domains are present as the most upstream of the DENN (differentially expressed in normal and neoplastic cells) domain. DENN domains contain an N-terminal longin domain and an additional C-terminal lobe (6). Many DENN-containing proteins have been shown to interact with small GTPases, functioning as RabGEFs (7). Structural and sequence analysis of DENN module proteins identified folliculin (FLCN), folliculin interacting protein-1 and 2 (FNIP1/2), NPR2-like (NPRL2) and NPR3-like (NPRL3), C9orf72 and Smith–Magenis syndrome chromosomal region candidate gene 8 (SMCR8) as DENN module proteins homologs (8). The common presence of an N-terminal longin domain in these proteins and its structural similarity to the roadblock domain of MglB first suggested that they might interact with small GTPases (8).

Further exploration into the interaction between longin domain containing proteins and small GTPases revealed some function as GTPase activating proteins (GAPs) in mammals (9, 10) and yeast (11–13). This emerging family of longin domain containing proteins complexes with GTPase activating activity, termed longin domain GAPs, play critical roles in nutrient dependent regulation within the cell. Thus far, three protein complexes have been grouped into the longin domain family; FLCN and FNIP1/2 in the eponymous complex, NPRL2 and NPRL3 in the GATOR1 (GAP activity towards Rags 1) complex, and C9orf72 and SMCR8 in the C9orf72:SMCR8:WDR41 (CSW) complex. FLCN:FNIP1/2 and GATOR1 regulate the mTORC1 nutrient signaling pathway through their GAP activity towards the Rag GTPases (9, 10). The CSW complex is a GAP for the ARF family of proteins (14, 15), which have extensive roles in membrane organization and vesicle traffic (16).

The catalytic units of the complexes listed above are, respectively, FLCN, NPRL2, and SMCR8 (14, 17, 18). The longin domains of these three proteins all contain functional arginine fingers. The Arg finger was first defined structurally in the crystal structure of the human GAP, p120GAP, which it was proposed to stabilize the transition state during cleavage of the γ-phosphate group by a water molecule (19). The Arg finger of a GAP works in conjunction with a catalytic Gln residue in the nucleotide binding site of small GTPases that positions the nucleophilic water for hydrolysis. Over the past few years, structural and functional investigations into longin domain GAPs have provided detailed mechanisms for their GAP activity and a framework for developing therapeutics that alter

their function. Here, we present an overview of each protein complex in the longin domain GAP family, connecting the current mechanistic understanding with related diseases and highlighting the scope of potential for therapeutic applications.

Longin Domain GAPs for Rag GTPases that regulate mTORC1: FLCN and GATOR1

Cells respond to changes in nutrient availability by selectively coordinating cellular growth, proliferation, metabolism, and autophagy. The mechanistic target of rapamycin complex 1 (mTORC1) is central to nutrient-dependent regulation and phosphorylates key substrates that control these processes in response to nutrient fluctuations. mTORC1 is recruited to the lysosomal membrane by the Rag small GTPases. The Rags are outliers among small GTPases, whose phylogenetic relationship to the main group of small GTPases in the Ras superfamily is unclear (20). Rag GTPases lack the lipid modifications present on nearly all other small GTPases. Instead, they contain roadblock domains that bind to the pentameric Ragulator complex, which is responsible for their localization to lysosomal membranes (21, 22). Rag GTPases intermediate between Ragulator and mTORC1 to localize the latter to lysosomal membranes under high nutrient conditions (22–24). The Rag GTPases function as an obligate dimer. Rag dimerization occurs through the C-terminal roadblock domain and contain one molecule of Rag A or B and one of Rag C or D. Two longin domain-containing GAP complexes, FLCN:FNIP1/2 and GATOR1, convert the Rag GTPases between two stable states. The inactive dimer (RagA or B^{GDP}:RagC or D^{GTP}) is present under low nutrient conditions, and the active dimer under high nutrient conditions (RagA or B^{GTP}:RagC or D^{GDP}) (25).

Structural insights into FLCN:FNIP regulation of Rag GTPases

The FLCN:FNIP complex functions as a GAP for RagC/D, transitioning the Rag-Ragulator complex from an inactive to active state under high nutrients conditions (10). Two forms of FNIP are expressed in most cell types, FNIP1 and FNIP2, and both complexes are active against RagC/D (10). FLCN and RagC/D^{GDP} are uniquely important for a subset of mTORC1 substrates, the MiT-TFE transcription factors (18, 26). The transcription factors EB and E3 (TFEB/TFE3) are the best known members of this family. The phosphorylation of other well-known mTORC1 substrates, as well as mTORC1 localization to lysosomes, is essentially independent of RagC/D nucleotide state (18, 26) (Figure 1B). Phosphorylation of TFEB/TFE3 promotes its sequestration to the cytosol, preventing transcription of genes important for autophagy and lysosome biogenesis (Figure 1B). Thus, FLCN and RagC/D^{GDP} selectively regulate a “non-canonical” subset of mTORC1 substrates.

A series of cryo-EM structures have provided snapshots of FLCN:FNIP2 interacting with Rag-Ragulator presenting two binding modes to elucidate the GAP mechanism and nuances of FLCN:FNIP2 regulation (Figure 2A). Although FLCN:FNIP2 functions as a RagC/D GAP, presumably at lysosomes, under high nutrient conditions, paradoxically, it localizes most strongly to the lysosome under conditions of amino acid starvation (27) (Figure 1A). The cryo-EM structure of FLCN in complex with Rag-Ragulator, termed the lysosomal folliculin complex (LFC), demonstrated that this binding mode was a GAP inhibitory

binding mode where FLCN rests in the cleft between RagA and RagC making substantial contacts with both Rag GTPases (18). In this binding mode, the catalytic Arg finger residue, Arg-164, is positioned more than 20 Å away from the RagC nucleotide binding pocket, confirming FLCN is inactive. To accommodate FLCN in this position, when inactive, the Rags undergo a conformational change, rotating to increase the size of the cleft by nearly double that of the active state (18).

The lack of FLCN lysosomal localization under high nutrients suggested that the GAP active binding mode for FLCN was transient. The transient active RagC GAP was trapped and the cryo-EM structure of the “active FLCN complex” (AFC) was determined (Figure 3A). In the AFC, the catalytic Arg-164 is positioned in the nucleotide binding pocket in a manner appropriate to promote GTP hydrolysis (28) (Figure 3B). In comparison to FLCN binding in the LFC, FLCN rotates ~90 degrees to interact solely with RagC, breaking all contact with RagA (Figure 2A). The structure revealed hydrophobic residues in FLCN and FNIP2 that bind to RagC in the AFC but not the inhibitory LFC. Mutation of these residues blocks mTORC1 phosphorylation of TFE3, but not canonical mTORC1 substrates, consistent with their unique role in RagC GAP activity (29).

The transition between the inhibitory binding state and active binding mode is triggered by the presence of cellular nutrients. A nutrient sensor in the mTORC1 pathway, the amino acid transporter solute carrier family 38 member 9 (SLC38A9), was shown to stimulate this conversion upon increase in cellular nutrients (30, 31). When lysosomal arginine levels are low, the N-terminal tail of SLC38A9 is nestled in the transmembrane portion of the transporter. As arginine levels increase, binding of arginine to SLC38A9 outcompetes binding of the N-terminal tail ($SLC38A9^{NT}$) and liberates it on the cytosolic face of the lysosomal membrane. The liberated $SLC38A9^{NT}$ then binds to the Rag dimer inter-domain cleft (32) (Figure 1B). $SLC38A9^{NT}$ binds in such a way that it competes with FLCN as it binds to the inactive LFC, so breaking up the LFC (32). However, the bound $SLC38A9^{NT}$ is compatible with the active AFC, thus its presence promotes RagC/D GAP activity. (Figure 1B). While FLCN:FNIP activation by SLC38A9 in response to lysosomal arginine levels is now understood in great structural and mechanistic detail, it is likely that a variety of other mechanisms also regulate FLCN:FNIP activity. For example, FNIP1 (although not FNIP2) is selectively ubiquitinated and degraded under reduction stress (33).

Structural insights into GATOR1 regulation of Rag GTPases

$RagA/B^{GTP}$ makes the major direct contact with mTORC1 involved in its lysosomal localization, and as such, it is critical for activation. The GAP for RagA/B is the GATOR1 complex. GATOR1 consists of three subunits NPRL2, NPRL3 and DEPDC5. GATOR1 inactivates the Rag dimer by converting $RagA/B^{GTP}$ to the $RagA/B^{GDP}$ under low nutrient conditions. Under these conditions, mTORC1 is inactive with respect to all of its substrates, canonical and non-canonical (Figure 1A).

In a striking parallel to the FLCN:FNIP complex, cryo-EM structures of GATOR1 bound to Rag dimers have revealed both a GAP inhibitory and an active GAP binding mode (34, 35) (Figure 2B). In the inhibitory binding mode, GATOR1 interacts with RagA predominately through the non-catalytic DEPDC5 domain (35). In this conformation, there is no interaction

between the catalytic finger Arg-78 of NPRL2 and RagA (17). The Rag GTPases remain in their active form with the cleft in its open conformation. The GAP active binding mode occurs when GATOR1 is reoriented such that the catalytic domains NPRL2-NPRL3 interact with RagA and the catalytic Arg-78 is positioned in the nucleotide binding pocket (34) (Figure 3C and D). In this binding mode, GATOR1 DEPDC5 domain also makes substantial contacts with RagC. This contrast FLCN, where in its GAP active binding mode, it only interacts with the GTPase it is stimulating hydrolysis of, RagC. The stimuli that trigger the conversion between the inhibitory and active binding modes have yet to be determined.

The answer may lie within the identified regulators that interact directly with GATOR1. A handful of GATOR1 regulators have been uncovered such as GATOR2, KICSTOR and the nutrient sensor SAMTOR (9, 36, 37). GATOR2 is a negative regulator of GATOR1. The GATOR2 complex is massive, consisting of five subunits (WDR24, MIOS, WDR59, SEH1L and SEC13) that adopt an open cage-like structure with an octagonal scaffold (38). Extending from the scaffold are eight pairs of WD40 β-propellers that could mediate interactions between GATOR1, KICSTOR and additional nutrients sensors in the pathway (38).

The membrane-associated protein complex KICSTOR is comprised of a large scaffold-like protein SZT2 and three additional subunits KPTN, ITFG2, and KICS2 (37). The large size and limited structural homology of SZT2 with other proteins has posed challenges for gaining structural insight into the nature of this complex. GATOR1 is thought to interact with the complex through SZT2, but all four KICSTOR subunits are necessary for GAP activity (37, 39). It remains unclear if GATOR2 is required to mediate the interaction between GATOR1 and KICSTOR. It is possible that KICSTOR functions as a nutrient sensor or interacts with GATOR2 and/or additional nutrient sensors to trigger the conversion between the GATOR1 binding modes. Additional studies into the interactions between GATOR1, GATOR2 and KICSTOR are necessary for determining the mechanism of each within the pathway in response to fluctuations in nutrients

A longin domain GAP complex in membrane traffic and neurodegeneration: C9orf72 and SMRC8

Membrane trafficking, or the transport of proteins and lipids between distinct organelle compartments, is critical for maintaining cellular homeostasis. Cells rely on membrane trafficking to grow and recycle key building blocks. Neurons, with their long axonal projections, are especially dependent on long-distance membrane traffic. The ADP-ribosylation factor (ARF) GTPases are particularly central to vesicular transport and are required for axonal regeneration (40, 41). Given their importance, ARF GTPases are tightly regulated by numerous guanine exchange factors (GEFs) and more than twenty GAPs (16). An intronic repeat expansion in C9ORF72 is the most common mutation in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The repeat expansion is associated with a toxic gain-of-function, but also results in a loss-of-function due to reduced C9orf72 protein expression (42–45).

The C9orf72 protein exists in cells as a stable complex with Smith-Magenis chromosome regions 8 (SMCR8) and WD repeat-containing protein 41 (WDR41), referred to as the "CSW" complex. Both C9orf72 and SMCR8 are DENN domain proteins and as such contain N-terminal longin domains. The architecture of this complex resembles an eye slip hook, with the WDR41 β- propeller acting as the eye and SMCR8 nestled in between that and C9orf72 (15, 46) (Figure 3E). The orientation of the longin domains is reminiscent of FLCN:FNIP, which suggested it might possess GAP activity. Additionally, expression of inactive ARF6 was found to rescue a C9orf72-dependent actin defect in axons (47). Taken together, this led to the hypothesis that the CSW complex might be an ARF GAP (15), which was confirmed biochemically using purified proteins (15).

A cryo-EM structure of active CSW trapped in complex with ARF1 revealed that SMCR8 interacts with ARF1 through its longin domain, similar to FLCN and NPRL2 in FLCN:FNIP2 and GATOR1(14). Just like FLCN and NPRL2 in these other two complexes, CSW stimulates GTP hydrolysis through a catalytic Arg finger, Arg-147 of SMCR8 (14) (Figure 3E and F). While CSW is completely inactive against Rag GTPases (15), it displayed activity towards both Arf and Rab GTPases. (46). RAB and ARF proteins belong to different branches of the Ras superfamily, which in general, each have their own GAPs. Side-by-side comparison of CSW GAP activity towards ARF1 and RAB8A revealed that CSW has more than 10-fold lower GAP activity towards RAB8A compared to ARFs (14). In addition, it has so far not been possible to capture an ordered structure of RAB8A in complex with active CSW.

The reversible interaction between CSW and the lysosomal membrane adds an additional level of mechanistic complexity and parallels that of FLCN:FNIP. Under nutrient deprivation, the WDR41 subunit, located distal to the catalytic domain of the protein complex, tethers the CSW complex to the cationic amino acid transporter PQLC2, which resides within the lysosomal membrane (Figure 1C) (48, 49). The physiological function of amino acid starvation dependent lysosomal localization of CSW remains unclear. Since ARF GTPases are not known to reside on the lysosomal membrane (50) it has been proposed that tethering to the lysosome sequesters that GAP away from its substrates in an inhibitory fashion. Another possibility, based on the catalytic domain being located distally to WDR41, is that anchoring CSW to lysosomes orients that it for GAP activity towards ARF GTPases localized on nearby membranes (Figure 1C). Additional studies into the nature of CSW ARF GAP activity at lysosomes and by nutrients will be required.

Longin domain GAPs and disease

Longin domain GAPs are important in maintaining cellular homeostasis through their roles in nutrient signaling and membrane trafficking. Consistent with the importance of their role, mutations in this class of proteins lead to disease phenotypes ranging from cancer to neurodegenerative diseases. Targeting these proteins poses an intriguing potential and largely unexplored therapeutic avenue for treating these conditions.

The tumor suppressor FLCN

FLCN was discovered and so-named because loss of function mutations with the *FLCN* gene were responsible for Birt Hogg-Dubé (BHD) syndrome (51). Patients with BHD develop benign skin tumors, lung cysts and are predisposed to renal cancer. As a result, *FLCN* was identified as a tumor suppressor. Recently, it has been discovered that FLCN is a specific regulator of mTORC1 phosphorylation of the MiT-TFE family of transcription factors, including TFEB (18, 26). TFEB activation drives transcriptional changes that lead to mTORC1 hyperactivation, in turn driving tumorigenesis (26). Most FLCN mutations isolated from BHD patients result in truncation of the protein leading to decreased protein stability or inability to interact with FNIP1/2 (52). A select few mutations can be mapped directly to the longin domain and may directly disrupt the structural motif necessary for GAP activity (52, 53).

All these loss of function mutations directly or indirectly reduce FLCN GAP activity, which results in retained nuclear localization of TFEB/TFE3. In turn, target genes of TFEB/TFE3, such as Rag C and Rag D, are expressed. Higher expression of Rag C/D, even in the absence of FLCN leads to increase mTORC1 activity seen through increased phosphorylation of key mTORC1 substrates S6K and 4E-BP1. Tumors that are observed in BHD patients and mouse models are reliant on mTORC1 activity to grow (26, 54–56). The link between TFEB nuclear localization and mTORC1 activity is critical for the progression of renal tumors. Determining a therapeutic that directly works to enhance or mimic FLCN GAP activity by downregulating $RagC/D^{GTP}$ could provide a targeted approach to reducing mTORC1 hyperactivity for these patients where renal tumors result from FLCN-deficiency. Mitigating downstream effects such as reducing TFEB/TFE3 nuclear localization by another means could be another therapeutic avenue.

Regulation of FLCN/TFEB axis presents itself as an attractive drug target for not only renal cancer, but for the treatment of LSDs and neurodegenerative diseases. Nuclear localization and activation of TFEB/TFE3, controlled by FLCN GAP activity, has been shown to upregulate the clearance and quality control mechanisms of autophagy and lysosome biogenesis (57, 58). In cells, chemical activation of TFEB has been shown to reduce the presence of aggregated α-synuclein, a hallmark of Parkinson’s disease (59). Therefore, inhibiting FLCN GAP activity to prevent translocation of TFEB/TFE3 to the cytoplasm could be a valuable drug approach for selectively upregulating autophagy and treating neurodegenerative diseases characteristic of reduced autophagic clearance. Due to the opposing roles of FLCN GAP activity in tumorigenesis and neurodegenerative diseases, careful consideration is needed in the development of drugs that are designed to alter FLCN GAP activity. These therapeutics need to balance obtaining desired effects in patients without promoting other negative conditions.

GATOR1 and epilepsy

Mutations in all three subunits of GATOR1 have been found in patients with focal epilepsy. The first mutations were identified in the DEPDC5 subunit (60, 61). Mutations in remaining two subunits, NPRL2 and NPRL3, have since been identified (62, 63). In most of these cases, these mutations cause truncation of the protein resulting in loss of function and

presumably prevents GATOR1 complex formation. A handful of mutations map to the NRPL2 and NPRL3 longin domains, suggesting that GAP function could be directly impaired without necessarily affecting complex stability. The best example is a mutation in which the catalytic Arg finger residue of NPRL2 is replaced by Cys (29). Analysis of DEPDC5 variants and brain tissue of patients containing DEPDC5 mutations show hyperactivation of mTORC1, consistent with the known role of the GATOR1 GAP as a negative regulator of mTORC1 (64, 65). Seizures resulting from GATOR1-related focal epilepsy patients have a differing clinical presentation from ion channel related mutations as these seizures emerge from variable foci. GATOR1 subunit mutations present a different pathological mechanism that may require a unique treatment approach. One plausible therapeutic approach to treating GATOR1-related epilepsy conditions is upregulating residual GATOR1 GAP activity where the loss of function is partial. In contrast to the situation with FLCN, our understanding of the detailed mechanisms of GATOR1 GAP regulation is still primitive. Further exploration of these mechanisms is urgently needed.

C9orf72 and neurodegenerative disease

Hexanucleotide-repeat expansions in the non-coding region of the C9orf72 gene are a major cause of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (66). The involvement of C9orf72 in pathways that are disrupted in ALS and FTD, including membrane trafficking, autophagy, and actin dynamics suggests that understanding the molecular mechanism of C9orf72's cellular function is critical to our ability to target and treat the effects of these mutations. Identification of the CSW complex as a GAP for ARF GTPases suggests that loss of function mutations in C9orf72 should result in local accumulation of ARF^{GTP} at sites of CSW activity (14). This hypothesis remains to be tested. However, this would imply that elevated $Arf1^{GTP}$ levels may be associated with pathogenesis of disease. Thus, reducing ARF^{GTP} levels in ALS and FTD patients is a potential therapeutic approach for restoring these cellular functions to a normal function. Two main routes can be taken to accomplish this task: enhancing ARF GAP activity or reducing ARF GEF activity. Additional investigation into specific ARF GEFs is required for a detailed understanding of target proteins in this pathway. Thus far, there has been one report that targeting ARF GEFs can protect motor neurons against degradation (67), providing a proof of concept for this approach. However, additional investigation into specific ARF GEF's is required for a detailed understanding of target proteins in this pathway.

Conclusions and Perspectives:

Longin domain GAPs are an emerging family of protein complexes consisting in mammals of the FLCN:FNIP, GATOR1 and CSW complexes. Each of these protein complex shares similar structural features and functional roles as GAPs. Recent advances have determined the structure of each complex, highlighted their common mechanisms of catalysis and their unique substrates and regulatory mechanisms. The progress in the field has opened many new questions regarding regulatory mechanisms. In the nutrient signaling area, it remains unclear what are the physiological stimuli for GATOR1 to transition from its inhibitory binding state to its GAP active binding mode. In an exciting advance, cholesterol binding

to the lysosomal membrane protein LYCHOS was shown to modulate GATOR1 activity (68). Further understanding will likely require consideration of the roles of the GATOR2 and KICSTOR complexes, whose functions are still murky. The relative roles of FNIP1 and 2 in the FLCN:FNIP complex remain enigmatic. FNIP1 has been shown to act as a reduction stress sensor at mitochondria (33), and it is currently unclear how or if the mitochondrial role for FNIP1 is related to the lysosomal action of the FLCN:FNIP complex. For C9orf72, it is known that low amino acid levels trigger localization to the lysosomal membrane (69, 70), but the role of this localization and its relationship to ARF GAP activity remains unclear. Additional mechanistic details will be beneficial to refine therapeutic approaches and aid in the development of drugs to treat the various diseases that result from mutations in longin domain GAPs.

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Abbreviations:

4E-BP1	4E (eIF4E)-binding protein 1
AFC	active folliculin complex
ALS	amyotrophic lateral sclerosis
ARF	adenosine diphosphate-ribosylation factor
BHD	Birt-Hogg-Dubé
CSW	C9orf72:SMCR8:WDR41
DENN	differentially expressed in normal and neoplastic cells
DEPDC5	DEP Domain Containing 5
FLCN	folliculin
FNIP1	folliculin interacting protein 1
FNIP2	folliculin interacting protein 2
FTD	frontotemporal dementia
GAP	GTPase activating protein
GATOR1	GAP activity toward RAGs 1
GEF	guanine exchange factor
GTP	guanosine triphosphate
ITFG2	integrin alpha FG-GAP repeat containing 2

KICS2	KICSTOR subunit 2
KPTN	kaptin
LFC	lysosomal folliculin complex
LSD	lysosomal storage diseases
LYCHOS	lysosomal cholesterol signaling
MiT-TFE	microphthalmia
MIOS	meiosis regulator for oocyte development
mTORC1	mechanistic target of rapamycin complex 1
NPRL2	nitrogen permease regulator like-2
NPRL3	nitrogen permease regulator like-3
S6K	S6 kinase
SLC38A9	solute carrier family 38 member 9
SMCR8	Smith–Magenis syndrome chromosomal region candidate gene 8
SZT2	seizure threshold 2
TFE3	transcription factor E3
TFEB	transcription factor EB
WDR24	WD Repeat Domain 24
WDR41	WD Repeat Domain 41
WDR59	WD Repeat Domain 59

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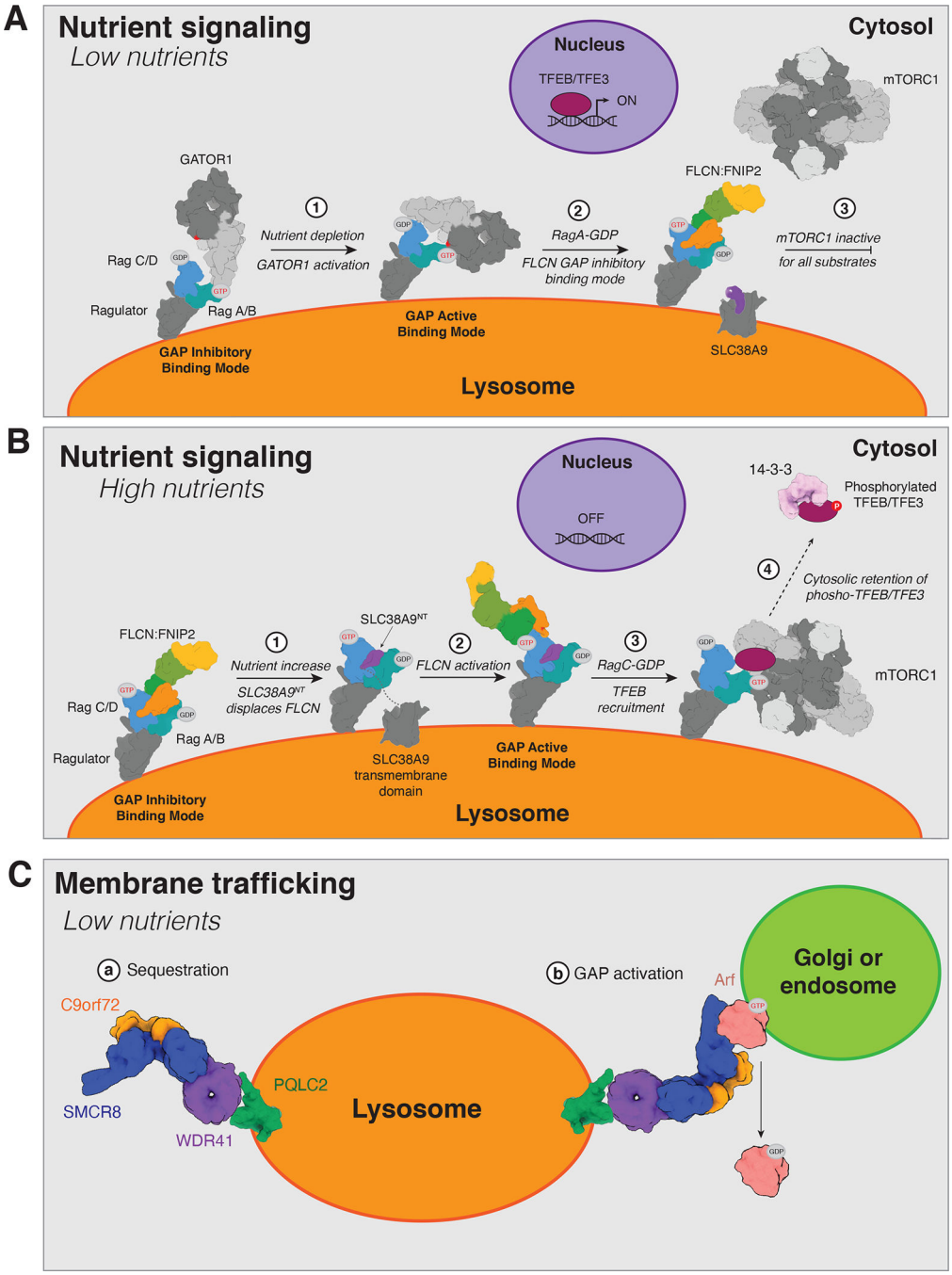

Figure 1. Longin domain GAPs role in nutrient-dependent pathways.

(A) Longin GAP involvement in nutrient-dependent signaling in the mTORC1 pathway under low nutrients. *(1)* Upon depletion of nutrients, GATOR1 transitions from its GAP inhibitory binding to its GAP active binding mode. *(2)* GATOR1 stimulates hydrolysis of RagA-GTP to form RagA-GDP. RagC spontaneously exchanges GDP for GTP generating the inactive Rags ($RagA^{GDP}$:$RagC^{GTP}$). FLCN:FNIP2 binds to inactive Rags in its GAP inhibitory binding mode. *(3)* Inactivation of Rags prevents mTORC1 recruitment and activation leading to no phosphorylation of all mTORC1 substrates. (B) Longin GAP

involvement in nutrient-dependent signaling in the mTORC1 pathway under high nutrients. *(1)* As cellular nutrients increase, the N-terminal of SLC38A9 ($SLC38A9^{NT}$) (purple) is freed from the transmembrane domain (dark grey) and displaces FLCN (orange):FNIP2 (green) in its GAP inhibitory binding mode. *(2)* FLCN:FNIP2 reorients to interact with the Rag-Ragulator complex (blue and grey) in its GAP active binding mode. *(3)* FLCN:FNIP2 stimulates the hydrolysis of RagC-GTP forming RagC-GDP. RagA-GDP undergoes spontaneous exchange to RagA-GTP generating active Rags ($RagA^{GTP}$:$RagC^{GDP}$). Active Rags recruit mTORC1 to the lysosomal membrane. *(4)* TFEB/TFE3 co-localizes with mTROC1 at the lysosomal membrane allowing mTORC1 to phosphorylate TFEB/TFE3. Phosphorylated TFEB/TFE3 is retained in the cytosol through interaction with 14-3-3 proteins. Not shown, following GATOR1 GAP inactivation, GDP to GTP exchange on RagA activates mTORC1 phosphorylation of canonical substrates, which include 4E-BP1, S6K, and ULK1. (C) Longin GAP involvement in nutrient-dependent membrane trafficking. In a state of low nutrients, C9orf72 (orange):SMCR8 (blue) is recruited to the lysosomal membrane through its WDR41 domain (purple). Recruitment to the lysosomal membrane could serve the purpose of *(a)* sequestration of the GAP from its substrates or *(b)* GAP activation where the GAP is positioned to stimulate hydrolysis of substrates on neighboring membranes.

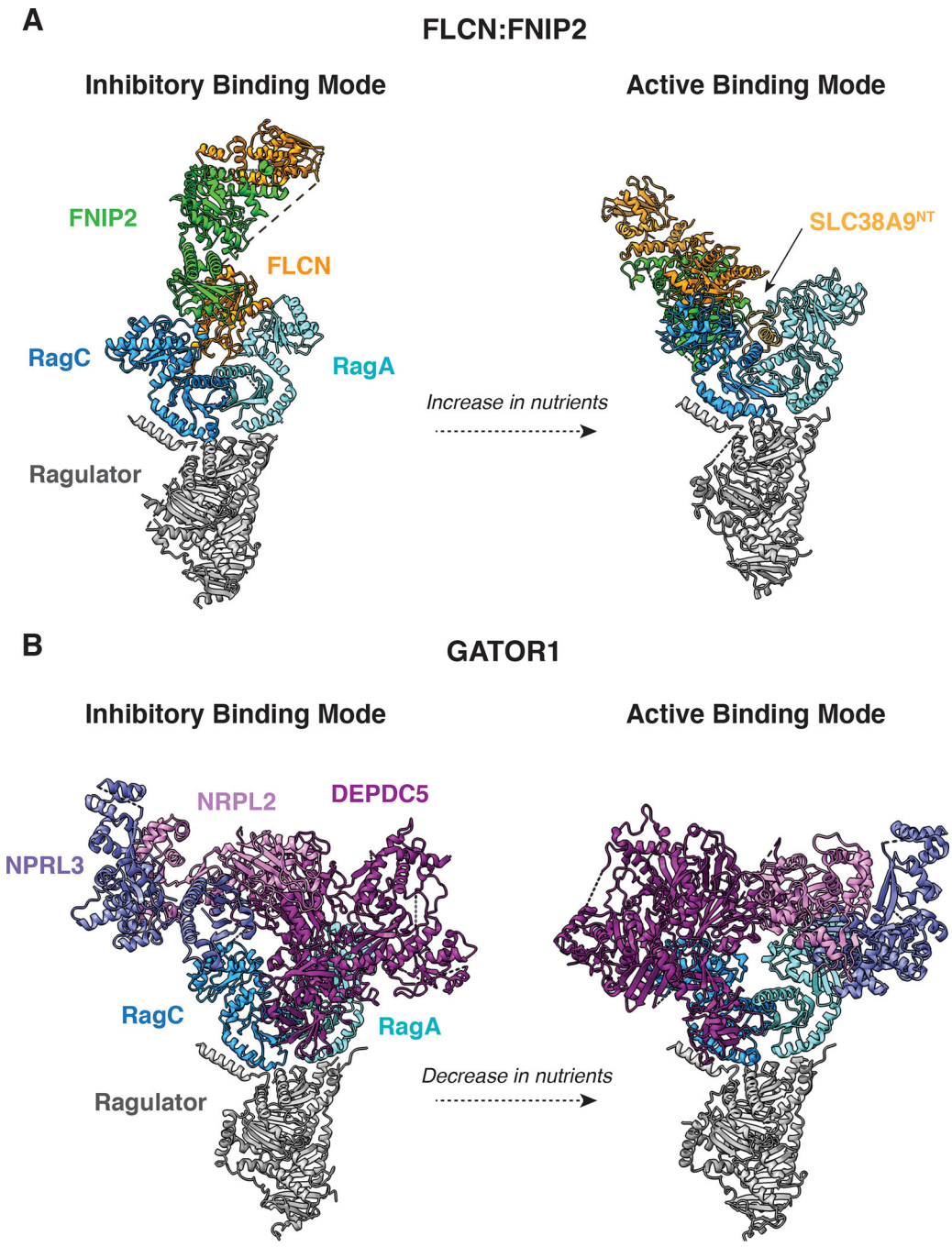

Figure 2. Dual binding modes of FLCN and GATOR1.

(A) Cryo-EM structures of FLCN:FNIP2 (orange and green) bound to the Rag-Ragulator complex (blue and grey) in two alternative binding modes (PDB: 6NZD and 8DHB). N-terminal tail of SLC38A9 ($SLC38A9^{NT}$) (yellow) bound in cleft between Rags in active binding mode. (B) Cryo-EM structures of GATOR1 complex containing NRPL2, NRPL3 and DEPDC5 (purple) bound to the Rag-Ragulator complex in two alternative binding modes (PDB: 7T3A and 7T3B).

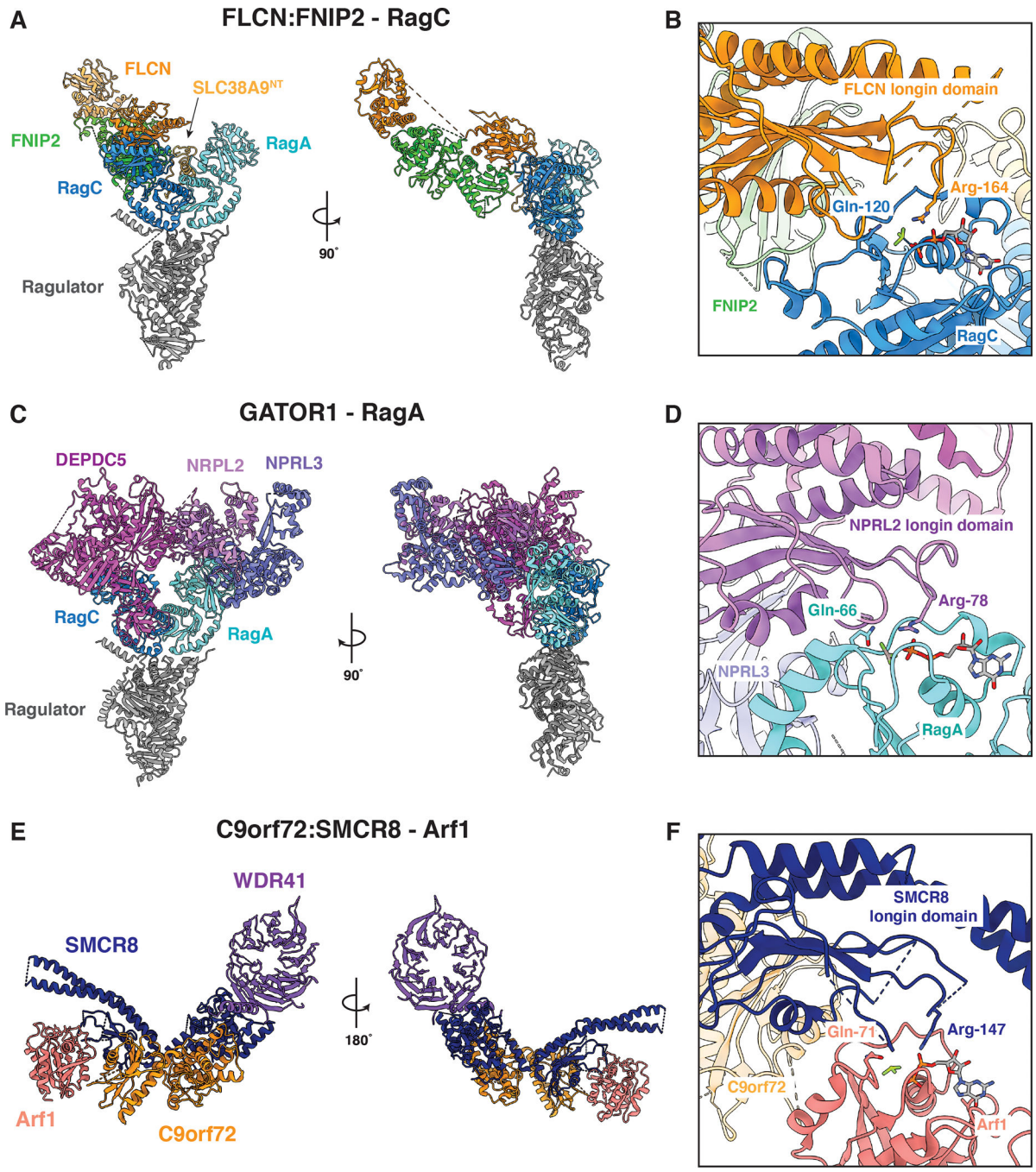

Figure 3. Longin domain GAPs interaction with substrates.

(A) Overall cryo-EM structure of FLCN (orange):FNIP2 (green) in complex with Rag-Ragulator (blue and grey) in active GAP binding mode (PDB: 8DHB). (B) Close-up view of FLCN interaction with RagC nucleotide binding site. Shown in green is the BeF_3 mimicking a γ-phosphate. (C) Overall cryo-EM structure of GATOR1 complex containing NPRL2 (light purple), NRPL3 (blue-purple) and DEPDC5 (magenta) in complex with Rag-Ragulator (blue and grey) in active GAP binding mode (PDB: 7T3B). (D) Close-up view of GATOR1 interaction with RagA nucleotide binding site. (E) Overall cryo-EM structure

C9orf72 (orange): SMCR8 (dark blue) in complex with membrane scaffold WDR41 (purple) and Arf1 (salmon) (PDB: 7MGE). (F) Close-up view of GATOR1 interaction with RagA nucleotide binding site. Shown in green is the AlF_3 mimicking a γ-phosphate.