Structural Insights into the Unusually Strong ATPase Activity of the AAA Domain of the Caenorhabditis elegans Fidgetin-like 1 (FIGL-1) Protein*

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The FIGL-1 (fidgetin like-1) protein is a homolog of fidgetin, a protein whose mutation leads to multiple developmental defects. The FIGL-1 protein contains an AAA (ATPase associated with various activities) domain and belongs to the AAA superfamily. However, the biological functions and developmental implications of this protein remain unknown. Here, we show that the AAA domain of the Caenorhabditis elegans FIGL-1 protein (CeFIGL-1-AAA), in clear contrast to homologous AAA domains, has an unusually high ATPase activity and forms a hexamer in solution. By determining the crystal structure of CeFIGL-1-AAA, we found that the loop linking helices α9 and α10 folds into the short helix α9a, which has an acidic surface and interacts with a positively charged surface of the neighboring subunit. Disruption of this charge interaction by mutagenesis diminishes both the ATPase activity and oligomerization capacity of the protein. Interestingly, the acidic residues in helix α9a of CeFIGL-1-AAA are not conserved in other homologous AAA domains that have relatively low ATPase activities. These results demonstrate that the sequence of CeFIGL-1-AAA has adapted to establish an intersubunit charge interaction, which contributes to its strong oligomerization and ATPase activity. These unique properties of CeFIGL-1-AAA distinguish it from other homologous proteins, suggesting that CeFIGL-1 may have a distinct biological function.

Background: Little is known about the structure and activity of the FIGL-1 protein, whose homolog fidgetin is involved in various developmental processes in mammals.

Results: The Caenorhabditis elegans FIGL-1 AAA domain has strong charge interaction between subunits.

Conclusion: The strong ATPase activity of the C. elegans FIGL-1 AAA domain is contributed by its strong intersubunit interaction.

Significance: These unique properties suggest that the C. elegans FIGL-1 protein possesses a distinct biological function.

Small eyes, polydactyly, and reduced or absent semicircular canals (1–4). However, the linkage between fidgetin mutation and the phenotype of fidget mice is unclear. Sequence analysis shows that fidgetin contains an AAA (ATPase associated with various activities) domain and thus belongs to the AAA superfamily (1). The members of this extensive superfamily possess either one or two conserved AAA domains in their C terminus, and they use the energy derived from ATP hydrolysis mediated by the AAA domain to perform various biological functions, including protein unfolding, nucleosome remodeling, and microtubule disassembly (5–7). To do so, AAA proteins often assemble into ring-shaped hexamers through interaction between AAA domains of each subunit. Although the AAA domain of these proteins serves as the site of ATP hydrolysis and hexamer formation, the region N-terminal to the AAA domain is often the substrate- or partner-binding site, the conformation of which is tightly coupled to the nucleotide state of the AAA domain (6, 8).

Structurally, the AAA domain consists of a large and a small subdomain, with the cleft between them serving as the nucleotide-binding and hydrolysis sites. Several conserved motifs, including Walker A, Walker B, sensor 1, sensor 2, and Arg finger, have been found to be important for the binding and hydrolysis of ATP (7, 8). A series of studies have established that ATP hydrolysis in one AAA domain is dependent on the binding of the neighboring AAA domain that is likely mediated by the direct contact of the conserved Arg finger motif in the neighboring AAA domain (7, 8). Thus, oligomerization is generally required for the ATP hydrolysis activity of AAA proteins.

Within the AAA superfamily, fidgetin is classified as a member of the subgroup that includes spastin and katanin (9); these three proteins have all been shown to have microtubule-severing activity and to be involved in multiple microtubule-related biological events, such as axon branching, cytokinesis, and ciliogenesis (10–13). Among these, spastin has been studied the most extensively because mutation of spastin accounts for ~40% of all cases of autosomal dominant hereditary spastic paraplegia (14). The crystal structure of the AAA domain of spastin has been determined, and the critical residues have been...
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Extensively studied in the context of the functional spastin protein (11, 15). In contrast, the biological functions of katanin and fidgetin are far less clear (12).

The FIGL-1 (fidgetin-like-1) protein was identified as a homolog of fidgetin through genome database searching (1). Thereafter, FIGL-1 was found to be important for mitotic progression of the germ line in Caenorhabditis elegans (16) and may be involved in testis development in mice (17). Recently, C. elegans FIGL-1 was found to bind to SUMO (18). Although some critical residues have been identified in C. elegans FIGL-1 (19, 20), its direct target and biological activity have not been identified, and the structure of FIGL-1 is not known yet. Thus far, only the structure of the AAA domain of human FIGL-1 has been determined (Protein Data Bank code 3D88), and it has been shown to have very weak ATPase activity (21).

To better understand the function of FIGL-1, we expressed the AAA domain of C. elegans FIGL-1 (CeFIGL-1-AAA) in Escherichia coli and studied its structure and ATPase activity. We found that CeFIGL-1-AAA has a strong intrinsic ATPase activity and is oligomeric in solution, as shown by gel filtration chromatography and analytical ultracentrifugation. The crystal structure of the AAA domain was then determined in nucleotide-free and ADP-binding states. Although the overall structure of CeFIGL-1-AAA is highly similar to that of other AAA proteins, CeFIGL-1-AAA contains a charge interaction at the potential interface between subunits. Interruption of this charge interaction by mutagenesis diminished both the ATPase activity and oligomerization of CeFIGL-1-AAA. These results demonstrate that the oligomerization interaction between subunits is essential for the ATP hydrolysis activity of CeFIGL-1. Furthermore, this unusually strong intersubunit interaction of CeFIGL-1 may be related to its unique physiological function.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—CeFIGL-1 cDNA was kindly provided by Dr. Kunitoshi Yamanaka (Kumamoto University, Kumamoto, Japan). The Ser-261→Arg-594 coding region was cloned into the pET-28a vector and expressed in the BL21 Star (DE3) strain. The recombinant protein was purified using HisTrap and Mono Q columns (GE Healthcare). Mutations were introduced using the QuikChange method. For purification of the D540K/E543K double mutant, a Mono S column was used instead of the Mono Q column.

ATPase Activity Assay—The steady-state ATPase activity of 1 μM CeFIGL-1-AAA was measured using an enzyme-coupled assay in 50 mM Tris-HCl (pH 8.2), 75 mM NaCl, 1 mM DTT, 2 mM MgCl₂, 1 mM EGTA, and various concentrations of ATP at 25 °C as described previously (22). The turnover numbers per subunit were calculated. ATPase activity was fitted using the Michaelis-Menten equation with the Hill coefficient as follows:

\[ v = (k_{cat} \times [F] \times [ATP]^{b})/(K_m^h + [ATP]^{b}), \]

where \( [F] \) is the concentration of CeFIGL-1-AAA, \( K_m \) is the Michaelis constant, and \( h \) is the Hill coefficient.

4The abbreviations used are: CeFIGL-1-AAA, C. elegans FIGL-1 AAA domain; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; AMP-PNP, adenosine 5′-β,γ-imino)triphosphate.

Size Exclusion Chromatography—Wild-type and mutant CeFIGL-1-AAA proteins were analyzed using a Superdex 200 10/300 GL column (GE Healthcare) in 20 mM Tris-HCl (pH 8.2), 50 mM NaCl, and 0.5 mM β-mercaptoethanol. The flow rate was 0.5 ml/min.

Analytical Ultracentrifugation—Determination of the sedimentation velocity was performed in a Beckman Coulter Optima XL-I analytical ultracentrifuge using double-sector and sapphire windows. Before the experiments, an additional protein purification step was performed using a Superdex 200 size exclusion column (GE Healthcare) in 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl. The experiment was performed at 40,000 rpm and 285 K using interference detection, and double-sector cells were loaded with ~2 mg/ml protein in the absence or presence of 3 mM nucleotide. The buffer composition (density and viscosity) and the protein partial specific volume were calculated using the SEDFIT and SEDPHAT programs (available through the Boston Biomedical Research Institute). The data were analyzed using the SEDFIT and SEDPHAT programs (23, 24).

Crystallization and Data Collection—Crystals of nucleotide-free and ADP-bound CeFIGL-1-AAA were grown using the vapor diffusion method by mixing 1 μl of protein solution with 1 μl of reservoir solution at 293 K and a protein concentration of 2 mg/ml. Nucleotide-free crystals were produced in a reservoir solution of 0.1 M Tris-HCl (pH 7.0), 0.5 M sodium malonate (pH 6.0), and 0.3% PEG 10,000 by the sitting drop method. After 3 days, the crystals were harvested and frozen in Paratone-N. To obtain crystals of ADP-bound CeFIGL-1-AAA, 5 mM ADP was added to the protein solution for crystallization, and crystals were produced in a reservoir solution of 0.1 M BisTris (pH 6.5) and 0.5 mM sodium acetate by the hanging drop method. After 1 week, the crystals were harvested and frozen in a cryoprotectant consisting of the reservoir solution supplemented with 25% glycerol.

The data from nucleotide-free and ADP-bound crystals were collected by in-house x-ray and the Shanghai Synchrotron Radiation Facility, respectively. Both crystals belong to the space group P6₁. The nucleotide-free crystals diffracted to 2.6 Å with unit cell dimensions of \( a = b = 108.5 \) Å and \( c = 55.0 \) Å. The ADP-bound crystals diffracted to 2.8 Å with unit cell dimensions of \( a = b = 113.5 \) Å and \( c = 58.1 \) Å. Both datasets were processed and scaled using the HKL2000 software package (25).

Structure Determination and Refinement—The initial phases of the CeFIGL-1-AAA structures were determined by the program PHASER (26) using the structure of human FIGL-1 (Protein Data Bank code 3D88) as a template. The residues were built manually using the Coot program (27) based on 2\( F_{\text{obs}} - F_{\text{calc}} \) and \( F_{\text{obs}} - F_{\text{calc}} \) difference Fourier maps. The structural model was refined using the PHENIX program (28, 29). The final structure of nucleotide-free CeFIGL-1-AAA had an \( R_{\text{cryst}} \) value of 19.0% and an \( R_{\text{free}} \) value of 24.5%. The final structure of ADP-bound CeFIGL-1-AAA had an \( R_{\text{cryst}} \) value of 20.2% and an \( R_{\text{free}} \) value of 23.9%. Detailed data collection and refinement statistics are summarized in Table 2.

RESULTS

CeFIGL-1-AAA Is a Hexameric Protein with Strong ATPase Activity—CeFIGL-1 is composed of 594 amino acid residues (NCBI accession number NP_504197). Based on sequence align-
ment with homologous AAA proteins (Fig. 1), the fragment spanning Ser-261–Arg-594 at the C terminus of CeFIGL-1 was defined as its AAA domain and was cloned and expressed in E. coli. Surprisingly, the recombinant CeFIGL-1-AAA protein has a strong intrinsic ATPase activity, with a $k_{cat}$ of $5.75 \text{s}^{-1}$ (Fig. 2A and Table 1). This activity is at least 2 orders of magnitude higher than the activity of the AAA domain of Drosophila spastin, which was reported to be only $0.028 \text{s}^{-1}$ (11). A similarly low ATPase activity ($0.006 \text{s}^{-1}$) was observed for the AAA domain of human FIGL-1 (21). Given the high sequence homology of these AAA domains, the striking difference in their ATPase activities was unexpected and was thus further explored.

Because it has been established that ATP hydrolysis in AAA proteins involves interaction between neighboring subunits (7, 8), it was possible that the high ATPase activity of CeFIGL-1-AAA was related to intersubunit interactions. Indeed, the ATP hydrolysis velocity of CeFIGL-1-AAA showed significant cooperativity against ATP concentration, indicating communication between its subunits (Fig. 2A and Table 1). Furthermore, when we assessed the oligomeric state of CeFIGL-1-AAA by size exclusion chromatography, the protein eluted at the position corresponding to $\sim 220 \text{kDa}$ (theoretical mass of 39 kDa per CeFIGL-1-AAA monomer), indicating that CeFIGL-1-AAA forms a hexamer in solution (Fig. 2B).

To further characterize CeFIGL-1-AAA in solution, CeFIGL-1-AAA was analyzed by analytical ultracentrifugation under different nucleotide conditions (Fig. 2C). The results show that CeFIGL-1-AAA formed tight hexamers in the presence of the
ATP analog AMP-PNP, giving a molecular mass of 219 kDa. In the nucleotide-free state, the molecular mass of CeFIGL-1-AAA was slightly lower than that in the presence of AMP-PNP, probably due to partial dissociation or deformation of the hexamer. However, when bound by ADP, CeFIGL-1-AAA was less oligomeric, existing primarily as a dimer and tetramer. This implies that the hexameric assembly of CeFIGL-1-AAA is regulated by the nucleotide state of the protein.

Crystal Structure of CeFIGL-1-AAA—To further explore the mechanism of CeFIGL-1-AAA activity, we determined its crystal structure. Crystals were obtained in nucleotide-free and ADP-bound states, and the structures were determined by molecular replacement as described under “Experimental Procedures” and refined to 2.6 and 2.8 Å, respectively (Table 2). Both crystals belong to the P6_5 space group, with one molecule of CeFIGL-1-AAA in one asymmetric unit. The final structural model of nucleotide-free CeFIGL-1-AAA contains residues 286–588, with the exception of residues 424–429 (Fig. 3A), whereas ADP-bound CeFIGL-1-AAA contains residues 288–587, with the exception of residues 423–429 (Fig. 3B). The structure of ADP-bound CeFIGL-1-AAA is very similar to that of the nucleotide-free state, with a root mean square deviation of 0.924 Å between 289 Cα atoms of these two structures (Fig. 3C).

The overall structure of CeFIGL-1-AAA shows a typical AAA domain architecture with two subdomains (Fig. 3A and B). The large subdomain, also referred to as the nucleotide-binding domain, has a central five-stranded parallel β-sheet flanked by two or three α-helices at each side, whereas the small subdomain exists mainly as a four-helix bundle. Like the structures of the AAA domains of proteins in the spastin subfamily, CeFIGL-1-AAA has an extra N-terminal α-helix (α1) and an extra C-terminal helix (α11) covering each edge of the central β-sheet plane (see Ref. 11 for the nomenclature of the secondary structure).

In both crystals, CeFIGL-1-AAA does not exist in a closed hexameric assembly, but instead shows a 6-fold helical packing (Fig. 3D and E) that has been observed for different AAA proteins (11, 30–32). This 6-fold symmetry strongly implies that when assembled into a closed ring hexamer, the large subdomain forms the inner ring, whereas the small subdomain forms the outer ring. In particular, the small subdomain of one subunit interacts intensively with the large subdomain of the neighboring subunit (an interface area of 622 Å²), which comprises most of the intersubunit interaction (a total interface area of 944 Å²).

Nucleotide-binding Site—The structure of CeFIGL-1-AAA is very similar to that of the AAA domain of Drosophila spastin (11), whose function has been extensively studied. However, in time corresponds to an apparent molecular mass of ~300 kDa. mAU, milliabsorbance units. C, the molecular mass distribution of CeFIGL-1-AAA in different nucleotide-binding states determined by sedimentation velocity.

| ATPase activities of wild-type and mutant CeFIGL-1-AAA proteins | CeFIGL-1-AAA | k$_{cat}$ | K$_{m}$ | Hill coefficient |
|---|---|---|---|---|
| Wild-type | 5.75 ± 0.16 | 0.51 ± 0.04 | 2.4 |
| D540K | 3.05 ± 0.62 | 2.34 ± 1.07 | 1.1 |
| D540K/E543K | 0.59 ± 0.15 | 1.91 ± 0.95 | 1.3 |

**FIGURE 2.** Biochemical properties of CeFIGL-1-AAA. A, the ATPase activities of wild-type and two mutant CeFIGL-1-AAA proteins are shown as a function of ATP concentration. Each ATPase curve is a single representative experiment of reproducible measurements from two (for mutant proteins) or three (for the wild-type protein) different protein preparations. The curves were fit as described under “Experimental Procedures,” and the data are listed in Table 1. B, gel filtration elution peaks of the wild-type and mutant CeFIGL-1-AAA proteins. The elution times of proteins with known molecular masses are indicated by arrows. The tubulin-RB3 complex (T2R) has a molecular mass of 217 kDa but exists in a rod shape rather than a globular shape, so its elution OD$_{280}$ corresponds to an apparent molecular mass of 300 kDa. mAU, milliabsorbance units. C, the molecular mass distribution of CeFIGL-1-AAA in different nucleotide-binding states determined by sedimentation velocity.
Crystal structure of CeFIGL-1-AAA.

In the CeFIGL-1-AAA structure, the C-terminal helix α11 is better defined, although the last seven residues are still invisible. This helix has been found to be important for the activity of spastin and other AAA proteins (11, 33, 34). The structure of Drosophila spastin reveals that helix α11 has a hydrophobic face that interacts with the phosphate-binding loop (P-loop, referred to as the Walker A motif in AAA proteins) and that a conserved aromatic residue (Tyr-753 in spastin) is indispensable for the activity of spastin (11). However, this residue, as well as the last turn of helix α11, is not visible in the structure of the spastin AAA domain. The structure of CeFIGL-1-AAA shows that three highly conserved aromatic residues of helix α11, Tyr-577, Trp-580, and Phe-584 (corresponding to Tyr-753 in spastin), pack against a hydrophobic surface near the P-loop, probably stabilizing the orientation of the C-terminal peptide of contrast to the fact that there is no nucleotide bound in the spastin AAA domain crystal, the structure of ADP-bound CeFIGL-1-AAA clearly shows the nucleotide-binding site located at the cleft between the two subdomains (Fig. 4, A and B). ADP forms an intensive hydrogen bond network with the Walker A motif and Arg-524 in the sensor 2 motif; however, no direct contact with the Walker B motif and the sensor 1 motif was observed. In addition, the adenosine ring forms hydrogen bonds with the main chain of Ala-319 and the side chain of Asp-317, which are located in the loop linking helices α1 and α2 (Fig. 4C). This implies that the nucleotide state of CeFIGL-1-AAA could be related to the conformation of the extra N-terminal helix α1, as well as the N-terminal domain of CeFIGL-1-AAA.

The data collection and refinement statistics are summarized in Table 2.

### Table 2: Data collection and refinement statistics

|                        | Nucleotide-free (apo) | ADP-bound |
|------------------------|-----------------------|-----------|
| **Data collection**     |                       |           |
| Space group            | P6_3                  | P6_3      |
| Unit cell (Å)          | a = b = 108.5, c = 55.0 | a = b = 113.5, c = 58.1 |
| Wavelength (Å)         | 1.5418                | 0.9793    |
| Resolution range (Å)   | 50–2.6 (2.6–2.69)     | 50–2.8 (2.9–2.8) |
| No. of unique reflections | 11,503              | 10,429    |
| Redundancy             | 10.9 (5.0)            | 10.2 (5.0) |
| Rsym (%)               | 12.1 (78.1)           | 24.5 (78.1) |
| I/σr (%)               | 20.8 (2.8)            | 31.1 (2.3) |
| Completeness (%)        | 100.0 (100.0)         | 97.9 (84.0) |
| **Refinement**         |                       |           |
| Resolution range (Å)   | 38.7–2.6              | 37.2–2.8  |
| Rcryst (%)             | 19.0                  | 20.2      |
| Rfree (%)              | 24.5                  | 23.9      |
| r.m.s.d. bond (Å)      | 0.009                 | 0.01      |
| r.m.s.d. angle         | 1.2°                  | 1.3°      |
| Ligand                 | Tris                  | ADP       |
| Number of atoms        | Protein 2325          | 2266      |
|                        | Ligand 11             | 27        |
|                        | Solvent 24            | 29        |
| Ramachandran plot (%)  | Most favored 88.5     | 85.2      |
|                        | Additionally allowed 11.5 | 14.1        |
|                        | Generously allowed 0  | 0.8       |
|                        | Disallowed 0          | 0         |
| Average B factor (Å^2) | Protein 49.6           | 77.2      |
|                        | Ligand 70.7            | 87.7      |
|                        | Solvent 37.0           | 67.6      |

* Data in parentheses are for the highest resolution shell.
* Rsym = Σ||Fobs|−|Fcalc||/Σ|Fobs|.
* Rcryst = Σ||Fobs|−|Fcalc||/Σ|Fobs|.
* Rfree was calculated by the same method used for Rcryst but from a test set containing 5% of the data excluded from the refinement calculation.
* r.m.s.d., root mean square deviation.

**FIGURE 3. Crystal structure of CeFIGL-1-AAA.** A and B, schematic representations of the structures of CeFIGL-1-AAA in the nucleotide-free (apo) and ADP-bound states, respectively. C, superimposed structures of CeFIGL-1-AAA in the nucleotide-free (orange) and ADP-bound (green) states. D and E, schematic representations of the side and top views of the CeFIGL-1-AAA hexamer, respectively. The small and large subdomains are colored orange and green, respectively. The extra N-terminal helix α1, C-terminal helix α11, and helix α9a are colored blue, red, and gray, respectively.
the protein (Fig. 5, A and B). As helix α11 extends close to the neighboring subunit (Fig. 3, D and E), the most C-terminal peptide may favor the hexamerization of AAA proteins in this subfamily and thus play a role in regulating the activity of the protein.

**Role of Charge Interaction between Subunits**—The structure of CeFIGL-1-AAA reveals another loop of interest between helices α9 and α10, which is invisible in all of the known structures of the AAA domains of *Drosophila* spastin, human spastin, and human FIGL-1. In CeFIGL-1-AAA, this loop folds as a one-and-a-half turn helix (here named α9a) and interacts with helix α2 of the adjacent subunit (Fig. 6A). In particular, two acidic residues in this loop, Asp-540 and Glu-543, which form a negatively charged surface, are positioned against a positively charged surface formed by three basic residues, Arg-329, Lys-337, and Arg-338, in helix α2 of the neighboring subunit (Fig. 6B), thus establishing charge-charge interactions between the two subunits.

For some AAA proteins, the small subdomain changes its orientation relative to the large subdomain of the same subunit during the working cycle while maintaining its interaction with the large subdomain of the neighboring subunit (31, 35–38). This interaction correlates with intersubunit binding strength and the ATPase activity of AAA proteins. Consistently, the charged residues involved in the potential charge interaction exist only in CeFIGL-1 and are not present in the corresponding positions in homologous AAA domains with low ATPase activity (Fig. 1).

To determine whether the intersubunit charge interaction observed in CeFIGL-1-AAA contributes to the interaction between CeFIGL-1-AAA subunits in solution and thus to its activity, the two acidic residues in helix α9a, Asp-540 and Glu-543, were replaced with Lys. Indeed, the ATPase activity of CeFIGL-1-AAA-D540K was remarkably lower than that of wild-type CeFIGL-1-AAA, and the double mutation (D540K/E543K) further decreased the ATPase activity (Fig. 2A). Accordingly, CeFIGL-1-AAA-D540K/E543K eluted significantly later than wild-type CeFIGL-1-AAA (Fig. 2B), suggesting that the mutant protein is much less oligomeric in solution. These results demonstrate that a charge interaction between the small subdomain and the neighboring large subdomain observed in crystal packing may very well exist in solution and contribute to the strong intersubunit interaction of CeFIGL-1-AAA, which correlates with the ATP hydrolysis activity of the protein.

**DISCUSSION**

In this study, we biochemically and structurally analyzed the AAA domain of CeFIGL-1 and found that the high ATPase activity of CeFIGL-1-AAA is correlated with its tight intersubunit interaction. This correlation coincides well with the find-
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that is supported by the presence of the conserved fundamental ATP-binding motifs (Walker A, Walker B, and Arg finger) in CeFIGL-1 (Fig. 1).

However, the strong intersubunit interaction observed in CeFIGL-1-AAA in solution is rather unique, as the AAA domains of homologous proteins (human FIGL-1-AAA and Drosophila spastin) have been found to be monomeric in solution (11, 21). The charged residues that mediate the charge interactions between subunits, as revealed by the crystal structure of CeFIGL-1-AAA (Fig. 6B) and mutagenesis (Fig. 2), are present only in CeFIGL-1 (Fig. 1). This unique property of CeFIGL-1 is probably indicative of a distinct biological function, although this function remains unknown.

In addition, the high ATPase activity of CeFIGL-1-AAA is of particular interest. According to the study of full-length CeFIGL-1 (20), the ATPase activity of the full-length CeFIGL-1 protein (225 nmol/mg/min) corresponds to 0.26 s⁻¹, which is ~20 times lower than the activity of its AAA domain. This suggests that the high intrinsic ATPase activity of the AAA domain is inhibited by the N-terminal fragment of the protein and that this inhibition is released when CeFIGL-1 binds to its substrate or partner. Further structural analysis will be needed to elucidate the interaction between the N-terminal fragment and the AAA domain of CeFIGL-1.

In summary, we found that the AAA domain of CeFIGL-1 has unusually high ATPase activity, which correlates with its strong intersubunit interaction. This interaction can be largely attributed to charge interactions mediated by two acidic residues in helix α9a, a short helix that is observed in the crystal structures of CeFIGL-1-AAA. These unique biochemical properties of CeFIGL-1 distinguish it from other homologous AAA proteins, implying that CeFIGL-1 may have a distinct biological function that remains to be identified.

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REFERENCES
1. Cox, G. A., Mahaffey, C. L., Nystuen, A., Letts, V. A., and Frankel, W. N. (2000) The mouse fidgetin gene defines a new role for AAA family proteins in mammalian developmental. Nat. Genet. 26, 198–202
2. Konyukhov, B. V., and Sazhina, M. V. (1976) The cell cycle and retinal histogenesis fidget mutant mice. J. Genet. 45, 22–28
3. Grueneberg, H. (1943) Two new mutant genes in the house mouse. J. Genet. 45, 13–22
4. Truslove, G. M. (1956) The anatomy and development of the fidget mouse. J. Genet. 54, 64–86
5. Hanson, P. L., and Whiteheart, S. W. (2005) AAA + proteins: have engine, will work. Nat. Rev. Mol. Cell Biol. 6, 519–529
6. Snider, J., and Houy, W. A. (2008) AAA+ proteins: diversity in function, similarity in structure. Biochem. Soc. Trans. 36, 72–77
7. Tucker, P. A., and Sallai, L. (2007) The AAA+ superfamily—a myriad of motions. Curr. Opin. Struct. Biol. 17, 641–652
8. Wendler, P., Ciniawsky, S., Kock, M., and Kube, S. (2012) Structure and function of the AAA+ nucleotide binding pocket. Biochim. Biophys. Acta 1823, 2–14
9. Frickey, T., and Lupas, A. N. (2004) Phylogenetic analysis of AAA proteins. J. Struct. Biol. 146, 2–10
10. McNally, F. J., and Vale, R. D. (1993) Identification of katanin, an ATPase

FIGURE 5. A, hydrophobic interaction between the extra C-terminal helix α11 and P-loop. Carbon, nitrogen, and oxygen atoms are colored white, blue, and red, respectively. B, sequence alignment of helix α11. Highly conserved aromatic residues are highlighted in red. Hs, Homo sapiens; Mm, Mus musculus; Dm, Drosophila melanogaster.

FIGURE 6. Interaction between two adjacent CeFIGL-1-AAA proteins. A, ribbon representation of dimeric ADP-bound CeFIGL-1-AAA. B, the electrostatic surfaces of helix α2 from one subunit and helix α9a from the adjacent subunit are shown and colored according to their electrostatic potential, ranging from deep blue (positive charge, + 9 kT/e) to red (negative charge, −9 kT/e).

ings of a previous study of the full-length CeFIGL-1 protein (20) and with the overall understanding of AAA proteins in general (7, 8). This implies that CeFIGL-1 shares the same ATP hydrolysis mechanism as other AAA proteins, a conclusion
that severs and disassembles stable microtubules. *Cell* **75**, 419–429
11. Roll-Meca, A., and Vale, R. D. (2008) Structural basis of microtubule sev
ering by the hereditary paraspinospar gene spinatin. *Nature* **451**, 363–367
12. Sharph, D. J., and Ross, J. L. (2012) Microtubule-severing enzymes at the
cutting edge. *J. Cell Sci.* **125**, 2561–2569
13. Mukherjee, S., Diaz Valencia, J. D., Steward, S., Metz, J., Monnier, S.,
Rath, U., Asenjo, A. B., Charafedine, R. A., Sosa, H. J., Ross, J. L., Ma, A.,
and Sharp, D. J. (2012) Human Fidgetin is a microtubule sev
ering enzyme and minus-end depolymerase that regulates mitosis. *Cell Cycle*
**11**, 2359–2366
14. Hazan, J., Fonknechten, N., Mavel, D., Paternotte, C., Samson, D., Ar
tiguenave, F., Davoine, C. S., Cruaud, C., Durr, A., Wincker, P., Bro	ttier, P., Cattolico, L., Barbe, V., Burgunder, J. M., Prudhomme, J. F., Br
tico, A., Fontaine, B., Heilig, B., and Weissenbach, J. (1999) Spastin, a new AAA
protein, is altered in the most frequent form of autosomal dominant spastic
paraplegia. *Nat. Genet.* **23**, 296–303
15. Taylor, J. L., White, S. R., Lauring, B., and Kull, F. J. (2012) Crystal structure
of the human spinatin AAA domain. *J. Struct. Biol.* **179**, 133–137
16. Luke-Glaser, S., Pintard, L., Tyers, M., and Peter, M. (2007) The AAA-
ATPase FIGL-1 controls mitotic progression, and its levels are regulated
by the CUL-3MEL-26 E3 ligase in the *Caenorhabditis elegans* germline. *J. Cell Sci.*
**120**, 3179–3187
17. L'Hôte, D., Vatin, M., Auer, J., Castille, J., Passet, B., Montagutelli, X.,
Serres, C., and Vaiman, D. (2011) Fidgetin-like 1 is a strong candidate for
a dynamic impairment of male meiosis leading to reduced testis weight in
mice. *PLoS ONE* **6**, e27582
18. Onitake, A., Yamanaka, K., Esaki, M., and Ogura, T. (2012) *Caenorhabditis
elegans* fidgetin homolog FIGL-1, a nuclear-localized AAA ATPase, binds
to SUMO. *J. Struct. Biol.* **179**, 143–151
19. Yakushiji, Y., Yamanaka, K., and Ogura, T. (2004) Identification of a cytosine residue important for the ATPase activity of *C. elegans* fidgetin homolog.
*FEBS Lett.* **578**, 191–197
20. Yakushiji, Y., Nishikori, S., Yamanaka, K., and Ogura, T. (2006) Mutational
analysis of the functional motifs in the ATPase domain of *Caenorhabditis
elegans* fidgetin homologue FIGL-1: evidence for an intersubunit catalytic process of ATP hydrolysis by AAA ATPases. *J. Struct. Biol.* **156**, 93–100
21. Peng, W., and Wang, C. (2011) Recombinant expression and activity analysis
of the AAA domain of human fidgetin like-1. *J. Shanghai Norm. University Nat. Sci.* **40**, 533–539
22. Wang, W., Jiang, Q., Argentini, M., Cornu, D., Gigant, B., Knossow, M.,
and Wang, C. (2012) Kid2C minimal functional domain has unusual nuclceotide
binding properties that are adapted to microtubule depolymerization. *J. Biol. Chem.* **287**, 15143–15153
23. Schuck, P. (2000) Size-distribution analysis of macromolecules by sedi-
mentation velocity analytical ultracentrifugation and Lamm equation modeling.
*Biophys. J.* **78**, 1606–1619
24. Schuck, P. (2003) On the analysis of protein self-association by sedimen-
tation velocity analytical ultracentrifugation. *Anal. Biochem.* **320**, 104–124
25. Otwinowski, Z., and Minor, W. (1997) Processing of x-ray diffraction data
collected in oscillation mode. *Methods Enzymol.* **276**, 307–326
26. McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Sto	toni, L. C., and Read, R. J. (2007) Phaser crystallographic software. *J. Appl.
Crystallogr.* **40**, 658–674
27. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecu-
lar graphics. *Acta Crystallogr.* **D Biol. Crystallogr.* **60**, 2126–2132
28. Zwart, P. H., Afonine, P. V., Grosse-Kunstleve, R. W., Hung, L. W., Joerger,
T. R., McCoy, A. J., McKee, E., Moriarty, N. W., Read, R. J., Sacchettini,
J. C., Sauter, N. K., Storoni, L. C., Terwilliger, T. C., and Adams, P. D.
(2008) Automated structure solution with the PHENIX suite. *Methods Mol.
Biol.* **426**, 419–435
29. Terwilliger, T. C., Grosse-Kunstleve, R. W., Afonine, P. V., Moriarty,
N. W., Zwart, P. H., Hung, L. W., Read, R. J., and Adams, P. D. (2008)
Iterative model building, structure refinement and density modification
with the PHENIX AutoBuild wizard. *Acta Crystallogr. D Biol. Crystallogr.*
**64**, 61–69
30. Guo, F., Maurizi, M. R., Esser, L., and Xia, D. (2002) Crystal structure of
CtPA, an Hsp100 chaperone and regulator of ClpAP protease. *J. Biol.
Chem.* **277**, 46743–46752
31. Mueller-Cajar, O., Stotz, M., Wendler, P., Hartl, F. U., Bracher, A., and
Hayer-Hartl, M. (2011) Structure and function of the AAA+ protein CbbX, a red-type Rubisco activase. *Nature* **479**, 194–199
32. Niwa, H., Tsuchiya, D., Makio, H., Yoshida, M., and Morikawa, K. (2002)
Hexameric ring structure of the ATPase domain of the membrane-inte-
grated metalloprotease FtsH from *Thermus thermophilus* HB8. *Structure* **10**, 1415–1423
33. Mackay, R. G., Helsen, C. W., Tkach, J. M., and Glover, J. R. (2008) The C-terminal extension of *Saccharomyces cerevisiae* Hsp104 plays a role in oligomer assembly. *Biochemistry* **47**, 1918–1927
34. Vajjhala, P. R., Nguyen, C. H., Landsberg, M. J., Kistler, C., Gan, A. L., King,
G. F., Hankamer, B., and Munn, A. L. (2008) The Vps4 C-terminal helix is a critical determinant for assembly and ATPase activity and has elements conserved in other members of the meiotic clade of AAA ATPases. *FEBS J.* **275**, 1427–1449
35. Glynn, B. E., Nager, A. R., Baker, T. A., and Sauer, R. T. (2012) Dynamic and static components power unfolding in topologically closed rings of a AAA + proteolytic machine. *Nat. Struct. Mol. Biol.* **19**, 616–622
36. Glynn, B. E., Martin, A., Nager, A. R., Baker, T. A., and Sauer, R. T. (2009)
Structures of asymmetric ClpX hexamers reveal nucleotide-dependent
motions in a AAA + protein- unfoldng machine. *Cell* **139**, 744–756
37. Wang, J., Song, J. I., Franklin, M. C., Kamtekar, S., Im, Y. J., Rho, S. H.,
Seong, I. S., Lee, C. S., Chung, C. H., and Eom, S. H. (2001) Crystal structures of the HsLVU peptidase-ATPase complex reveal an ATP-dependent proteolysis mechanism. *Structure* **9**, 177–184
38. Carter, A. P., Cho, C., Jin, L., and Vale, R. D. (2011) Crystal structure of the
dynein motor domain. *Science* **331**, 1159–1165