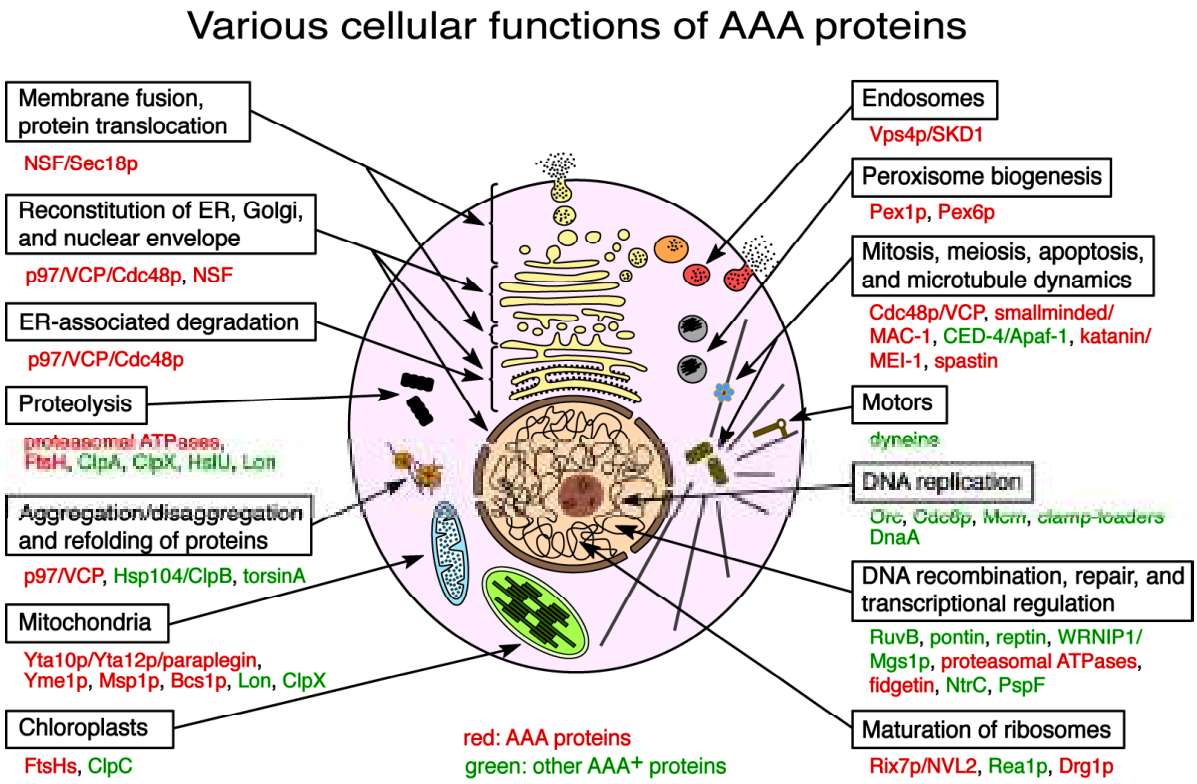
分子細胞制御分野
Department of Molecular Cell Biology

AAA ファミリータンパク質は、タンパク質分解、膜融合、細胞骨格の動態、オルガネラの形成・再構築など様々な細胞機能に関わる分子シャペロンである。リング状のオリゴマーを形成し、ATP のエネルギーを使って基質タンパク質の立体構造を解きほぐしたり、タンパク質複合体を脱会合したりする。神経変性疾患などヒト疾患に関連する AAA タンパク質が相次いで同定されている。分子細胞制御分野では、様々な AAA タンパク質の細胞機能、分子機構、ナノ動態について研究している。

AAA family proteins are molecular chaperones involved in a variety of cellular processes including protein degradation, membrane biogenesis/fusion, dynamics of cytoskeleton, and maintenance/reconstitution of organelles. They form ring-shaped oligomers. Upon ATP hydrolysis, they unfold and translocate substrate proteins or disassemble protein complexes. Recent studies have also implicated AAA proteins in a number of human genetic diseases including neurodegenerative disorders. We have been investigating the cellular functions, molecular mechanisms, and nanodynamics of various AAA proteins for decades.



AAA タンパク質の多彩な細胞機能

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研究概略　Projects

AAA ファミリータンパク質は、Walker 型 ATPase で、リング状 6 量体を形成して機能する。タンパク質やその複合体の立体構造をエネルギー依存的に変換する分子シャペロンである。真正細菌からヒトに至るまで普遍的に存在し、真核生物では酵母からヒトまで 20 数個のほぼ同じセットの AAA タンパク質が存在する。私たちは AAA ファミリータンパク質の共通分子基盤と多彩な細胞機能の解明をめざしている。

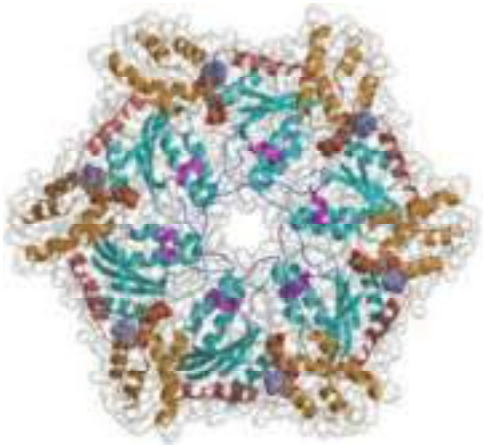

図 1. AAA タンパク質のリング状 6 量体構造

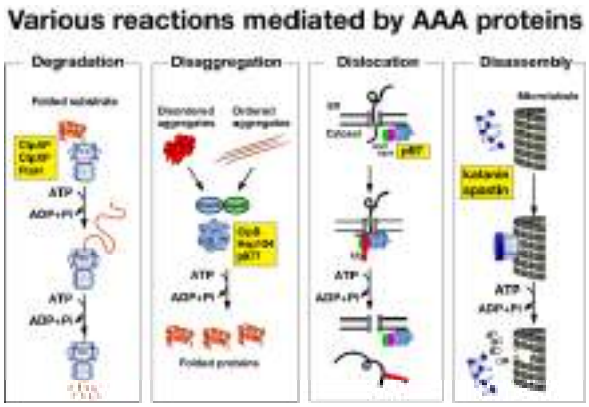

図 2. AAA タンパク質の基質構造変換作用

近年、ヒト遺伝性疾患に関わる AAA タンパク質が次々に同定されてきた（図 3）。このうち、筋萎縮性側索硬化症（amyotrophic lateral sclerosis）や骨パジェット病と前頭側頭葉型認知症を伴う封入体筋炎（IBMPFD）に関わる p97/VCP、遺伝性痙性対麻痺（hereditary spastic paraplegia）に関わる spastin、呼吸鎖複合体 III 不全症、GRACILE 症候群及び Björnstad 症候群に関わる BCS1L などに注目して細胞機能と分子機構を解析してきた。

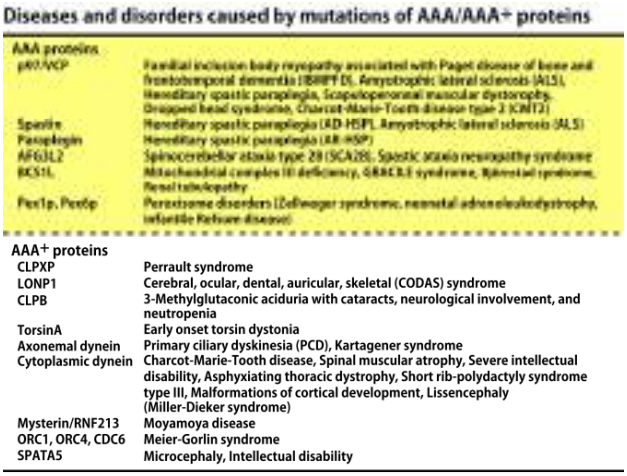

図 3. AAA タンパク質に起因する疾患

1．AAA ファミリーATPase の分子機構の解明

微小管切断酵素 spastin・katanin の 6 量体リング構造の中央の孔に位置する保存された芳香族残基を含むポアモチーフや周辺の塩基性残基が機能に重要であることを証明した（Matsushita-Ishiodori et al., 2009; Johjima et al., 2015）。また、katanin による切断には、微小管を形成するαおよびβチューブリン双方の C 末端酸性残基領域が必須であることがわかった（Johjima et al., 2015）。

酵母の p97 ホモログ Cdc48 の D1 AAA ドメインはポアモチーフの芳香族残基を欠失している。ポアモチーフの芳香族残基は AAA 型シャペロンが基質タンパク質をリングの孔に通すようにしてアンフォールドする活性に重要であると考えられている（糸通しモデル）。酵母 Cdc48 の D1 AAA ドメインのポアモチーフに芳香族残基を導入すると、致死となり、これはアンフォールディング活性の上昇した Cdc48 と 20S プロテアソーム複合体により、細胞増殖に必須な未知のタンパク質基質の分解が促進されたためと考えられる（Esaki et al., 2017）。

2．AAA タンパク質の作用のナノ動態観察

AAA タンパク質およびその基質の動的変化を、金沢大学の安藤研究室により開発された高速原子間力顕微鏡（AFM）を用いて観察し、ナノ動

態解析から分子機構を解明している。ATP 加水分解のサイクルで、p97 がどのような構造変化をするか、高速 AFM で観察したところ、ATP 存在下で、N-D1 リングが D2 リングに対して、25° 時計回りに回転し、すぐに元の位置に戻るという構造変化を繰り返し起こすことが分かった。変異体を用いた解析から、この構造変化には ATP の結合が必須で、加水分解は必要ないことを明らかにした。このような p97 の運動とアンフォールディング活性の関係を今後明らかにしていく。

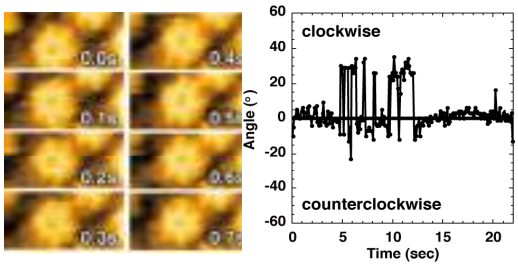

図 4. p97 の連続 AFM 画像（左）と
対称軸の継時的変化（右）

26S プロテアソームは、19S 制御ユニットに 6 種類の AAA タンパク質があり、基質タンパク質をアンフォールドし、プロテアーゼ活性を持つβリングに送り込むと考えられている。ポリユビキチン化基質が、プロテアソームに結合し、脱ユビキチン化されて、分解される過程を高速 AFM で観察し、プロテアソームによる基質タンパク質の全プロセスの可視化に取り組んでいる【山形大学奥野准教授らとの共同研究】。現在のところ、基質の結合頻度が低く、シャトリング因子 Rad23 などによる促進効果、Rad23 と基質タンパク質の結合などについても解析を進めている。

p97 と同様に 2 つの AAA ドメインをタンデムに持ち、タンパク質凝集体の脱凝集活性を持つ ClpB の ATP 依存的構造変化についても AFM 解析し、閉じたフラットなリング構造とらせん構造が観察され、機能との関連が推測される【東京農工大学養王田教授らとの共同研究】。

AAA タンパク質以外の分子シャペロンの高速 AFM 観察もいくつか挑戦している。小胞体でタンパク質のジスルフィド結合の形成を制御して、タンパク質のフォールディングを助ける PDI (Protein Disulfide Isomerase)ファミリータンパク質のうち、ミスフォールドタンパク質の間違ったジスルフィド結合を切断し、基質の分解反応を促進する ERdj5 のドメイン間のダイナミクスと機能の相関を明らかにした【東北大学稲葉教授らとの共同研究】（Maegawa et al., 2017）。PDI が基質タンパク質と結合して、2 量体を形成する様子などについても明らかにした。

３．AAA タンパク質の細胞機能

線虫の p97 ホモログ CDC-48 とアダプター分子群：p97 ホモログは、細胞分裂後の小胞体、ゴルジ体、核膜の再構築に働くほか、ユビキチン・プロテアソーム経路や小胞体関連分解（ERAD）など様々な細胞機能に関与している。アポトーシスやオートファジーへの関与やポリグルタミン結合能なども指摘されている。多数のアダプタータンパク質が p97 に結合して、これらの多彩な機能が発揮される。ヒト p97 ホモログ VCP は ALS や IBMPFD の原因因子として同定されている。

図 5. 多機能 AAA タンパク質 p97

線虫には、p97 ホモログが 2 個存在し、機能が重複する必須因子であることを証明している。小胞体関連分解、性決定、精子形成、染色体凝縮、染色体分配（Sasagawa et al., 2012）などにおける p97 の役割を明らかにした。染色体分配においては、CDC-48 が AuroraB キナーゼの時空間的制御を通して、染色体の解離制御を行っている。また CDC-48 の C 末端アダプターの 1 つである UFD-3 は、ポリグルタミン凝集体形成を制御することを明らかにし、その過程において、ポリグルタミン凝集体の毒性に関して、蛍光顕微鏡で観察できる凝集体よりもその形成過程で生じる中間体分子の方の毒性が高いという結果も得た（Murayama et al., 2015）。

線虫の fidgetin ホモログ FIGL-1：マウスの頭部（特に内耳や眼）の形成が異常となる *fidget* 変異の原因因子として同定された fidgetin の線虫のホモログ FIGL-1 について解析し、FIGL-1 が翻訳後修飾因子 SUMO と特異的に相互作用し、線虫の発生、特に生殖腺形成に重要な役割を担っていることを明らかにした（Onitake et al., 2012）。

酵母の BCS1L ホモログ Bcs1：BCS1L はミトコンドリアの呼吸鎖複合体 III の会合に必要で、Rieske 鉄イオウタンパク質を複合体に挿入するシャペロンと考えられている。酵母を用いた遺伝的解析により、Bcs1 の N 末端の膜間配列が機能に重要であることを明らかにした（Sawamura et al., 2014）。

酵母の p97 ホモログ Cdc48：酵母の p97 ホモログ Cdc48 が、ミトコンドリアの融合反応に必須であり, 融合反応を直接司る Fzo1 の分解に寄与することを見出した（Esaki & Ogura, 2012; Miyazaki et al., 2014）。

Cdc48 (p97)-20S プロテソーム：上に述べたように、Cdc48 が 20S プロテアソームに結合した新規プロテアソームの存在を明らかにした（図 6）（Esaki et al., 2017）。Cdc48-20S プロテアソームの細胞内基質として、Sod1 (superoxide dismutase) などいくつかのタンパク質を同定し、in vitro で Cdc48-20S プロテアソームによる Sod1 の分解を確認した。Sod1 の変異は、ALS の原因として同定されており、ヒト p97 ホモログ VCP の変異も ALS の原因となることから、p97-20S プロテアソームが ALS と関連する可能性が考えられる。

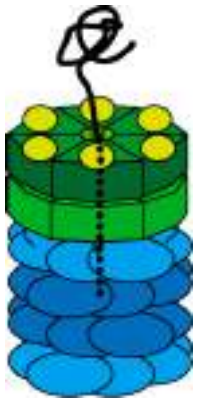

図 6. Cdc48－20S プロテアソームによる基質分解

４．アミロイド線維と分子シャペロン

ヒト p97 ホモログ VCP の TDP-43 アミロイド線維への結合：VCP の変異によって発症する ALS や IBMPFD では、RNA 結合核タンパク質 TDP-43 の凝集体が細胞内に蓄積する。VCP が TDP-43 に直接作用して凝集体形成を制御する可能性について検討し、VCP は ATP 非存在下で TDP-43 凝集体に結合すること、ATP 存在下では結合しないこと、AFM 観察で、VCP が TDP-43 のアミロイド線維には結合するが、不定形の凝集体にはほとんど結合しないことを明らかにした。

家族性アミロイドーシスの線虫モデル作製及びそれを用いた治療薬の探索：トランスサイレチンの細胞外アミロイドの沈着により引き起こされる家族性アミロイドーシスの線虫モデルを作製し、それを用いた治療薬の探索に向けた研究を開始した【本学生命科学研究部安東教授らとの共同研究】。

機能性アミロイドバイオフィルムと分子シャペロン：固体表面に付着した細菌は、自らが産生する細胞外マトリクス（タンパク質、多糖や核酸など）に覆われながら、バイオフィルムを形成する。バイオフィルム中の細菌は抗生物質や宿主免疫からまぬがれ、慢性感染症を引き起こす。大腸菌を用いた遺伝的解析から、分子シャペロン DnaK がバイオフィルム形成に必須であることを見いだし、植物由来フラボノイドの 1 つミリセチンがバイオフィルム形成に重要な細胞外マトリクスの 1 種である curli 産生を抑制して、バイオフィルム形成を抑制することを明らかにした（Arita-Morioka et al., 2015）。Curli は、細菌にとって有益なアミロイド線維であることから、機能性アミロイドと呼ばれる。さらに DnaK がどのような機構で curli 産生を制御しているかについて研究を進め、次のことを明らかにした。①2 つの転写因子 RpoS と CsgD の質的量的制御により、curli 主要構成因子 CsgA の発現を制御している。②CsgA に直接結合して膜輸送を制御している（Sugimoto et al., in press）。また、大気圧走査電子顕微鏡（ASEM）という新しい電子顕微鏡を用いて、バイオフィルム形成過程や内部構造の観察に成功した（Sugimoto et al., 2016）。アミロイド線維の検出試薬として汎用されているチオフラビン T が RNA にも結合することを見出し、この性質を利用してポリ A 合成酵素や RNA 分解酵素の活性をリアルタイムで定量し、大腸菌の細胞内 RNA の量的変動を可視化することに成功した（Sugimoto et al.,

2015)。【東京慈恵会医科大学杉本准教授らとの共同研究】

AAA family proteins are a subfamily of the Walker-type ATPases. Their functional architecture is a hexameric ring with a central pore. Recent studies have indicated that they represent a novel class of molecular chaperones, which unfold proteins and disassemble protein complexes. They are highly conserved from prokaryotes to humans. Interestingly, the number of AAA members and their functional distribution are similar in eukaryotes as diverse as yeast, worms, flies, and humans. We are interested in elucidating whether there is a common molecular mechanism underlying the diverse functions of the family members. Some AAA proteins are involved in human genetic diseases, e.g., p97/VCP in amyotrophic lateral sclerosis (ALS) and inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD), spastin in hereditary spastic paraplegia (HSP), and BCS1L in respiratory complex III deficiency, GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death) syndrome. We have studied the molecular mechanisms and cellular functions of several AAA family proteins, especially those related to human genetic diseases.

1. Common molecular basis of the AAA family ATPases

Katanin and spastin are AAA proteins, which possess microtubule-severing activity. It should be interesting to ask whether or not the pore of these AAA proteins is also functionally important. We have found that conserved aromatic and basic amino acid residues in the pore region of the *C. elegans* spastin homolog, SPAS-1, and sea urchin katanin are important for microtubule-severing (Matsushita-Ishiodori et al., 2009; Johjima et al., 2015). In addition, both of acidic C-terminal regions of α and β tubulins are essential for the severing by katanin (Johjima et al., 2015).

p97 (also known as VCP in human, CDC-48 in *C. elegans*, and Cdc48 in yeasts) consists of the N-terminal domain and two homologous AAA ATPase domains, D1 and D2, and D1 lacks the aromatic residue in the pore motif. It has been proposed that the conserved aromatic residue of the pore motif is important for unfolding activity of AAA chaperones by threading substrate polypeptides through the pore. We have found that introduction of an aromatic residue to the pore mitif of D1 of yeast Cdc48 becomes lethal, suggesting that it is most likely that unnecessary degradation of a certain essential protein(s) by the Cdc48-20S proteasome complex with an elevated unfolding activity causes lethal effect (Esaki et al., 2017).

2. Nanodynamics imaging of AAA proteins in action by high-speed atomic force microscopy

Dynamic movements of AAA proteins and their complexes with substrates are also being analyzed by using a high-speed atomic force microscope, which was developed by Prof. T. Ando at Kanazawa Univ. To understand the molecular mechanism of p97, we visualize conformational changes of *C. elegans* p97 homolog CDC-48.1 hexamers, using high-speed atomic force microscopy (HS-AFM). In the presence of ATP, the N-D1 ring repeatedly rotates ~25° clockwise relative to the D2 ring (Noi et al., 2013). Mutational analysis reveals that this rotation is induced by ATP-binding to the D2 domain. Repeated back and forward rotational movements of p97 may account for, at least in part, mechanical force in substrate processing.

The 26S protease consists of 20S catalytic and 19S regulatory particles, and the 19S particle contain six distinct AAA proteins, which form a ring with a narrow central pore, through which substrate proteins are unfolded and translocated to the proteolytic center of the catalytic β ring. We are trying to observe the whole process of degradation of polyubiquitylated substrate proteins by the 26S proteasome; binding of substrates to the proteasome, deubiquitylation, and degradation [collaboration with Dr. T. Okuno of Yamagata Univ.]. So far, it has been found that substrates bind to the 26S proteasome inefficiently. We are also investigating a stimulation effect of a shuttling factor Rad23 and complex formation of Rad23 and a substrate protein.

We are also observing ATP-dependent conformational changes of ClpB, which has a disaggregating activity, by HS-AFM. Both closed flat ring and closed spiral ring have been observed [collaboration with Prof. M. Yohda of Tokyo Univ. of Agr. and Tech.].

Several non-AAA chaperones have also been observed by HS-AFM. PDI (Protein Disulfide Isomerase) family proteins regulate disulfide bonds of client proteins in the ER, and assist their proper folding. HS-AFM observation of ERdj5, which cleaves incorrect disulfide bonds formed in misfolded proteins, and accelerates their degradation, revealed its highly dynamic property and correlation between dynamics and function [collaboration with Prof. K. Inaba of Tohoku Univ.] (Maegawa et al., 2016). Currently, we have also revealed dimer

formation of PDI upon binding to a substrate.

3. Cellular functions of AAA proteins

p97 homologs in *C. elegans* (CDC-48.1 and CDC-48.2) and adaptors: p97 is a multifunctional AAA protein, which is involved in the reconstitution of endoplasmic reticulum (ER), Golgi apparatus, and nuclear envelope, the ubiquitin-proteasome pathway, and ER-associated degradation (ERAD). It is also implicated in apoptosis, autophagy, and polyglutamine diseases. Diverse functions of p97 are regulated by differential binding of adaptor proteins. Mutations in human p97 homologue VCP cause ALS and IBMPFD.

RNAi assays of *C. elegans* p97 homologs, CDC-48.1 and CDC-48.2, have revealed that simultaneous disruption of both genes is lethal at the embryo stage. We have revealed the roles of *C. elegans* p97 homologs in ERAD, sex determination, spermatogenesis, chromosome condensation, and chromosome segregation (Sasagawa et al., 2012). In the chromosome segregation mechanism, CDC-48 controls chromosome separation through spatiotemporal regulation of AuroraB kinase. We have also revealed the importance of UFD-3, a C-terminal adaptor of CDC-48, in polyglutamine aggregate formation. In this study, we have obtained the results indicating that the toxicity of large aggregates is weaker than intermediate molecules formed during the process of aggregate formation (Murayama et al., 2015).

The fidgetin homologue (FIGL-1) in *C. elegans*: Fidgetin has been identified as a causative factor of the 'fidget' phenotype in mice, which is characterized by reduced or absent semicircular canals and small eyes. We have found that FIGL-1 specifically interacts with SUMO, which is a posttranslational modification factor (Onitake et al., 2012).

Bcs1, a BCS1L homolog in yeast: BCS1L is involved in the assembly of complex III of the respiratory chain in mitochondria, presumably inserting Rieske Fe/S protein into precursors to assemble complex III. Genetic analysis has revealed that the N-terminal intermembrane segment of Bcs1 is important for its function (Sawamura et al., 2014).

Cdc48, a p97 homolog in yeast: We have found that Cdc48 is essential for fusion of mitochondria, and is involved in degradation of Fzo1 responsible for the fusion process (Esaki & Ogura, 2012; Miyazaki et a., 2014).

Cdc48 (p97)-20S proteasome: We have identified several candidate substrates which are degraded by the new proteasome, Cdc48-20S. One of them is Sod1, superoxide dismutase, and in vitro experiments showed degradation of Sod1 by the Cdc48-20S proteasome. Mutations in Sod1 as well as VCP, a human p97 homolog, cause ALS. It is interesting to address the possibility that degradation of Sod1 by the VCP-20S proteasome may somehow relate to ALS.

4. Amyloid filaments and molecular chaperones

Binding of VCP to TDP-43 amyloid filaments: Muscular and neuronal cells of ALS and IBMPFD patients contain abnormal aggregates of TAR-DNA binding protein 43 (TDP-43), an RNA-binding nuclear protein. We have investigated the possibility that VCP interacts with TDP-43 to modulate aggregate formation. It has been indicated that VCP binds to TDP-43 amyloid filaments, but not amorphous aggregates, in the absence of ATP, and that this interaction is not observed in the presence of ATP.

Establishment of a *C. elegans* model of familial amyloidosis polyneuropathy and its application for drug screening: Familial amyloidosis polyneuropathy (FAP) is caused by deposition of transthyretin amyloids. We have developed a *C. elegans* model of FAP, and started research to screen drugs using it [collaboration with Prof. Ando of Fac. Life Sci., Kumamoto Univ.].

Functional amyloids curli and molecular chaperones: Bacteria on solid surface form biofilms with self-produced extracellular substances such as proteins, lipopolysaccharides, and nucleic acids. Bacteria embedded in biofilms become resistant to antibiotics and host immune responses, resulting in persistent infections. Genetic analysis of *E. coli* indicated the importance of DnaK (Hsp70) in biofilm formation. It was found that Myricetin, a natural flavonoid, suppresses the formation of biofilm by inhibiting the biogenesis of curli, the major component of the extracellular matrix (Arita-Morioka et al., 2015). Curli are referred to as functional amyloids. Further analysis has revealed that 1) DnaK regulates expression of CsgA, a major component of curli, via control of two transcription factors, RpoS and CsgD, and 2) DnaK also regulates translocation of CsgA through the membrane by directly binding to CsgA (Sugmimoto et al., in press). Bacterial multicellular behavior in biofilms has been imaged by atmospheric scanning electron microscopy (Sugimoto et al., 2016). We also succeeded to monitor RNA metabolism in vitro and in vivo by using the amyloid-binding probe Thioflavin T (Sugimoto et al., 2015). [collaboration with Dr. S. Sugimoto of Jikei Univ. Sch. of Med.]

論文目録　Publications

1. Sugimoto, S., Arita-Morioka, K., Terao, A., Yamanaka, K., Ogura, T., and Mizunoe, Y. Multitasking of Hsp70 chaperone in the biogenesis of bacterial functional amyloids. ***Communications Biology*** (in press)

2. Fukuda, N., Noi, K., Weng, L., Kobashigawa, Y., Miyazaki, H., Wakeyama, Y., Takaki, M., Nakahara, Y., Tatsuno, Y., Uchida-Kamekura, M., Suwa, Y., Sato, T., Ichikawa-Tomikawa, N., Nomizu, M., Fujiwara, Y., Ohsaka, F., Saitoh, T., Maenaka, K., Kumeta, H., Shinya, S., Kojima, C., Ogura, T., and Morioka, H., Production of single-chain Fv antibodies specific for GA-pyridine, an advanced glycation end-product (AGE), with reduced inter-domain motion. ***Molecules*** 22, 1695, 2017.

3. Esaki, M., Islam, Md. T., Tani, N., and Ogura, T. Deviation of the typical AAA substrate-threading pore prevents fatal protein degradation in yeast Cdc48. ***Sci. Rep.*** 7, 5475, 2017.

4. Maegawa, K., Watanabe, S., Noi, K., Okumura, M., Amagai, Y., Inoue, M., Ushioda, R., Nagata, K., Ogura, T., and Inaba, K. The highly dynamic nature of ERdj5 is key to efficient elimination of aberrant protein oligomers through ER-associated degradation. ***Structure*** 25, 6, 846-857, 2017.

5. Yamamoto, D., Noi K., and Ogura, T. A substrate for observing histidine-tagged proteins by high-speed atomic force microscopy. ***Fukuoka Univ. Sci. Rep***. 47, 1-6, 2017.

6. Esaki, M. Cdc48 AAA ATPase regulates protein dynamics and turnover in mitochondria. In ***AUTOPHAGY: Cancer, Other Pathologies, Inflammation, Immunity, Infection and Aging*** (ed. M. Hayat) Vol 12, 161-173, 2017.

7. Sugimoto, S., Okuda,K., Miyakawa, R., Sato, M., Arita-Morioka, K., Chiba, A., Yamanaka, K., Ogura, T., Mizunoe, Y. and Sato, C. Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy. ***Sci. Rep.*** 6, 25889, 2016.

8. Sugimoto, S., Arita-Morioka, K., Mizunoe, Y., Yamanaka, K., and Ogura, T. Thioflavin T as a fluorescence probe for monitoring RNA metabolism at molecular and cellular levels. ***Nucl. Acids Res.*** 14, e92, 2015.

9. Johjima, A., Noi, K., Nishikori, S., Ogi, H., Esaki, M., and Ogura, T. Microtubule severing by katanin p60 AAA+ ATPase requires the C-terminal acidic tails of both α- and β-tubulins and basic amino acid residues in the AAA+ ring pore. ***J. Biol. Chem.*** 290, 11762-11770, 2015.

10. Murayama, Y., Ogura, T., and Yamanaka, K. Characterization of C-terminal adaptors, UFD-2 and UFD-3, of CDC-48 on the polyglutamine aggregation in *C. elegans*. ***Biochem. Biophys. Res. Commun.*** 459, 154-160, 2015.

11. Arita-Morioka, K., Yamanaka, K., Mizunoe, Y., Ogura, T., and Sugimoto, S. Novel strategy for biofilm inhibition by using small molecules targeting molecular chaperone DnaK. ***Antimicrob. Agents Chemother.*** 59, 633-641, 2015.

12. Matsuyama, S., Moriuchi, M., Siuco M.A., Yano, S., Morino-Koga, S., Shuto, T., Yamanaka, K., Kondo, T., Araki, E., and Kai, H. Mild electrical stimulation increases stress resistance and suppresses fat accumulation via activation of LKB1-AMPK signaling pathway in *C. elegans*. ***PLoS ONE*** 9, e114690, 2014.

13. Miyazaki, N., Esaki, M., Ogura, T., and Murata, K. Serial block-face scanning electron microscopy for three-dimensional analysis of morphological changes in mitochondria regulated by Cdc48p/p97 ATPase. ***J. Struct. Biol.*** 187, 187-193, 2014.

14. Murata, K., Esaki, M., Ogura, T., Arai, S., Yamamoto, Y., and Tanaka, N. Whole-cell imaging of the budding yeast Saccharomyces cerevisiae by high-voltage scanning transmission electron tomography. ***Ultramicroscopy*** 146, 39-45, 2014.

15. Sawamura, R., Ogura, T., and Esaki, M. A

conserved α helix of Bcs1, a mitochondrial AAA chaperone, is required for the Respiratory Complex III maturation. ***Biochem. Biophys. Res. Commun.*** 443, 997-1002, 2014.

16. Noi, K., Yamamoto, D., Nishikori, S., Arita-Morioka, K., Kato, T., Ando, T., and Ogura, T. High-speed atomic force microscopic observation of ATP-dependent rotation of the AAA+ chaperone p97. ***Structure*** 21, 1992-2002, 2013.

17. Okuno, T., and Ogura, T. FtsH protease-mediated regulation of various cellular functions. ***Subcell. Biochem.*** 66, 53-69, Regulated proteolysis in microorganisms. D. A. Dougan (ed.) Springer, 2013.

18. Ogura, T., Okuno, T., Suno, R., and Akiyama, Y. FtsH protease. In ***Handbook of Proteolytic Enzymes, 3rd edition,*** N. Rawlings and G. Salvesen (ed.) Elsevier Ltd., pp 685-692, 2013.

19. Terabayashi, T., Sakaguchi, M., Shinmyozu, K., Ohshima,T., Johjima,A., Ogura, T., Miki, H., Nishinakamura, R. Phosphorylation of Kif26b promotes Its polyubiquitination and subsequent proteasomal degradation during kidney development. ***PLoS ONE*** 7, e39714, 2012.

20. Sasagawa, Y., Higashitani, A., Urano, T., Ogura, T., and Yamanaka, K. CDC-48/p97 is required for proper meiotic chromosome segregation via controlling AIR-2/Aurora B kinase localization in *Caenorhabditis elegans*. ***J. Struct. Biol.*** 179, 104-111, 2012.

21. Esaki, M., and Ogura, T. Cdc48p/p97-mediated regulation of mitochondrial morphology is Vms1p-independent. ***J. Struct. Biol.*** 179, 112-120, 2012.

22. Onitake, A., Matsushita-Ishiodori, Y., Johjima, A., Esaki, M., Ogura, T., and Yamanaka, K. The C-terminal α-helix of SPAS-1, a *Caenorhabditis elegans* spastin homologue, is crucial for microtubule severing. ***J. Struct. Biol.*** 179, 138-142, 2012.

23. Onitake, A., Yamanaka, K., Esaki, M., and Ogura, T. *Caenorhabditis elegans* fidgetin homolog FIGL-1, a nuclear-localized AAA ATPase, binds to SUMO. ***J. Struct. Biol.*** 179, 143-151, 2012.

24. Yamanaka, K., Sasagawa, Y., and Ogura, T. Recent advances in p97/VCP/Cdc48 cellular functions. ***Biochim. Biophys. Acta*** 1823, 130-137, 2012.

学会発表目録　Meeting Presentations

1. 杉本 真也、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：ミリセチン類縁体による菌体外アミロイド線維依存的バイオフィルムの制御、第 91 回日本細菌学会総会、3 月 27 日−29 日、2018、福岡.

2. Tsuda, Y., Ueda, M., Yamanaka, K., Masuda, T., Misumi, Y., Ogura, T., Ando, Y. A transgenic *Caenorhabditis elegans* expressing transthyretin as a model in transthyretin amyloidosis. The XVIth International Symposium on Amyloidosis, 26-29 March, 2018, Kumamoto.

3. Hirayama, C., Okumura, M., Noi, K., Ogura, T., Machida, K., Imataka, H., and Inaba, K. : Molecular Actions of the PDI-family Enzymes on Nascent Polypeptide Chains in the Catalysis of Oxidative Protein Folding、CLS, Tokyo Tech. International Forum 2018、March 4-5、2018、Tokyo.

4. Islam, Md.T., Kaneko, S., Ogura, T., and Esaki, M. : AAA molecular chaperone Cdc48 functions with the 20S proteasome to maintain protein homeostasis、第 40 回日本分子生物学会年会(ConBio 2017)、12 月 6 日−9 日、2017、神戸.

5. 杉本 真也、寺尾 明莉、山中 邦俊、小椋 光、水之江 義充：Hsp70 シャペロンによる AAA+ プロテアーゼ分解基質のトリアージ、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日−9 日、2017、神戸.

6. Mojumder, S., Sawamura, R., Ogura, T., and Yamanaka, K. : Elucidation of UBXN-6 function in CDC-48-regulated ERAD in *C.*

elegans、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

7. 村田 愛、小椋 光、江崎 雅俊：高速原子間力顕微鏡を用いたミトコンドリア膜の高分解能ダイナミクス観察、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

8. 森田 健斗、堀 綾佳、山本 陽平、宇野 裕子、野井 健太郎、小椋 光、Valpuesta Jose、野口 恵一、養王田 正文：好熱性真菌 *Chaetomium thermophilum* 由来プレフォルディンの機能構造解析、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

9. 篠原 恭介、井上 耀介、花園 祐矢、野井 健太郎、川本 晃大、竹田 一旗、岩政 菜津紀、野口 恵一、難波 啓一、小椋 光、三木 邦夫、養王田 正文：好熱性真菌由来の脱凝集因子 Hsp104 の機能構造解析、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

10. Chowdhury, A., Ogura, T., and Esaki, M. ：Roles of the Cdc48-cofactor complexes in regulation of mitochondrial morphology in yeast、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

11. Hirayama, C., Okumura, M., Noi, K., Ogura, T., Machida, K., Imataka, H., and Inaba, K. ：Molecular basis of oxidative folding of human serum albumin nascent chain catalyzed by PDI family enzymes、第 40 回日本分子生物学会年会（ConBio 2017）、12 月 6 日-9 日、2017、神戸.

12. 平山 千尋、奥村 正樹、町田 幸大、野井 健太郎、小椋 光、今高 寛晃、稲葉 謙次：翻訳合成初期の新生鎖に対する PDI と ERp46 のジスルフィド結合導入機構の解明、新学術領域研究「新生鎖の生物学」平成 29 年度第 1 回班会議、11 月、2017、別府.

13. 津田 幸元、植田 光晴、山中 邦俊、小椋 光、安東 由喜雄：TTR アミロイド沈着を来たす線虫モデルの開発に向けた検討、第 5 回日本アミロイドーシス研究会学術集会、8 月 19 日、2017、京都.

14. 奥村 正樹、野井 健太郎、金村 進吾、引間 孝明、秋山 修志、小椋 光、稲葉 謙次：酸化的フォールディングの触媒過程にみられる Protein Disulfide Isomerase の動的な結合と解離、第 17 回日本蛋白質科学会年会、6 月 20 日-22 日、2017、仙台.

15. 奥村 正樹、平山 千尋、野井 健太郎、町田 幸大、今高 寛晃、小椋 光、稲葉 謙次：新生鎖の酸化的フォールディングを触媒する PDI ファミリーの作用機構、第 17 回日本蛋白質科学会年会、6 月 20 日-22 日、2017、仙台.

16. 杉本 真也、寺尾 明莉、山中 邦俊、小椋 光、水之江 義充：Hsp70 シャペロンをハブとした AAA+プロテアーゼ分解基質のトリアージ、第 14 回 21 世紀大腸菌研究会、6 月 8 日-9 日、2017、熱海.

17. Inoue, Y., Hanazono, Y., Noi, K., Kawamoto, A., Takeda, K., Noguchi, K., Namba, K., Ogura, T., Miki, K., Shinohara, K., Yohda, M. High resolution structural studies and direct visual observation of Hsp104 from *Chaetomium thermophilum* suggest the role of the spiral architecture. EMBO Conference on Protein quality control: Success and failure in health and disease, 14-19 May, 2017, Sant Feliu de Guixols, Spain.

18. 杉本 真也、宮川 玲奈、寺尾 明莉、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：細胞外アミロイド線維形成タンパク質の細胞内品質管理機構、第 39 回日本分子生物学会年会、11 月 30 日-12 月 2 日、2016、横浜.

19. 奥村 正樹、野井 健太郎、金村 進吾、引間 孝明、秋山 修志、小椋 光、稲葉 謙次：酸化的フォールディングの触媒過程にみられる PDI の動的な結合・解離、第 39 回日本分子生物学会年会、11 月 30 日-12 月 2 日、2016、横浜.

20. 江崎 雅俊、Md.Tanvir Islam、小椋 光：多機能 AAA タンパク質 Cdc48 はいかにして基質タンパク質に作用するのか？、第 39 回日本分子生物学会年会、11 月 30 日-12 月 2 日、2016、横浜.

21. 江崎 雅俊、Abhijit Chowdhury、小椋 光：Cdc48 と複数のアダプタータンパク質によ

るミトコンドリア融合反応制御、第 89 回日本生化学会大会、9 月 25 日-27 日、2016、仙台.

22. 野井 健太郎、小椋　光：分子シャペロン VCP/p97 の TDP-43 及びαシヌクレインアミロイド線維への作用機構、第 89 回日本生化学会大会、9 月 25 日-27 日、2016、仙台.

23. 森田 健斗、堀 綾佳、山本 陽平、宇野 裕子、野井 健太郎、小椋 光、野口 恵一、養王田 正文：好熱性真菌 *Chaetomium thermophilum* 由来プレフォルディンの機能構造解析、平成 28 年度 日本生化学会関東支部例会、6 月 11 日、2016、東京.

24. 野井 健太郎、小椋 光：TDP-43 アミロイド線維に対する分子シャペロン VCP/p97 の作用機構の解明、第 16 回日本蛋白質科学会年会、6 月 7 日-9 日、2016、福岡.

25. 福田 夏希、宮崎 広海、分山 結加里、中原 悠介、佐藤 卓史、小橋川 敬博, 逢坂 文那、齊藤 貴士、前仲 勝実、野井 健太郎、小椋 光、中村 照也、山縣 ゆり子、森岡 弘志：生体内 AGE 化蛋白質検出系の構築に向けた一本鎖抗体（scFv）の創製と評価、第 16 回日本蛋白質科学会年会、6 月 7 日-9 日、2016、福岡.

26. 奥村 正樹、野井 健太郎、金村 進吾、引間 孝明、秋山 修志、小椋 光、稲葉 謙次：酸化的フォールディングを支える PDI ファミリー酵素の新規触媒機構の解明、第 16 回日本蛋白質科学会年会、6 月 7 日-9 日、2016、福岡.

27. 森田 健斗、山本 陽平、宇野 裕子、野井 健太郎、小椋 光、野口 恵一、養王田 正文：好熱性真菌 *Chaetomium thermophilum* 由来プレフォルディンの機能構造解析、第 16 回日本蛋白質科学会年会、6 月 7 日-9 日、2016、福岡.

28. 杉本 真也、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：分子シャペロン DnaK によるバイオフィルムの形成制御メカニズム、第 13 回 21 世紀大腸菌研究会、6 月 2 日-3 日、2016、阿蘇.

29. 野中 桃子、柿本 衿菜、原 怜、久堀 徹、小椋 光、坂本 亘、天野 豊己：FtsH プロテアーゼの基質タンパク質との親和性、第 7 回日本光合成学会年会およびシンポジウム、5 月 27 日-28 日、2016、東京.

30. 佐藤 恵春、三塚 綾音、北 潔、矢幡 一英、金子 修、小椋 光：熱帯熱マラリア原虫のプラスチドゲノムがコードする ClpC の解析、第 85 回日本寄生虫学会大会、3 月 19 日-20 日、2016、宮崎.

31. 宮崎 広海、分山 結加里、中原 悠介、福田 夏希、佐藤 卓史、小橋川 敬博、逢坂 文那、齊藤 貴士、前仲 勝実、野井 健太郎、小椋 光、中村 照也、山縣 ゆり子、森岡 弘志：生体内 AGE 化タンパク質の検出系構築に向けた一本鎖抗体の創製、第 3 回生命分子科学研究会、3 月 15 日、2016、札幌.

32. 森田 健斗、山本 陽平、宇野 裕子、野井 健太郎、小椋 光、野口 恵一、養王田 正文：好熱性真菌 *Chaetomium thermophilum* 由来プレフォルディンの機能構造解析、第 4 回日本生物工学会東日本支部コロキウム、3 月 1 日、2016、東京.

33. 奥村 正樹、野井 健太郎、金村 進吾、秋山 修志、小椋 光、稲葉 謙次：Structural basis of disulfide bond formation in the mammalian endoplasmic reticulum、第 15 回東北大学多元物質科学研究所研究発表会、12 月 22 日、2015、宮城.

34. 柿本 衿菜、原 怜、久堀 徹、小椋 光、坂本 亘、天野 豊己：植物由来 FtsH の ATPase ドメインにおける親和性変化、日本生体エネルギー研究会 第 41 回討論会、12 月 22 日、2015、東京.

35. 杉本 真也、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：細胞外アミロイド産生におけるコシャペロン非依存的な分子シャペロン DnaK の機能、第 38 回日本分子生物学会年会/第 88 回日本生化学会大会 合同大会、12 月 1 日-4 日、2015、神戸.

36. 澤村 理英、山中 邦俊：*C. elegans* における CRISPR/Cas9 system によるゲノム編集法の確立、第 38 回日本分子生物学会年会/第 88 回日本生化学会大会 合同大会、12 月 1 日-4 日、2015、神戸.

37. 大枝 弘明、加藤 裕介、坂本 亘、久堀 徹、小椋 光、天野 豊己：植物由来ヘテロ 6 量体 FtsH の大腸菌での共発現系の構築とそ

の機能解析、第 38 回日本分子生物学会年会/第 88 回日本生化学会大会 合同大会、12 月 1 日-4 日、2015、神戸.

38. 奥村 正樹、野井 健太郎、金村 進吾、秋山 修志、小椋 光、稲葉 謙次：高速 AFM が明らかにした PDI による基質の酸化的フォールディング触媒機構の解明、第 38 回日本分子生物学会年会/第88回日本生化学会大会 合同大会、12 月 1 日-4 日、2015、神戸.

39. 江崎 雅俊、Abhijit Chowdhury、小椋 光：Cdc48 AAA ATPase によるミトコンドリア融合反応の制御、第 38 回日本分子生物学会年会/第 88 回日本生化学会大会 合同大会、12 月 1 日-4 日、2015、神戸.

40. 山本 大輔、野井 健太郎、有田（森岡） 健一、小椋 光：高速原子力間顕微鏡により観察された AAA シャペロン p97 の主要 ATPase リングの構造変化、第 53 回日本生物物理学会年会、9 月 13 日ー15 日、2015、金沢.

41. 奥野 貴士、野井 健太郎、有田（森岡） 健一、土屋 光、佐伯 泰、高橋 一暢、伊野部 智由、斎藤 貴洋、山中 邦俊、小椋 光：26S プロテアソームの高速 AFM 観察、第 53 回日本生物物理学会年会、9 月 13 日ー15 日、2015、金沢.

42. 奥村 正樹、野井 健太郎、金村 進吾、増井 翔史、引間 孝明、秋山 修志、小椋 光、稲葉 謙次：高速 AFM が明らかにする PDI の酸化的フォールディングの触媒機構の解明、第 53 回日本生物物理学会年会、9 月 13 日ー15 日、2015、金沢.

43. 豊島 梨沙、植田 光晴、山中 邦俊、小椋 光、安東 由喜雄：トランスサイレチンを発現する線虫モデルの作成と解析、第 3 回アミロイドーシス研究会学術集会、8 月 21 日、2015、東京.

44. 杉本 真也、有田（森岡）健一、水之江 義充、山中 邦俊、小椋 光：蛍光プローブチオフラビン T による分子レベル・細胞レベルの RNA 代謝の高感度モニター、第 29 回日本バイオフィルム学会学術集会、7 月 10 日ー11 日、2015、蒲郡.

45. 野井 健太郎、小椋 光：AAA 型シャペロン VCP/p97 の分子機構の高速原子間力顕微鏡による解明 (Elucidation of molecular mechanisms of a AAA chaperone VCP/p97 by high-speed AFM). 第 15 回日本蛋白質科学会年会、6 月 24 日-26 日、2015、徳島市 あわぎんホール.

46. 奥村 正樹、野井 健太郎、金村 進吾、増井 翔史、引間 孝明、秋山 修志、小椋 光、稲葉 謙次：高速 AFM が明らかにする PDI による酸化的フォールディングの触媒機構 （Direct observation of PDI-catalyzed oxidative protein folding by high-speed atomic force microscopy). 第 15 回日本蛋白質科学会年会、6 月 24 日-26 日、2015, 徳島市 あわぎんホール.

47. 杉本 真也、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：DnaK/Hsp70 シャペロンシステムは VIII 型分泌装置の発現と品質を制御する、第 12 回 21 世紀大腸菌研究会、6 月 4 日-5 日、2015、大津.

48. 有田（森岡） 健一、 山中 邦俊、水之江 義充、小椋 光、杉本 真也：分子シャペロン DnaK をターゲットにした小分子化合物を用いた新規バイオフィルム阻害法の開発、第 12 回 21 世紀大腸菌研究会、6 月 4 日-5 日、2015、大津.

49. Okumura, M., Noi, K., Kanemura, S., Masui, S., Hikima, T., Akiyama, S., Ogura, T., and Inaba, K. Real-time monitoring of PDI-catalyzed oxidative protein folding by high-speed atomic force microscopy. ER&Redox Club Meeting, 16 April, 2015, Venice, Italy.

50. 鈴木 香於里、大枝 弘明、加藤 裕介、有田（森岡）健一、野井 健太郎、原 怜、山本 亘、小椋 光、久堀 徹、天野 豊己：ホウレンソウ由来天然 FtsH の精製と解析、日本生体エネルギー研究会 第 40 回討論会、12 月 12 日-13 日、2014、松山.

51. 澤村 理英、江崎 雅俊、山中 邦俊、小椋 光：ヒト疾患関連 AAA タンパク質 BCS1L の酵母ホモログ Bcs1p の機能解析、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、横浜.

52. 村山 佑樹、小椋 光、山中 邦俊：ポリグルタミン凝集体形成における線虫 CDC-48 C 末端アダプターの役割、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、

横浜.

53. 奥村 正樹、野井 健太郎、金村 進吾、増井 翔史、小椋 光、稲葉 謙次：高速原子間力顕微鏡を用いた PDI ファミリータンパク質の酸化還元状態に依存した動的構造解析、第37回日本分子生物学会年会、11月 25 日-27 日、2014、横浜.

54. 城島 愛、野井 健太郎、錦織 伸吾、荻 博次、江崎 雅俊、小椋 光：AAA タンパク質 katanin による微小管切断における AAA ドメインの pore 領域の残基と tubulin C 末端配列の重要性、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、横浜.

55. 江崎 雅俊、宮崎 直幸、村田 和義、小椋 光：Cdc48/p97 は Fzo1 の分解促進によってミトコンドリア融合反応を制御する、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、横浜.

56. 杉本 真也、有田（森岡） 健一、山中 邦俊、小椋 光、水之江 義充：分子シャペロン DnaK はタンパク質のフォールディングと局在化を介してバイオフィルム形成を制御する、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、横浜.

57. 有田（森岡）健一、山中 邦俊、水之江 義充、小椋 光、杉本 真也：分子シャペロン DnaK を標的とする低分子化合物を用いた新たなバイオフィルム阻害法の開発、第 37 回日本分子生物学会年会. 11 月 25 日-27 日、2014、横浜.

58. 山本 大輔、野井 健太郎、有田（森岡） 健一、小椋 光：高速原子間力顕微鏡による AAA シャペロン p97 の主要 ATPase(D2)リングの構造変化の直接観察、第 52 回 日本生物物理学会年会. 9月25日-27日、2014、札幌.

59. 有田（森岡） 健一、山中 邦俊、水之江 義充、小椋 光、杉本 真也：DnaK 阻害剤を用いた慢性細菌感染症の制御戦略、第 66 回 日本生物工学会大会. 9月9日-11日、2014、札幌.

60. Okumura, M., Noi, K., Masui, S., Kanemura, S., Ogura, T., and Inaba, K. Structural basis of disulfide bond formation in the mammalian endoplasmic reticulum. Key Forum: From Stem Cells to Organs, 4-5 September, 2014, Kumamoto Japan.

61. Okuno, T., Noi, K., Okawa, A., Tsuchiya, H., Saeki, Y., Takahashi, K., Inobe, T., Yamanaka, K., and Ogura, T. Visualization of dynamics of the 26S proteasome and proteasome-substrate complexes by high-speed AFM. Key Forum: From Stem Cells to Organs, 4-5 September, 2014, Kumamoto Japan.

62. 山本 大輔、野井 健太郎、有田（森岡） 健一、小椋 光：高速原子間力顕微鏡による AAA シャペロン p97 の主要 ATPase(D2)リングの直接観察、第 14 回 日本蛋白質科学会年会. 6 月 25 日-27 日、2014、横浜.

63. Yamanaka, K., Murayama, Y., and Ogura, T. : Adaptors of p97 and disease-associated p97 mutations. 231st IMEG Seminars & Minisymposium、February 20-21, 2014、Kumamoto University IMEG、Kumamoto.

64. Esaki, M., Noi, K., and Ogura, T. : Yeast Cdc48-20S proteasome complex in vitro and in vivo. 231st IMEG Seminars & Minisymposium、February 20-21, 2014、Kumamoto University IMEG、Kumamoto.

65. Okuno, T., Noi, K., Okawa, A., Tsuchiya, H., Saeki, Y., Takahashi, K., Inobe, T., Yamanaka, K., and Ogura, T.: High-speed AFM imaging of the 26S proteasome dynamics. 231st IMEG Seminars & Minisymposium、February 20-21, 2014 、 Kumamoto University IMEG 、 Kumamoto.

66. Yamamoto, D., Noi, K., Arita-Morioka, K, Ando, T., and Ogura, T. : Observation of AAA chaperone p97 by high-speed AFM. 231st IMEG Seminars & Minisymposium、February 20-21, 2014、Kumamoto University IMEG、Kumamoto.

67. Karata, K., and Ogura, T.: Clamp loading by Gamma complex. 231st IMEG Seminars & Minisymposium、February 20-21, 2014、Kumamoto University IMEG、Kumamoto.

68. Ueda, E., Noi, K., Arita-Morioka, K., Ogura, T., Sakamoto, W., Hisabori, T., and Amano, T.: Biochemical properties of FtsH protease from plants. 231st IMEG Seminars & Minisymposium、February 20-21, 2014、Kumamoto University IMEG、Kumamoto.

69. Noi, K., Yamamoto, D., Arita-Morioka, K., Ando, T., and Ogura, T. : High-speed AFM observation of ATP-dependent rotation of the AAA chaperone p97 and its interaction with TDP-43 amyloid fibrils. 231st IMEG Seminars & Minisymposium、February 20-21, 2014 、 Kumamoto University IMEG 、 Kumamoto.

70. Niwa, H., Ogura, T., and Fujiki, Y. : AFM study of peroxisomal membrane protein import. 231st IMEG Seminars & Minisymposium 、 February 20-21, 2014 、 Kumamoto University IMEG、Kumamoto.

71. 斉藤 勝広、植田 依里、柿本 衿菜、 鈴木 香於里、原 怜、久堀 徹、加藤 裕介、坂本 亘、野井 健太郎、有田 健一、小椋 光、天野 豊己：植物由来 FtsH プロテアーゼの分子機構. 日本生体エネルギー研究会 第 39 回討論会、12 月 18 日-20 日、2013、静岡.

72. 山中 邦俊、村山 佑樹、小椋 光：線虫を用いた疾患型変異 CDC-48/p97/VCP の機能解析. 第 36 回日本分子生物学会年会、12 月 3 日-6 日、2013、神戸.

73. 江崎 雅俊、野井 健太郎、佐伯 泰、小椋 光：In vivo において Cdc48 は 20S プロテアソーム複合体と相互作用する、 第 36 回日本分子生物学会年会、12 月 3 日-6 日、2013、神戸.

74. 澤村 理英、江崎 雅俊、小椋 光：呼吸鎖複合体 III 形成における AAA 分子シャペロン Bcs1p の N 末端の機能解析. 第 36 回日本分子生物学会年会、12 月 3 日-6 日、2013、神戸.

75. 奥野 貴士、野井 健太郎、大川 阿紀子、土屋 光、佐伯 泰、高橋 一暢、伊野部 智由、山中 邦俊、小椋 光：高速 AFM による 26S プロテアソームのダイナミクス解析、第 36 回日本分子生物学会年会、12 月 3 日-6 日、2013、神戸.

76. Ogura, T., Noi, K., Okuno, T., Arita-Morioka, K., Yamamoto, D., Okawa, A., Tsuchiya, H., Saeki, Y., Ando, T., Esaki, M., and Yamanaka, K. Dynamics of p97 and the 26S proteasome in action revealed by high-speed atomic force microscopy. EMBO Workshop on AAA+ Proteins: from mechanism and disease to targets, 15-19 September, 2013, Neuss, Germany.

77. Yamanaka, K., Murayama, Y., and Ogura, T. Functional analysis of disease-associated mutant CDC-48/p97/VCP in *C. elegans*. EMBO Workshop on AAA+ Proteins: from mechanism and disease to targets, 15-19 September, 2013, Neuss, Germany.

78. Esaki, M., and Ogura, T. Non-conservation of the aromatic residue in the D1 pore loop of yeast Cdc48 is essential for cell viability. EEMBO Workshop on AAA+ Proteins: from mechanism and disease to targets, 15-19 September, 2013, Neuss, Germany.

79. Noi, K., Arita-Morioka, K., Ogi, H., Hirao, M., and Ogura, T. Biochemical analysis of molecular mechanisms of the interaction between VCP/p97 and amyloid fibrils of TDP-43. EMBO Workshop on AAA+ Proteins: from mechanism and disease to targets, 15-19 September, 2013, Neuss, Germany.

80. Sawamura, R., Esaki, M., and Ogura, T. The N-terminal region of Bcs1p is important for the respiratory chain complex III assembly. EMBO Workshop on AAA+ Proteins: from mechanism and disease to targets, 15-19 September, 2013, Neuss, Germany.

81. 野井 健太郎、有田（森岡） 健一、小椋 光 ：分子シャペロン VCP/p97 と TDP-43 アミロイド線維の相互作用の分子機構の解析. 第 13 回日本蛋白質科学会年会、6 月 12 日-14 日、2013、鳥取市.

82. 宮崎 直幸、江崎 雅俊、小椋 光、村田 和義：Serial Block-Face SEM による酵母ミトコンドリアの形態変化の観察. 日本顕微鏡学会 第 69 回学術講演会、5 月 20 日-22 日、2013、吹田市.

83. 村田 和義、江崎 雅俊、小椋 光、荒井 重勇、山本 悠太、丹司 敬義、田中 信夫：超高圧 STEM トモグラフィーによる細胞まるごとの三次元構造解析. 日本顕微鏡学会 第 69 回学術講演会、5 月 20 日-22 日、2013, 吹田市.

84. 村田 和義、宮崎 直幸、江崎 雅俊、小椋

光、箕田 弘喜、新井 善博、永山 國昭：GFP-CL を用いた光顕・電顕相関観察法の試み. 日本顕微鏡学会 第69回学術講演会、5月 20 日-22 日、2013、吹田市.

85. Ogura, T., Noi, K., Arita-Morioka, K., Yamamoto, D., Ando, T., Nishikori, S., Esaki, M., and Yamanaka, K. AAA chaperones, focusing on the molecular mechanism of p97. EMBO Conference: The biology of molecular chaperones: From molecules, organelles and cells to misfolding diseases, 17-22 May, 2013, Santa Margherita de Pula, Italy.

86. 野井 健太郎、有田（森岡） 健一、小椋 光 ：分子シャペロン VCP/p97 と TDP-43 アミロイド線維の相互作用の分子機構の解析. 第85回日本生化学会大会、12月 14 日-16 日、2012、福岡.

87. Noi, K., Yamamoto, D., Arita-Morioka, K., Nishikori, S., Ando, T., and Ogura, T. High-speed atomic force microscopy of ATP-dependent rotational movements of p97/VCP and its interaction with amyloid fibrils of TDP-43. 3rd Kanazawa Bio-AFM Workshop, 5-8 November, 2012, Kanazawa, Japan.

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Production of Single-Chain Fv Antibodies Specific for GA-Pyridine, an Advanced Glycation End-Product (AGE), with Reduced Inter-Domain Motion

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Abstract: Due to their lower production cost compared with monoclonal antibodies, single-chain variable fragments (scFvs) have potential for use in several applications, such as for diagnosis and treatment of a range of diseases, and as sensor elements. However, the usefulness of scFvs is limited by inhomogeneity through the formation of dimers, trimers, and larger oligomers. The scFv protein is assumed to be in equilibrium between the closed and open states formed by assembly or disassembly of VH and VL domains. Therefore, the production of an scFv with equilibrium biased

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Deviation of the typical AAA substrate-threading pore prevents fatal protein degradation in yeast Cdc48

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Yeast Cdc48 is a well-conserved, essential chaperone of ATPases associated with diverse cellular activity (AAA) proteins, which recognizes substrate proteins and modulates their conformations to carry out many cellular processes. However, the fundamental mechanisms underlying the diverse pivotal roles of Cdc48 remain unknown. Almost all AAA proteins form a ring-shaped structure with a conserved aromatic amino acid residue that is essential for proper function. The threading mechanism hypothesis suggests that this residue guides the intrusion of substrate proteins into a narrow pore of the AAA ring, thereby becoming unfolded. By contrast, the aromatic residue in one of the two AAA rings of Cdc48 has been eliminated through evolution. Here, we show that artificial retrieval of this aromatic residue in Cdc48 is lethal, and essential features to support the threading mechanism are required to exhibit the lethal phenotype. In particular, genetic and biochemical analyses of the Cdc48 lethal mutant strongly suggested that when in complex with the 20S proteasome, essential proteins are abnormally forced to thread through the Cdc48 pore to become degraded, which was not detected in wild-type Cdc48. Thus, the widely applicable threading model is less effective for wild-type Cdc48; rather, Cdc48 might function predominantly through an as-yet-undetermined mechanism.

ATPases associated with diverse cellular activities (AAA+) proteins convert the chemical energy of ATP to mechanical forces to carry out diverse functions such as unfolding of their substrate proteins, disassembly of protein complexes and microfibers such as microtubules and amyloid fibrils, and the transport of macromolecules by walking on cytoskeletons, among others[1–3]. AAA proteins are defined as a subfamily of AAA+ proteins based on possession of the second region of homology (SRH) domain[4]. Bacteria and archaea harbor a few AAA proteins, most of which function as unfoldases. Eukaryotes contain 20–24 highly conserved AAA proteins, which function at multiple intracellular regions, including the nucleus, mitochondria, and cytosol. All AAA+ proteins contain one or two AAA+ modules as a mechanochemical engine, which typically form a hexameric ring structure with a central narrow pore. The ring structure has been shown to be essential for ATP hydrolysis and the proper functions of many AAA+ proteins.

In order to uncover the nature of biological reactions, the underlying fundamental mechanisms as well as the molecular architectures of essential enzymes must be elucidated. A threading mechanism has been proposed as a key and common molecular mechanism driving the reactions of several AAA+ proteins, which was mainly based on data obtained using bacterial AAA+ module-regulated proteases[5]. The bacterial AAA protease FtsH forms a AAA hexameric ring followed by a proteolytic chamber[6]. Since the AAA pore is interconnected to the interior of the proteolytic chamber, substrate proteins must first pass through the narrow pore of the AAA ring to reach the proteolysis site to be degraded. During such threading through the AAA pore, substrate proteins undergo unfolding by a function of the AAA module. The loop structure protruding into the pore (pore loop) is essential for this

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CellPress

Structure
Article

The Highly Dynamic Nature of ERdj5 Is Key to Efficient Elimination of Aberrant Protein Oligomers through ER-Associated Degradation

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SUMMARY

ERdj5, composed of an N-terminal J domain followed by six thioredoxin-like domains, is the largest protein disulfide isomerase family member and functions as an ER-localized disulfide reductase that enhances ER-associated degradation (ERAD). Our previous studies indicated that ERdj5 comprises two regions, the N- and C-terminal clusters, separated by a linker loop and with distinct functional roles in ERAD. We here present a new crystal structure of ERdj5 with a largely different cluster arrangement relative to that in the original crystal structure. Single-molecule observation by high-speed atomic force microscopy visualized rapid cluster movement around the flexible linker loop, indicating the highly dynamic nature of ERdj5 in solution. ERdj5 mutants with a fixed-cluster orientation compromised the ERAD enhancement activity, likely because of less-efficient reduction of aberrantly formed disulfide bonds and prevented substrate transfer in the ERdj5-mediated ERAD pathway. We propose a significant role of ERdj5 conformational dynamics in ERAD of disulfide-linked oligomers.

INTRODUCTION

The endoplasmic reticulum (ER) is an essential organelle that has evolved stringent quality control systems for secretory and cell surface membrane proteins. Glycosylation and disulfide bond formation take place co- or post-translationally in this compartment as critical events for promoting native protein folding and editing non-native protein structure. Nevertheless, a significant fraction of newly synthesized proteins is terminally misfolded and must be eliminated efficiently to negate cellular stresses. N-linked glycans serve as marking tags distinguishing correctly from incorrectly folded proteins. A UDP-glucose:glycoprotein glucosyltransferase selectively transfers monoglucose onto misfolded glycoproteins, which in turn interact with the lectin chaperones calnexin and/or calreticulin to restore native structure (Ellgaard and Frickel, 2003; Samuelson and Robbins, 2015; Tannous et al., 2015). Furthermore, mammalian ER harbors more than 20 protein disulfide isomerase (PDI) family members (Appenzeller-Herzog and Ellgaard, 2008; Hatahet and Ruddock, 2009; Sato and Inaba, 2012). The enzymes individually or cooperatively introduce native disulfide bonds, isomerize non-native disulfide bonds, and sometimes reduce aberrantly formed intermolecular disulfide bonds for acceleration of the ER-associated degradation (ERAD), leading to the maintenance of protein homeostasis in this organelle (Okumura et al., 2015).

ERdj5 (alternatively termed JPDI) is not only a member of the PDI family but also a member of the ER-localized DNAJ family since the enzyme comprises both a J domain and multiple redox-active thioredoxin (Trx)-like domains in a single polypeptide (Cunnea et al., 2003; Hosoda et al., 2003). ERdj5 functions as a disulfide reductase and acts in concert with ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1) and binding immunoglobulin protein (BiP) to accelerate the degradation of misfolded glycoprotein oligomers (Ushioda et al., 2008). EDEM1 selectively recruits misfolded glycoproteins (Hosokawa et al., 2001; Molinari et al., 2003; Oda et al., 2003) and binds ERdj5 to promote reduction of non-native disulfide bonds. Consequently, polypeptides are extended, transferred from ERdj5 to BiP in an ATP-dependent manner, and finally transported back from the ER to the cytosol through an unidentified dislocon channel to be degraded by the proteasome (Hagiwara et al., 2011).

We previously determined the crystal structure of full-length ERdj5 from *Mus musculus*, which comprised an N-terminal J domain followed by six (four redox-active and two redox-inactive) Trx-like domains (Trx1, Trxb1, Trxb2, Trx2, Trx3, and Trx4) (Hagiwara et al., 2011). The overall fold of ERdj5 can be separated into N- and C-terminal clusters at a linker region flanked by Trx2 and Trx3. With the exception of a salt bridge formed between Asp552 in Trx2 and Arg603 in Trx3, there is little contact at the interface between the two clusters, suggesting cluster mobility within the intact ERdj5. Systematic functional studies based on the structural information indicated that, while the N-terminal cluster is essential for binding to BiP through the

A Substrate for Observing Histidine-Tagged Proteins by High-Speed Atomic Force Microscopy

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Abstract

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1 Introduction

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CHAPTER

5

Cdc48 AAA ATPase Regulates Protein Dynamics and Turnover in Mitochondria

Masatoshi Esaki

OUTLINE

Abstract

Protein degradation contributes to not only the removal of abnormal proteins but also the regulation of biological reactions. The AAA ATPase Cdc48 and the proteasome are widely involved in protein degradation in the cytosol. Cdc48 and the proteasome also regulate quality control of mitochondrial outer membrane proteins including antiapoptotic factors and mitofusins that are responsible for tethering of the mitochondrial outer membrane during fusion events. ATPase-deficient mutants of Cdc48 inhibit mitochondrial fusion and collectively, it has been proposed that mitofusin degradation is required for the progression of mitochondrial membrane fusion. In contrast, recent evidence suggests that Cdc48 may mediate mitochondrial fusion by disassembly of mitofusin tethering complexes. The cytosolic AAA protein thus controls mitochondrial integrity by regulating not only protein stability but also complex formation. This review provides a detailed exploration of how Cdc48 functions to regulate mitochondrial protein quality and morphology.

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Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy

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Biofilms are complex communities of microbes that attach to biotic or abiotic surfaces causing chronic infectious diseases. Within a biofilm, microbes are embedded in a self-produced soft extracellular matrix (ECM), which protects them from the host immune system and antibiotics. The nanoscale visualisation of delicate biofilms in liquid is challenging. Here, we develop atmospheric scanning electron microscopy (ASEM) to visualise Gram-positive and -negative bacterial biofilms immersed in aqueous solution. Biofilms cultured on electron-transparent film were directly imaged from below using the inverted SEM, allowing the formation of the region near the substrate to be studied at high resolution. We visualised intercellular nanostructures and the exocytosis of membrane vesicles, and linked the latter to the trafficking of cargos, including cytoplasmic proteins and the toxins hemolysin and coagulase. A thick dendritic nanotube network was observed between microbes, suggesting multicellular communication in biofilms. A universal immuno-labelling system was developed for biofilms and tested on various examples, including *S. aureus* biofilms. In the ECM, fine DNA and protein networks were visualised and the precise distribution of protein complexes was determined (*e.g.*, straight curli, flagella, and excreted cytoplasmic molecular chaperones). Our observations provide structural insights into bacteria-substratum interactions, biofilm development and the internal microbe community.

Biofilms are highly organized microbial communities on surfaces, such as the surfaces of medical implants and host organisms. The microbes in them, are embedded in a self-produced extracellular matrix (ECM)[1] consisting of proteins[2], polysaccharides[3] and/or extracellular DNA (eDNA)[4]. The ECM has diverse functions to maintain the structural integrity of the biofilm and adapt to surrounding environments[5]. The resistance it confers to antimicrobial agents and host immune systems[6] is a deleterious property, causative of various chronic human infectious diseases, including periodontal disease, bacteremia, pneumonia and meningitis. Biofilm-embedded bacteria cooperate and communicate with each other[5]. Thus, biofilms act as multi-cellular organisms, allowing microbes to survive in various environments and attack their host organisms[1,6]. Biofilms also cause familiar problems, such as those encountered in the maintenance of drinking water distribution systems.

Direct observation of the structure of biofilms is essential to understand their development and functions. Generally, optical microscopy (OM) is employed to observe bacterial cells or macro-organelles, and confocal laser scanning microscopy (CLSM) has made a large contribution to biofilm research[7]. Using fluorescence probes, CLSM can visualise the entire expanse of a biofilm, describe its overall structure and localize ECM constituents. However, the resolution of diffraction-limited OM is restricted to approximately 200 nm. Super-resolution OM overcomes this limitation[8–11], and is providing exciting information about biofilms[12]. Nevertheless, this method

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Thioflavin T as a fluorescence probe for monitoring RNA metabolism at molecular and cellular levels

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ABSTRACT

The intrinsically stochastic dynamics of mRNA metabolism have important consequences on gene regulation and non-genetic cell-to-cell variability; however, no generally applicable methods exist for studying such stochastic processes quantitatively. Here, we describe the use of the amyloid-binding probe Thioflavin T (ThT) for monitoring RNA metabolism *in vitro* and *in vivo*. ThT fluoresced strongly in complex with bacterial total RNA than with genomic DNA. ThT bound purine oligoribonucleotides preferentially over pyrimidine oligoribonucleotides and oligodeoxyribonucleotides. This property enabled quantitative real-time monitoring of poly(A) synthesis and phosphorolysis by polyribonucleotide phosphorylase *in vitro*. Cellular analyses, in combination with genetic approaches and the transcription-inhibitor rifampicin treatment, demonstrated that ThT mainly stained mRNA in actively dividing *Escherichia coli* cells. ThT also facilitated mRNA metabolism profiling at the single-cell level in diverse bacteria. Furthermore, ThT can also be used to visualise transitions between non-persister and persister cell states, a phenomenon of isogenic subpopulations of antibiotic-sensitive bacteria that acquire tolerance to multiple antibiotics due to stochastically induced dormant states. Collectively, these results suggest that probing mRNA dynamics with ThT is a broadly applicable approach ranging from the molecular level to the single-cell level.

INTRODUCTION

Defining temporal fluctuations in gene expression in specific cells within organisms ranging from bacteria to humans will provide molecular insights into diverse biological processes, including morphogenesis, development, differentiation and adaptation to environmental stresses (1–6). Recently, several techniques have become available that enable investigation of this central issue in individual living cells, rather than depending upon the averaged properties of large populations (7–10). Unfortunately, there are still no general methods for meeting this important challenge.

Bacterial persistence is an example of a biological process that results from the fluctuations in gene expression in specific cells (11,12). Bacterial persistence was initially reported in 1944 (13) and is currently being studied extensively (11,14–17). Persisters are slow-growing cells that tolerate treatment with multiple drugs. Persisters are rare bacterial cells that form stochastically and are thus genetically identical to the majority of the bacterial population (11,12). In persister cells, type II toxin–antitoxin (TA) systems are believed to be important for inducing the resting state. Ectopically induced toxins of TA modules inhibit replication, transcription or translation, leading to the arrest of cell growth and drug tolerance (18). Understanding the genesis of bacterial persisters is important not only for understanding bacterial physiology, but also for the eradication of these multi-drug tolerant bacteria. Distinguishing persister cells from normal cells has been challenging because they are minority populations (ranging from 10^{-6} to 10^{-1}) and a lack of information concerning persister-specific gene expression profiles exists. However, such information can be attained using recently developed imaging techniques such as microscopes, microfluidics and flow cytometry (19). Fluorescent protein-based reporter systems have also been developed to monitor persister cells (11,17,20). However, non-genetic approaches are also strongly desired in the case of studying organisms in which genetic manipulation has not yet been established and conventional fluorescent protein reporter systems do not provide adequate information.

Thioflavin T (ThT; 4-(3,6-dimethyl-1,3-benzothiazol-3-ium-2-yl)-N,N-dimethylaniline chloride), is a well-known fluorescence probe used for detecting amyloid fibrils (21).

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Microtubule Severing by Katanin p60 AAA+ ATPase Requires the C-terminal Acidic Tails of Both α- and β-Tubulins and Basic Amino Acid Residues in the AAA+ Ring Pore*

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Background: Katanin p60 is a protein that actively severs microtubules.
Results: Mutations within the AAA+ pore of katanin p60 and in the C-terminal regions of tubulins perturb efficient microtubule severing.
Conclusion: Interactions between the conserved residues in the katanin p60 pore and the acidic tails of both tubulins may be important.
Significance: Both tubulin molecules are essential for microtubule severing by katanin.

The microtubule (MT) network is highly dynamic and undergoes dramatic reorganizations during the cell cycle. Dimers of α- and β-tubulins rapidly polymerize to and depolymerize from the end of MT fibrils in an intrinsic GTP-dependent manner. MT severing by ATP-driven enzymes such as katanin and spastin contributes significantly to microtubule dynamics, and it has been shown that katanin p60, a AAA+ family protein, has ATPase and MT-severing activities. The mechanism of MT severing by katanin p60 is poorly understood, and the residues in katanin p60 and tubulins important for severing activity were therefore explored in this study. MT-severing activity, but not ATPase activity, was inhibited by mutations of the conserved aromatic residue and the flanking basic residues in the pore region of the katanin p60 hexameric ring. When the acidic residue-rich C-terminal unstructured segment of either α- or β-tubulin was removed, polymerized MTs were resistant to katanin p60 treatment. Interactions between katanin p60 and the mutant MTs, on the other hand, were unaffected. Taken together, these findings led us to propose that the interactions between the positively charged residues of katanin p60 and the acidic tails of both tubulins are essential for efficient severing of MTs.

Microtubules (MTs)[4] represent an essential component of the intracellular architecture; they function to separate chromosomes during mitosis and as rails of intracellular transport, and act as the cytoskeleton of the cell. MTs typically consist of 13 protofilaments, each of which is a polymer of α-tubulin and β-tubulin dimers (1). MTs are not static structures because they dramatically change in length, especially during mitosis. Tubulin dimers attach to and are released from the ends of MT filaments in an intrinsic GTPase-dependent manner. Various MT-associated proteins, furthermore, significantly contribute to the reorganization of MT networks (2). Three AAA+ family proteins (ATPases associated with diverse cellular activities), katanin, spastin, and fidgetin, have been identified as MT-severing enzymes (3, 4). These ATP-dependent MT-severing enzymes are found in many eukaryotes such as *Tetrahymena*, nematodes, fruit flies, and humans, but are absent in yeasts. Mutations in the human spastin gene have also been identified as the most common causes of autosomal dominant hereditary spastic paraplegia (5). It has been shown that during mitosis spastin and fidgetin sever MTs and facilitate depolymerization of the tubulin dimers from the minus-end of MTs, although the *Caenorhabditis elegans* homolog of fidgetin may not have MT-severing activity (6–8). Katanin is also required for control of spindle length during mitosis and meiosis by severing MTs and facilitating depolymerization from the plus-end of MTs (8–11). In neurons, both katanin and spastin play important roles in reconstitution of axonal branches (12). Katanin has also been shown to be essential for cilia biogenesis in *Tetrahymena* (13) and for flagella biogenesis in *Chlamydomonas* (14, 15). Katanin also plays a role in the cell motility of *Drosophila* cells by regulating the MT networks (16). Although the role of MT-severing enzymes in cells has been investigated extensively, the molecular mechanisms of MT severing are poorly understood.

Katanin is composed of a 60-kDa enzymatic subunit (p60) and an 80-kDa regulatory subunit (p80). Katanin p60 is a AAA+ protein and exhibits MT-severing activity in an ATPase-dependent manner (17, 18), whereas katanin p80 regulates the MT-severing activity of katanin p60 and determines the localization of the protein complex (19). Katanin p60 contains an N-terminal domain followed by a AAA+ domain. A recent NMR analysis revealed that the solution structure of the N-ter-

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[4] The abbreviations used are: MT, microtubule; AAA+, ATPases associated with diverse cellular activities; ATPγS, adenosine 5′-*O*-(thiotriphosphate).

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Characterization of C-terminal adaptors, UFD-2 and UFD-3, of CDC-48 on the polyglutamine aggregation in *C. elegans*

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ABSTRACT

CDC-48 (also called VCP or p97 in mammals and Cdc48p in yeast) is a AAA (ATPases associated with diverse cellular activities) chaperone and participates in a wide range of cellular activities including modulation of protein complexes and protein aggregates. UFD-2 and UFD-3, C-terminal adaptors for CDC-48, reportedly bind to CDC-48 in a mutually exclusive manner and they may modulate the fate of substrates for CDC-48. However, their cellular functions have not yet been elucidated. In this study, we found that CDC-48 preferentially interacts with UFD-3 in *Caenorhabditis elegans*. We also found that the number of polyglutamine (polyQ) aggregates was reduced in the *ufd-3* deletion mutant but not in the *ufd-2* deletion mutant. Furthermore, the lifespan and motility of the *ufd-3* deletion mutant, where polyQ40::GFP was expressed, were greatly decreased. Taken together, we propose that UFD-3 may promote the formation of polyQ aggregates to reduce the polyQ toxicity in *C. elegans*.

1. Introduction

CDC-48 (although it is also called VCP or p97 in mammals and Cdc48p in yeast, we use CDC-48 throughout this study) is a AAA (ATPases associated with diverse cellular activities) chaperone that converts the chemical energy generated from ATP hydrolysis into the mechanical force used for protein conformational changes such as the unfolding of proteins and disassembly of protein complexes. CDC-48 is involved in a wide variety of cellular processes, including the modulation of protein aggregation, cell-cycle control, organelle membrane fusion, endoplasmic reticulum-associated protein degradation and mitochondrial quality control [1–7]. It should be noted that two highly homologous CDC-48s, CDC-48.1 and CDC-48.2, exist in *Caenorhabditis elegans* and that their function is essential and redundant [8].

The functional diversity of CDC-48 is determined by a differential binding of a variety of adaptors. Several adaptors have been identified in *C. elegans*, e.g. NPL-4-UFD-1 and six different UBXN proteins, which bind to the N-terminal domain of CDC-48 [9], and UFD-2 and UFD-3, which bind to the C-terminal motif of CDC-48 [9–11]. In general, it is considered that N-terminal adaptors are involved in the determination of substrate proteins, whereas C-terminal adaptors may determine the fate of these substrate proteins [12,13]. UFD-2 is an E4 ubiquitin ligase, which extends the ubiquitin chains of ubiquitinated substrates to promote proteasomal degradation [13–15]. In contrast, neither enzymatic activity nor cellular function of UFD-3 is clarified yet. UFD-2 and UFD-3 reportedly bind to CDC-48 in a mutually exclusive manner [10,13].

Mutational expansion of CAG repeats encoding polyglutamine (polyQ) stretches is responsible for nine neurodegenerative diseases including Huntington's Disease, spinal and bulbar muscular atrophy, dentatorubral pallidoluysian atrophy and spinocerebellar ataxias types 1, 2, 3, 6, 7 and 17 [16,17]. PolyQ aggregates accumulate in nuclear or cytoplasmic inclusion bodies that are invariably associated with end-stage neurodegenerative disease in patients and model animals. It is previously considered that polyQ aggregates have cytotoxicity [18–22]. On the other hand, recently, it has been increasingly suggested that oligomeric forms of polyQ proteins are more toxic and polyQ aggregates may act as a cellular coping mechanism to sequester levels of potentially toxic soluble monomeric and oligomeric species of polyQ [23–25]. To develop a therapeutic tactics including drugs, it is quite important to know exactly what causes the cytotoxicity, but unfortunately it is still contradictory on this issue.

It has been reported that CDC-48 co-localizes with polyQ aggregates in cultured cells and with intraneuronal inclusions in

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Novel Strategy for Biofilm Inhibition by Using Small Molecules Targeting Molecular Chaperone DnaK

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Biofilms are complex communities of microorganisms that attach to surfaces and are embedded in a self-produced extracellular matrix. Since these cells acquire increased tolerance against antimicrobial agents and host immune systems, biofilm-associated infectious diseases tend to become chronic. We show here that the molecular chaperone DnaK is important for biofilm formation and that chemical inhibition of DnaK cellular functions is effective in preventing biofilm development. Genetic, microbial, and microscopic analyses revealed that deletion of the *dnaK* gene markedly reduced the production of the extracellular functional amyloid curli, which contributes to the robustness of *Escherichia coli* biofilms. We tested the ability of DnaK inhibitors myricetin (Myr), telmisartan, pancuronium bromide, and zafirlukast to prevent biofilm formation of *E. coli*. Only Myr, a flavonol widely distributed in plants, inhibited biofilm formation in a concentration-dependent manner (50% inhibitory concentration [IC_{50}] = 46.2 μM); however, it did not affect growth. Transmission electron microscopy demonstrated that Myr inhibited the production of curli. Phenotypic analyses of thermosensitivity, cell division, intracellular level of RNA polymerase sigma factor RpoH, and vulnerability to vancomycin revealed that Myr altered the phenotype of *E. coli* wild-type cells to make them resemble those of the isogenic *dnaK* deletion mutant, indicating that Myr inhibits cellular functions of DnaK. These findings provide insights into the significance of DnaK in curli-dependent biofilm formation and indicate that DnaK is an ideal target for antibiofilm drugs.

Biofilms are communities of microorganisms enclosed in a self-produced polymeric matrix of extracellular polymer substances (EPS). These matrices contribute to bacterial accumulation in multiple layers and protect the embedded cells from antimicrobial agents and host immune systems (1). Therefore, once biofilms are formed on tissues or implanted medical devices (e.g., catheters and orthopedic devices), it becomes difficult to eradicate them by chemotherapeutic treatment. Biofilm-associated infections (e.g., catheter-related bloodstream infections, prosthetic-joint infections, and artificial-valve infections) tend to be intractable and chronic (2). To eradicate biofilm-associated infections, effective antimicrobial agents and novel strategies based on conceptual advances in understanding the mechanisms underlying biofilm development are needed.

Bacterial biofilm development proceeds in three steps: initial attachment to a surface, maturation, and dispersal. Biofilm-forming bacteria produce EPS such as extracellular polysaccharides, proteins, DNA, and others (3). These components play crucial roles in cell-to-surface adhesion for initial attachment and cell-to-cell cohesion during maturation. The composition of EPS varies depending on environmental conditions (e.g., temperature and salt concentration) and genetic background (4). After biofilm maturation, dispersal of biofilm-embedded cells occurs via self-produced EPS-destructing factors (e.g., D-amino acids, proteases, and phenol-soluble modulins) (5–7) and other yet-uncharacterized mechanisms. Consequently, dispersed cells can move to different niches in the body or in the environment.

Curli is the extracellular functional amyloid produced by many *Enterobacteriaceae*, such as *Escherichia coli* and *Salmonella enterica* (4). In concert with other EPS, such as type I pili (8), colanic acids (9), cellulose (10), and poly-*N*-acetylglucosamine (11), curli contributes to the initial attachment to a surface and to cell-to-cell cohesion (8). Particularly, curli-dependent biofilm is involved in urinary tract infectious diseases (12–14). Considering that curli, like amyloid fibrils in general, is extremely stable against proteolytic enzymes and detergents such as sodium dodecyl sulfate (SDS), a biofilm depending on curli biosynthesis is stable and its removal can be difficult. Therefore, the development of countermeasures against curli-dependent biofilms is an important challenge.

The components of curli biosynthesis are encoded by two curli-specific gene (*csg*) operons, *csgBAC* and *csgDEFG* (15). The structural components of curli, CsgA and CsgB, are synthesized in the cytoplasm, probably in an unfolded, soluble state, translocated to the periplasm through the inner membrane via the Sec translocon, and subsequently exported to the extracellular milieu by the CsgG channel embedded in the outer membrane (15). CsgE and CsgF support the transport of CsgA and CsgB. The exported CsgB anchors to the cell envelope and converts the unfolded state of CsgA to a β-sheet-rich amyloid polymer (16). Expression of the *csg* operons requires at least two major regulatory proteins, CsgD

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RESEARCH ARTICLE

Mild Electrical Stimulation Increases Stress Resistance and Suppresses Fat Accumulation *via* Activation of LKB1-AMPK Signaling Pathway in *C. elegans*

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Abstract

Electrical current at physiological strength has been applied as a therapeutic approach for various diseases. Several of our works showed that mild electrical stimulation (MES) at 0.1-ms pulse width has positive impact on organisms. But despite the growing evidence of the beneficial effects of MES, its effects on individual animals and the molecular underpinnings are poorly understood and rarely studied. Here, we examined the effects of MES on individual animal and its mechanisms by mainly using *Caenorhabditis elegans*, a powerful genetic model organism. Interestingly, MES increased stress resistance and suppressed excess fat accumulation in wild-type N2 worms but not in AMPK/AAK-2 and LKB1/PAR-4 mutant worms. MES promoted the nuclear localization of transcription factors DAF-16 and SKN-1 and consequently increased the expression of anti-stress genes, whereas MES inhibited the nuclear localization of SBP-1 and suppressed the expression of lipogenic genes. Moreover, we found that MES induced the activation of LKB1/PAR4-AMPK/AAK2 pathway in *C. elegans* and in several mammalian cell lines. The mitochondrial membrane potential and cellular ATP level were slightly and transiently decreased by MES leading to the activation of LKB1-AMPK signaling pathway. Together, we firstly and genetically demonstrated that MES exerts beneficial effects such as stress resistance and suppression of excess fat accumulation, via activation of LKB1-AMPK signaling pathway.

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Serial block-face scanning electron microscopy for three-dimensional analysis of morphological changes in mitochondria regulated by Cdc48p/p97 ATPase

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ABSTRACT

Cdc48p is a highly conserved cytosolic AAA chaperone that is involved in a wide range of cellular processes. It consists of two ATPase domains (D1 and D2), with regulatory regions at the N- and C-terminals. We have recently shown that Cdc48p regulates mitochondrial morphology, in that a loss of the ATPase activity or positive cooperativity in the D2 domain leads to severe fragmentations and aggregations of mitochondria in the cytoplasm. We have now used serial block-face scanning electron microscopy (SBF-SEM), an advanced three-dimensional (3D) electron microscopic technique to examine the structures and morphological changes of mitochondria in the yeast *Saccharomyces cerevisiae*. We found that mutants lacking ATPase activity of Cdc48p showed mitochondrial fragmentations and aggregations, without fusion of the outer membrane. This suggests that the ATPase activity of Cdc48p is necessary for fusion of the outer membranes of mitochondria. Our results also show that SBF-SEM has considerable advantages in morphological and quantitative studies on organelles and intracellular structures in entire cells.

1. Introduction

In *Saccharomyces cerevisiae*, the protein Cdc48p (which is also known as p97/VCP in mammals, TER94 in flies, and CDC-48.1 and CDC-48.2 in *Caenorhabditis elegans*) is one of the most abundant cytosolic proteins (Dalal and Hanson, 2001; Wang et al., 2004). It is a highly conserved AAA (ATPases associated with diverse cellular activities) chaperone that is found in all eukaryotes (for a reviews, see Stolz et al., 2011).

Cdc48p is involved in a broad range of cellular events and it uses the chemical energy generated by hydrolysis of ATP to induce various conformational changes (for reviews, see Vale, 2000; Ogura and Wilkinson, 2001). Cdc48p consists of an N-terminal regulatory domain followed by two conserved AAA (D1 and D2) domains and a short C-terminal segment. Each AAA domain consists of Walker A and B motifs, and a specific motif referred to as the second region of homology (SRH) (Ammelburg et al., 2006). The Walker A and B motifs mediate ATP binding and hydrolysis, respectively. Cdc48p assembles into a stable homo-hexamer, in which six domains each of D1 and D2 form two tandem rings with a central pore (Zhang et al., 2000; Davies et al., 2008), and the ATP active sites regulate each other within the whole complex and perform their biological activities cooperatively.

The C-terminal D2 domain exhibits essential ATPase activity in a coordinated fashion with positive cooperativity among subunits under physiological conditions (DeLaBarre et al., 2006; Esaki and Ogura, 2010; Nishikori et al., 2011; Song et al., 2003). The positive cooperativity of the D2 ATPase activity is vital for the functioning of Cdc48p, because a loss of this cooperativity leads to cell death, and the cell growth rate is strongly correlated with magnitude of positive cooperativity in Cdc48p (Nishikori et al., 2011).

In contrast, there is controversy regarding the functional role of the D1 domain. The ATPase activity of the N-terminal D1 domain is relatively low at physiological temperature (Briggs et al., 2008; DeLaBarre et al., 2006; Kobayashi et al., 2002; Song et al., 2003; Ye et al., 2003). Some groups have suggested that a mutation causing a defect in ATP binding of D1 strongly reduces the overall ATP-

Abbreviations: AAA, ATPases associated with diverse cellular activities; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; SEM, scanning electron microscopy; SBF-SEM, serial block-face SEM; TEM, transmission electron microscopy; MOM, mitochondrial outer membrane.

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Whole-cell imaging of the budding yeast *Saccharomyces cerevisiae* by high-voltage scanning transmission electron tomography

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ABSTRACT

Electron tomography using a high-voltage electron microscope (HVEM) provides three-dimensional information about cellular components in sections thicker than 1 μm, although in bright-field mode image degradation caused by multiple inelastic scattering of transmitted electrons limit the attainable resolution. Scanning transmission electron microscopy (STEM) is believed to give enhanced contrast and resolution compared to conventional transmission electron microscopy (CTEM). Samples up to 1 μm in thickness have been analyzed with an intermediate-voltage electron microscope because inelastic scattering is not a critical limitation, and probe broadening can be minimized. Here, we employed STEM at 1 MeV high-voltage to extend the useful specimen thickness for electron tomography, which we demonstrate by a seamless tomographic reconstruction of a whole, budding *Saccharomyces cerevisiae* yeast cell, which is ~3 μm in thickness. High-voltage STEM tomography, especially in the bright-field mode, demonstrated sufficiently enhanced contrast and intensity, compared to CTEM tomography, to permit segmentation of major organelles in the whole cell. STEM imaging also reduced specimen shrinkage during tilt-series acquisition. The fidelity of structural preservation was limited by cytoplasmic extraction, and the spatial resolution was limited by the relatively large convergence angle of the scanning probe. However, the new technique has potential to solve longstanding problems of image blurring in biological specimens beyond 1 μm in thickness, and may facilitate new research in cellular structural biology.

1. Introduction

Electron tomography (ET) is a method for obtaining nanometer-scale 3D reconstructions from tilt-series of images obtained by transmission electron microscopy (TEM) or scanning transmission electron microscopy (STEM). In biological applications, cellular ultrastructure and supramolecular assemblies can be visualized [1,2]. However, the penetrating power of electrons at typical accelerating voltages impedes the use of thick biological specimens. High-voltage electron microscopy (HVEM) was developed to extend the range of useful specimen thicknesses. Electrons accelerated by 1 MeV can penetrate to a depth of over 1 μm in biological samples embedded in resin [3]. Nevertheless, large numbers of multiply and inelastically scattered electrons are generated in thick specimens, thus degrading image quality [4].

Zero-loss energy filtering can remove inelastically scattered electrons [5], however, biological specimens in the micrometer thickness range transmit insufficient zero-loss electrons for a useful image. Most-probable-loss (MPL) imaging [6] can be used for improved imaging of thicker biological specimens. In this technique, images are formed by moving the energy-selection slit to the region of the energy-loss spectrum where majority of the (inelastically scattered) electrons can be found. MPL imaging has been successfully used to image Golgi-stained neuronal cells embedded in a resin section over 1 μm in thickness, using 300 keV electron microscope [6].

STEM has been shown to be an effective alternative technique for imaging thick biological specimens. In STEM, a focused electron probe is rastered over the specimen, and transmitted electrons are collected pixel-by-pixel on axial (bright-field) or off-axial (dark-field) detectors. These electrons have undergone multiple scattering and have lost energy, but since they need not be further imaged by electron lenses, image degradation due to lens aberrations does not

Abbreviations: STEM, scanning transmission electron microscopy; TEM, transmission electron microscopy; HVEM, high voltage electron microscopy; BF, bright-filed; ADF, annular dark-field; ET, electron tomography; MPL, most-probable-loss; HAADF, high-angle annular dark-field

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A conserved α helix of Bcs1, a mitochondrial AAA chaperone, is required for the Respiratory Complex III maturation

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ABSTRACT

Bcs1 is a transmembrane chaperone in the mitochondrial inner membrane, and is required for the mitochondrial Respiratory Chain Complex III assembly. It has been shown that the highly-conserved C-terminal region of Bcs1 including the AAA ATPase domain in the matrix side is essential for the chaperone function. Here we describe the importance of the N-terminal short segment located in the intermembrane space in the Bcs1 function. Among the N-terminal 44 amino acid residues of yeast Bcs1, the first 37 residues are dispensable whereas a hydrophobic amino acid in the residue 38 is essential for integration of Rieske Iron-sulfur Protein into the premature Complex III from the mitochondrial matrix. Substitution of the residue 38 by a hydrophilic amino acid residue affects conformation of Bcs1 and interactions with other proteins. The evolutionarily-conserved short α helix of Bcs1 in the intermembrane space is an essential element for the chaperone function.

1. Introduction

The respiratory chain, mainly constituted from five large protein complexes in the mitochondrial inner membrane, is vital for production of ATP, the major energy source required for most cellular processes. Respiratory Chain Complex III, also called as the cytochrome bc_1 complex, actively pumps protons across the inner membrane from the matrix to the intermembrane space upon electron transfer from Complexes I and II using ubiquinol to Complex IV via cytochrome *c*. Complex III is composed of three catalytic subunits, cytochrome *b*, cytochrome c_1, and Rieske Iron-sulfur Protein, and several structural subunits. Cytochrome *b* is encoded by mitochondrial DNA and synthesized in the mitochondrial matrix whereas all other subunits are encoded by nuclear DNA, synthesized in the cytosol, and imported into mitochondria. The assembly pathway of the Complex III subunits is well characterized in the yeast *Saccharomyces cerevisiae* [1]. Briefly, cytochrome *b* forms a core complex with Qcr7 and Qcr8. Cytochrome c_1, Cor1, Cor2, Qcr6, and Qcr9 are then assembled into the core complex to form the premature complex. Qcr10 and Rip1, the yeast homolog of Rieske Iron-sulfur Protein, are finally assembled to form the mature complex. At last, a homo-dimer of Complex III forms a supercomplex with Complex IV. Since Rip1 is essential for electron transfer within Complex III, the premature complex lacking Rip1 is non-functional.

It has been reported that molecular chaperones including Bca1, TTC19, Mzm1, and Bcs1 are involved in the Complex III assembly process [1]. Bca1, which is only found in fungi with no known orthologs in higher eukaryotes, may control formation and/or stability of the premature complex [2]. The Bca1 function can be bypassed and/or redundant with other chaperones, since requirement of Bca1 for yeast growth on non-fermentable carbon sources is limited [2]. TTC19 has been identified from patients suffering from progressive encephalopathy associated with profound Complex III deficiency [3]. Abnormal accumulation of an assemble intermediate composed of Cor1 and Cor2 in the mutant muscle suggests involvement of TTC19 in an early stage of the Complex III assembly process. Mzm1 was identified in yeast and shown to interact with Rip1 in the mitochondrial matrix and protects Rip1 from aggregation and degradation prior to integration into the mitochondrial inner membrane [4,5]. A human homolog of Mzm1, LYRM7/MZM1L, has recently been identified [6].

Bcs1 is the first identified chaperone required for the Complex III assembly in yeast two decades ago [7], and then a human homolog of Bcs1, BCS1L, was identified as a causative gene from patients suffering from fatal human diseases, the GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death) syndrome and the Complex III deficiency, and from

Abbreviations: AAA, ATPases associated with diverse cellular activities; BN-PAGE, blue-native polyacrylamide gel electrophoresis; DTSSP, 3,3′-dithiobis(sulfosuccinimidylpropionate).

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High-Speed Atomic Force Microscopic Observation of ATP-Dependent Rotation of the AAA+ Chaperone p97

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SUMMARY

p97 (also called VCP and CDC-48) is an AAA+ chaperone, which consists of a substrate/cofactor-binding N domain and two ATPase domains (D1 and D2), and forms a homo-hexameric ring. p97 plays crucial roles in a variety of cellular processes such as the ubiquitin-proteasome pathway, the endoplasmic reticulum-associated protein degradation, autophagy, and modulation of protein aggregates. Mutations in human p97 homolog VCP are linked to neurodegenerative diseases. The key mechanism of p97 in these various functions has been proposed to be the disassembly of protein complexes. To understand the molecular mechanism of p97, we studied the conformational changes of hexameric CDC-48.1, a *Caenorhabditis elegans* p97 homolog, using high-speed atomic force microscopy. In the presence of ATP, the N-D1 ring repeatedly rotates ~23 ± 8° clockwise and resets relative to the D2 ring. Mutational analysis reveals that this rotation is induced by ATP binding to the D2 domain.

INTRODUCTION

AAA+ (ATPases associated with diverse cellular activities) family proteins are ATP-dependent molecular chaperones, which exert mechanical work on macromolecular substrates, such as unfolding of proteins and disassembly of multisubunit protein complexes (Ogura and Wilkinson, 2001; Hanson and Whiteheart, 2005). It has been proposed that AAA+ chaperones undergo conformational changes during the cycle of ATP hydrolysis, and that the conformational changes provide their mechanical work on substrates. AAA+ proteins contain one (type I) or two (type II) conserved AAA+ domains, consisting of the ATPase domain with the Walker A (ATP binding) and Walker B (ATP hydrolysis) motifs followed by an α-helical C-domain (Ammelburg et al., 2006). AAA+ domains form a ring-shaped hexamer and thus there are 6 and 12 ATPase active sites in the type I and type II AAA+ proteins, respectively.

p97 (also known as VCP in mammals, CDC-48.1 and CDC-48.2 in *Caenorhabditis elegans*, and Cdc48p in yeast) is a highly conserved and abundant type II AAA+ protein. p97 plays essential roles in a number of cellular processes including membrane dynamics, cell cycle control, the ubiquitin-proteasome system, autophagy, and modulation of protein aggregates (for recent reviews, Stolz et al., 2011; Yamanaka et al., 2012; Meyer et al., 2012; Buchberger, 2013). Human p97 (VCP) has been linked to neurodegenerative disorders, hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (Watts et al., 2004; Weihl et al., 2009), and amyotrophic lateral sclerosis (Johnson et al., 2010). TAR DNA-binding protein 43-positive inclusions are accumulated in neuronal cells of the patients with both diseases (Johnson et al., 2010).

p97 consists of an N-terminal substrate/adaptor-binding domain followed by two AAA+ domains (D1 and D2) and forms a homo-hexameric double-ring structure. It has been shown that D2 mediates major ATPase activity (Song et al., 2003; DeLaBarre et al., 2006), and that the ATPase activity of D2 is critical for biological functions (Song et al., 2003; DeLaBarre et al., 2006; Esaki and Ogura, 2010; Nishikori et al., 2011). A quantitative analysis of nucleotide-binding to both D1 and D2 domains of rat p97 indicates that ATPγS, an ATP analog, binds to D1 and D2 with similar affinity, but ADP binds to D1 with higher affinity than to D2, which is consistent with the higher ATPase activity in D2 (Briggs et al., 2008). Stoichiometric measurements suggest that both ADP and ATPγS can saturate all six nucleotide binding sites in D1, but that only three to four of the six D2 sites can bind ATPγS simultaneously, and that ATPγS binding to the D2 domain triggers a downstream cooperative conformational change of at least three monomers (Briggs et al., 2008). The

Chapter 3
FtsH Protease-Mediated Regulation of Various Cellular Functions

Takashi Okuno and Teru Ogura

Abstract FtsH, a member of the AAA (ATPases associated with a variety of cellular activities) family of proteins, is an ATP-dependent protease of ~71 kDa anchored to the inner membrane. It plays crucial roles in a variety of cellular processes. It is responsible for the degradation of both membrane and cytoplasmic substrate proteins. Substrate proteins are translocated through the central pore of the AAA domain into the proteolytic chamber, where the polypeptide chains are processively degraded into short peptides. FtsH is not only involved in the proteolytic elimination of unnecessary proteins, but also in the proteolytic regulation of a number of cellular functions. Its role in proteolytic regulation is achieved by one of two approaches, either the selective degradation of a regulatory protein or the activity of a protein's substrate protein is modified by processing. In the latter case, protein processing results in the production of a stable domain with a new substrate. Since FtsH does not have a robust unfolding activity, this soluble fragment is sufficient to direct processive degradation of the protein – resulting in release of a stable protein fragment.

Keywords FtsH • Proteasome degradation • Protein processing • Protein quality control • Regulation of lipid synthesis

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PLoS one

Phosphorylation of Kif26b Promotes Its Polyubiquitination and Subsequent Proteasomal Degradation during Kidney Development

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Abstract

Kif26b, a member of the kinesin superfamily proteins (KIFs), is essential for kidney development. *Kif26b* expression is restricted to the metanephric mesenchyme, and its transcription is regulated by a zinc finger transcriptional regulator Sall1. However, the mechanism(s) by which Kif26b protein is regulated remain unknown. Here, we demonstrate phosphorylation and subsequent polyubiquitination of Kif26b in the developing kidney. We find that Kif26b interacts with an E3 ubiquitin ligase, neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4) in developing kidney. Phosphorylation of Kif26b at Thr-1859 and Ser-1962 by the cyclin-dependent kinases (CDKs) enhances the interaction of Kif26b with Nedd4. Nedd4 polyubiquitinates Kif26b and thereby promotes degradation of Kif26b via the ubiquitin-proteasome pathway. Furthermore, Kif26b lacks ATPase activity but does associate with microtubules. Nocodazole treatment not only disrupts the localization of Kif26b to microtubules but also promotes phosphorylation and polyubiquitination of Kif26b. These results suggest that the function of Kif26b is microtubule-based and that Kif26b degradation in the metanephric mesenchyme via the ubiquitin-proteasome pathway may be important for proper kidney development.

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Introduction

The kidney is composed of minimum units called nephrons, which maintain an appropriate homeostatic balance of water and salt levels and remove nitrogenous metabolic waste products. The tubular epithelial network of the nephron originates from two different tissues, the ureteric bud and the metanephric mesenchyme, that form the collecting duct system and the renal tubules, respectively [1,2]. The signals from the metanephric mesenchyme, such as glial cell line-derived neurotrophic factor (GDNF), induce sprouting of the ureteric bud from the caudal region of the Wolffian duct and invasion of the ureteric buds into the metanephric mesenchyme. Wnt9b secreted from the ureteric buds induces Wnt4 expression in the mesenchyme [3], and Wnt4 induces the pre-tubular aggregates of the condensed mesenchyme beneath the ureteric bud tips to form renal vesicles in a cell-autonomous manner [4]. Renal vesicles differentiate into each segment of the nephron, including the glomerulus, proximal tubule, loop of Henle, and distal tubule, to eventually form functional nephrons [1,2].

The kinesin superfamily proteins (KIFs) are known to be important molecular motors that are involved in the microtubule- and ATP-dependent transport of various cargos, including membranous organelles, protein complexes, and mRNAs, to specific destinations [5]. Accumulating evidence demonstrates the importance of KIFs in the regulation of many physiological events, including higher brain function, tumor suppression, and developmental patterning. Kif26b was originally identified by a database search of the mouse genome for DNA sequences that contained a motif similar to the kinesin motor domain [6]. Kif26b is classified to the Kinesin-11 family along with Kif26a, an unconventional kinesin that lacks microtubule-based motility [7]. Human KIF26A does not contain the conserved amino acid sequences that are required for motor activity but retains the microtubule-associating ability as well as other conserved KIFs. Smy1p, a Kinesin-11 family member from *Saccharomyces cerevisiae*, is thought not to be motile, especially along microtubules, due to the deviance in both a catalytic pocket for ATP hydrolysis and the microtubule-binding sites [8]. These reports suggest that Kif26b also does not function as a microtubule-based motor. Therefore, the biochemical and cellular functions of Kif26b remain to be clarified.

We recently reported that *Kif26b*-knockout mice exhibit kidney agenesis or hypoplasia [9]. In *Kif26b*-null embryos, the ureteric buds elongate and migrate in proximity to, but do not invade, the

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CDC-48/p97 is required for proper meiotic chromosome segregation via controlling AIR-2/Aurora B kinase localization in *Caenorhabditis elegans*

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ABSTRACT

CDC-48/p97 is a AAA (ATPases associated with diverse cellular activities) chaperone involved in protein conformational changes such as the disassembly of protein complexes. We previously reported that *Caenorhabditis elegans* CDC-48.1 and CDC-48.2 (CDC-48s) are essential for the progression of meiosis I metaphase. Here, we report that CDC-48s are required for proper chromosome segregation during meiosis in *C. elegans*. In wild-type worms, at the diakinesis phase, phosphorylation of histone H3, one of the known substrates of aurora B kinase (AIR-2), on meiosis I chromatids correlated with AIR-2 localization at the cohesion sites of homologous chromatids. Conversely, depletion of CDC-48s resulted in a significant expansion of signals for AIR-2 and phosphorylated histone H3 over the entire length of meiotic chromosomes, leading to defective chromosome segregation, while the total amount of AIR-2 in lysates was not changed by the depletion of CDC-48s. The defective segregation of meiotic chromosomes caused by the depletion of CDC-48s was suppressed by the simultaneous depletion of AIR-2 and is similar to that observed following the depletion of protein phosphatase 1 (PP1) phosphatases. However, the amount and localization of PP1 were not changed by the depletion of CDC-48s. These results suggest that CDC-48s control the restricted localization of AIR-2 to the cohesion sites of homologous chromatids in meiosis I.

1. Introduction

CDC-48/p97 is a ubiquitin-selective AAA (ATPases associated with diverse cellular activities) chaperone that converts the chemical energy generated from ATP hydrolysis into the mechanical force used for protein conformational changes such as the unfolding of proteins and disassembly of protein complexes (Jentsch and Rumpf, 2007; Shcherbik and Haines, 2007; Meyer et al., 2010; Yamanaka et al., 2012). *CDC48* was first identified in *Saccharomyces cerevisiae* as a cell division cycle gene (Moir and Botstein, 1982). It has been demonstrated that CDC-48/p97 has multiple functions during the progression of the mitotic M-phase (Moir and Botstein, 1982; Fu et al., 2003; Cao et al., 2003; Wojcik et al., 2004; Ikai and Yanagida, 2006; Ramadan et al., 2007; Dobrynin et al., 2011). We previously reported that *Caenorhabditis elegans* possesses two CDC-48/p97 homologs, CDC-48.1 and CDC-48.2 (hereafter referred to as CDC-48s) (Yamanaka et al., 2004), and that *C. elegans* CDC-48s play essential roles in chromosome condensation during meiotic processes as well as the progression of meiosis I metaphase (Sasagawa et al., 2007).

Chromosome segregation requires the regulated release of chromosome cohesion. During meiosis, the cohesion of homologous chromosomes is released at the end of meiosis I, whereas the association of sister chromatids has to be maintained until segregation in meiosis II. Meiotic chromosome cohesion is mediated by REC-8, a meiosis-specific subunit of cohesin. The loss of REC-8 from homologous chromosome cohesion in meiosis I and sister chromatid cohesion in meiosis II coordinates proper chromosome segregation during meiosis in yeast and *C. elegans* (Klein et al., 1999; Pasierbek et al., 2001). In *C. elegans*, aurora B kinase (AIR-2) is required for meiotic chromosome segregation and localizes to cohesion sites corresponding to the release of chromosomes in metaphase I and II (Hsu et al., 2000; Kaitna et al., 2002; Rogers et al., 2002). Other components of the AIR-2 complex, including a survivin homolog (BIR-1), an Incenp homolog (ICP-1), and CSC-1, also localize to the same regions as AIR-2 (Kaitna et al., 2002; Rogers et al., 2002; Romano et al., 2003). Furthermore, AIR-2 has been shown to phosphorylate REC-8 and function in the coordinated release of chromosome cohesion during meiosis in *C. elegans* (Rogers et al., 2002). The distribution of phosphorylated histone

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Cdc48p/p97-mediated regulation of mitochondrial morphology is Vms1p-independent

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ABSTRACT

Cdc48p/p97 is a cytosolic essential AAA chaperone, which regulates multiple cellular reactions in a ubiquitin-dependent manner. We have recently shown that Cdc48p exhibits positively cooperative ATPase activity and loss of the positive cooperativity results in yeast cell death. Here we show that loss of the positive cooperativity of the yeast Cdc48p ATPase activity led to severe mitochondrial aggregation. The actin cytoskeleton and distribution of the ER–mitochondria tethering complex (ERMES) were eliminated from the cause of the mitochondrial aggregation. Instead, a mitochondrial outer membrane protein Fzo1p, which is required for mitochondrial fusion, and components of ERMES, which is involved in mitochondrial morphology, were remarkably stabilized in the Cdc48p mutants. In the last couple of years, it was shown that Vms1p functions as a cofactor of Cdc48p for the function of protein degradation of mitochondrial outer membrane proteins. Nevertheless, we found that Vms1p was not involved in the Cdc48p-dependent mitochondrial aggregation and loss of Vms1p did not significantly affect degradation rates of proteins anchored to the mitochondrial outer membrane. These results suggest that Cdc48p controls mitochondrial morphology by regulating turnover of proteins involved in mitochondrial morphology in a Vms1p-independent manner.

1. Introduction

Cdc48p, also called as p97 and VCP, is a highly conserved AAA (ATPases associated with diverse cellular activities) chaperone found in all eukaryotes. Cdc48p is an essential protein and one of the most abundant intracellular proteins (Dalal and Hanson, 2001). Cdc48p is involved in many cellular processes including homotypic fusion of endoplasmic reticulum (ER) and Golgi membranes (Latterich et al., 1995; Patel et al., 1998; Rabouille et al., 1995; Roy et al., 2000), nuclear envelope reassembly (Hetzer et al., 2001), ER-associated protein degradation (ERAD) (Braun et al., 2002; Jarosch et al., 2002; Rabinovich et al., 2002; Ye et al., 2003), autophagosome formation (Ju et al., 2009; Krick et al., 2010; Tresse et al., 2010), transcription activation (Wang et al., 2004), DNA replication (Franz et al., 2011; Raman et al., 2011), meiotic progression (Sasagawa et al., 2007) and modification of protein aggregates (Nishikori et al., 2008). These diverse functions of Cdc48p are mainly regulated by multiple adaptor and cofactor proteins (Madsen et al., 2009; Stolz et al., 2011). For instance, the Npl4p-Ufd1p heterodimer and recently identified Vms1p (Ydr049p) are involved in ubiquitin-selective protein degradation during ERAD (Bays et al., 2001; Tran et al., 2011; Ye et al., 2001), and p37 and p47 are required for membrane fusion of Golgi complexes (Kondo et al., 1997; Uchiyama et al., 2006). Within the last couple of years, it has been revealed that Cdc48p is also involved in degradation of mitochondrial outer membrane proteins such as Mcl1, an anti-apoptotic protein of the Bcl2 family, and Fzo1p/mitofusin, which is required for mitochondrial fusion (Heo et al., 2010; Tanaka et al., 2010; Xu et al., 2011). The Vms1p-Npl4p heterodimer was identified as a cofactor of Cdc48p for degradation of Fzo1p (Heo et al., 2010). However, temperature-sensitive mutants of Cdc48p have no impact on mitochondrial fusion in the mating process (Nunnari et al., 1997). Thus, physiological significance of the involvement of Cdc48p in degradation of mitochondrial outer membrane proteins has not been clear.

Cdc48p is composed of an N-terminal domain followed by two AAA ATPase domains called as D1 and D2 and a short C-terminal segment. Adaptors/cofactors bind to either or both the N-terminal domain and the C-terminal segment (Madsen et al., 2009). Cdc48p arranges into a stable hexamer, in which the six D1 and D2 domains form two tandem rings with a central narrow pore (Davies et al., 2008). The hexameric complex seems to be sustained by the D1 ring (Song et al., 2003). ATPase activity of the D1 domain is relatively low at physiological temperature (Briggs et al., 2008;

Abbreviations: AAA, ATPases associated with diverse cellular activities; ERAD, ER-associated protein degradation; ERMES, ER–mitochondria tethering complex; HA, hemagglutinin.

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The C-terminal α-helix of SPAS-1, a *Caenorhabditis elegans* spastin homologue, is crucial for microtubule severing

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ABSTRACT

Spastin belongs to the meiotic subfamily, together with Vps4/SKD1, fidgetin and katanin, of AAA (ATPases associated with diverse cellular activities) proteins, and functions in microtubule severing. Interestingly, all members of this subgroup specifically contain an additional α-helix at the very C-terminal end. To understand the function of the C-terminal α-helix, we characterised its deletion mutants of SPAS-1, a *Caenorhabditis elegans* spastin homologue, in vitro and in vivo. We found that the C-terminal α-helix plays essential roles in ATP binding, ATP hydrolysing and microtubule severing activities. It is likely that the C-terminal α-helix is required for cellular functions of members of meiotic subgroup of AAA proteins, since the C-terminal α-helix of Vps4 is also important for assembly, ATPase activity and in vivo function mediated by ESCRT-III complexes.

1. Introduction

Spastin (SPAS-1 in *Caenorhabditis elegans*) is a member of the AAA (ATPases associated with various cellular activities) protein family (Hazan et al., 1999; Matsushita-Ishiodori et al., 2007). It has been demonstrated that spastin has an ATP-dependent microtubule severing activity (Roll-Mecak and McNally, 2010). Spastin is a causative agent for human disease, hereditary spastic paraplegia (HSP) (Hazan et al., 1999; Svenson et al., 2001) and defects in microtubule severing activity of mutant spastin are considered to be a cause of axonal degeneration in HSP McDermott et al., 2003; Sherwood et al., 2004; Trotta et al., 2004; Evans et al., 2005; Salinas et al., 2005; Wood et al., 2006; Yu et al., 2008a). AAA proteins generally assemble into a hexamer, whose formation is critical for their function. It has been demonstrated that human spastin forms a hexamer in an ATP-dependent (White et al., 2007) or -independent (Pantakani et al., 2008) manner and that *Drosophila* spastin and *C. elegans* SPAS-1 form a hexamer in an ATP-dependent manner (Roll-Mecak and Vale, 2008). In contrast, we recently found that SPAS-1 forms a hexamer in an ATP-independent manner, when highly concentrated SPAS-1 was analysed in vitro (Matsushita-Ishiodori et al., 2009). In addition, we demonstrated that SPAS-1 interacts with microtubules through MTBD (microtubule binding domain) of SPAS-1 and thus is enriched on microtubules, leading to form a hexamer in an ATP-independent manner (Matsushita-Ishiodori et al., 2009). It is noteworthy, however, that there is no direct evidence so far that a hexamer is the form bound to microtubules. It could be a hexamer or a superstructure of several hexamers.

Together with Vps4/SKD1 (VPS-4 in *C. elegans*), fidgetin (FIGL-1), and katanin (MEI-1), spastin (SPAS-1) belongs to the meiotic subfamily of AAA proteins (Fröhlich, 2001; Lupas and Martin, 2002). Spastin, katanin and fidgetin have been demonstrated to function in microtubule severing (McNally and Vale, 1993; Evans et al., 2005; Roll-Mecak and Vale, 2005; Zhang et al., 2007; Roll-Mecak and McNally, 2010). In contrast, Vps4 forms a dodecameric double-ring structure (Scott et al., 2005; Gonciarz et al., 2008; Yu et al., 2008b; Landsberg et al., 2009) and functions in endocytic trafficking, virus budding and cytokinesis mediated with ESCRT-III complexes (Hurley and Hanson, 2010). Interestingly, all members of this subgroup specifically contain an additional α-helix at the very C-terminal end (Scott et al., 2005). It has been revealed that the C-terminal α-helix is important for ATPase activity and microtubule severing of *Drosophila* spastin (Roll-Mecak and Vale, 2008) and for Vps4 assembly and ATPase activity in vitro and function in vivo (Vajjhala et al., 2008).

Here, we examined the importance of the C-terminal α-helix of the microtubule severing enzyme SPAS-1 of *C. elegans*. We demonstrate that the C-terminal α-helix plays an essential role in ATP binding, ATP hydrolysing and microtubule severing activities of SPAS-1.

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Caenorhabditis elegans fidgetin homolog FIGL-1, a nuclear-localized AAA ATPase, binds to SUMO

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ABSTRACT

Fidgetin is a member of the AAA (ATPases associated with diverse cellular activities) chaperones. It is well-known that the specific function of a given AAA protein primarily depends upon its subcellular localization and interacting partners. FIGL-1, a *Caenorhabditis elegans* homolog of mammalian fidgetin, is localized in the nucleus. Here, we identified that the N-terminal PKRVK sequence of FIGL-1 functions as a monopartite nuclear localization signal. Nuclear localization of FIGL-1 is required for its function. We also found that FIGL-1 specifically interacted with SMO-1, a *C. elegans* homolog of small ubiquitin-like modifier (SUMO), using a yeast two-hybrid assay. Furthermore, the direct physical interaction between FIGL-1 and SMO-1 was demonstrated by pull-down assay using purified proteins as well as immunoprecipitation assay using lysates from epitope-tagged SMO-1-expressing worms. Binding of FIGL-1 to SMO-1 is required for its function. The depletion of FIGL-1 and SMO-1 resulted in developmental defects in *C. elegans*. Taken altogether, our results indicate that FIGL-1 is a nuclear protein and that in concert with SMO-1, FIGL-1 plays an important role in the regulation of *C. elegans* development.

1. Introduction

Fidgetin is a member of the ATPases associated with diverse cellular activities (AAA) family of proteins, which are molecular chaperones mediating the assembly and disassembly of macro-protein complexes. AAA proteins are involved in a variety of cellular activities such as membrane fusion, vesicle-mediated transport, proteasome function, peroxisome biogenesis, and microtubule dynamics. Mechanistically, they are thought to exert their activity through the energy-dependent disassembly and unfolding of substrate proteins (Patel and Latterich, 1998; Vale, 2000; Ogura and Wilkinson, 2001; Lupas and Martin, 2002). Some AAA proteins are now known to be causative factors for genetic diseases. Fidgetin has been identified as a causative factor of the *fidget* mutant mouse (Cox et al., 2000). The mouse *fidget* mutation is an autosomal recessive mutation that affects multiple developmental processes (Gruneberg, 1943). These mice are characterized by side-to-side head shaking and circling, small eyes, and reduced growth of the retinal neural epithelium (Truslove, 1956; Konyukhov and Sazhina, 1976). Fidgetin is the first AAA protein whose mutation leads to mammalian developmental abnormalities and is speculated to play a role in the development of the eyes, inner ear, and some skeletal bones (Cox et al., 2000). Fidgetin is localized predominantly in the nucleus due to the presence of a bipartite nuclear localization signal in the middle portion of the protein (Yang et al., 2005).

Fidgetin reportedly associates with cAMP-dependent protein kinase A anchoring protein 95 kDa (AKAP95), which targets chromosomes throughout mitosis and may modulate a signal pathway underlying palatogenesis in mice (Yang et al., 2006). In *Drosophila melanogaster*, a fidgetin homolog (Dm-fidgetin; CG3326) is localized to the centrosome and is required for microtubule minus-end depolymerization (Zhang et al., 2007). We reported that a *Caenorhabditis elegans* fidgetin homolog, FIGL-1, possesses ATPase activity and forms homo-oligomers (Yakushiji et al., 2004, 2006) (see Supplemental Fig. S1). Depletion of FIGL-1 in *C. elegans* results in defective gonad formation and the sterile phenotype (Yakushiji et al., 2004; Luke-Glaser et al., 2007). The *figl-1* mutant shows the accumulation of mitotic nuclei in the proliferative zone of the *C. elegans* germline, and FIGL-1 is suggested to control mitotic progression in the germline and early embryos (Luke-Glaser et al., 2007). However, the specific cellular functions of fidgetin remain unknown.

Small ubiquitin-like modifier (SUMO) is a member of the ubiquitin-like superfamily whose members are structurally similar to ubiquitin (Bayer et al., 1998). Like ubiquitin, SUMO has been found to be covalently attached to certain lysine residues of specific

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Review

Recent advances in p97/VCP/Cdc48 cellular functions ☆

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ABSTRACT

p97/VCP/Cdc48 is one of the best-characterized type II AAA (ATPases associated with diverse cellular activities) ATPases. p97 is suggested to be a ubiquitin-selective chaperone and its key function is to disassemble protein complexes. p97 is involved in a wide variety of cellular activities. Recently, novel functions, namely autophagy and mitochondrial quality control, for p97 have been uncovered. p97 was identified as a causative factor for inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD) and more recently as a causative factor for amyotrophic lateral sclerosis (ALS). In this review, we will summarize and discuss recent progress and topics in p97 functions and the relationship to its associated diseases. This article is part of a Special Issue entitled: AAA ATPases: structure and function.

1. Introduction

p97—also called valosin-containing protein (VCP) in mammals, Cdc48p in yeast, or CDC-48 in *Caenorhabditis elegans*—is well conserved among all eukaryotes and is essential for growth in *Saccharomyces cerevisiae*[1], *Drosophila melanogaster*[2], *C. elegans*[3] and mice [4]. p97 is highly abundant, accounting for about 1% of the total cellular protein. p97 belongs to the type II AAA (ATPases associated with diverse cellular activities) family [5–7]. p97 is composed of a substrate and cofactor binding N domain followed by two AAA ATPase domains termed D1 and D2, and forms a hexameric double-ring structure [8,9]. It has been demonstrated that the two ATPase domains are not catalytically equivalent: D2 has the major ATPase activity at physiological temperatures, whereas D1 is involved in the regulation of heat-induced ATPase activity [10]. D1 also plays a major role in hexamerization [11,12]. Recently, Nishikori et al. reported that the high D2 ATPase activity is in a coordinated fashion with positive cooperativity [13]. Given that the growth rate of yeast cells strongly related to the positive cooperativity rather than the ATPase activity itself, it has been claimed that the positive cooperativity is critical for p97 function [13].

It has been suggested that p97 is a ubiquitin-selective chaperone and that its key function is to unfold proteins and disassemble protein complexes [14]. p97 converts chemical energy generated from ATP hydrolysis into mechanical force used for conformational changes of target proteins as a unfoldase or a segregase [6,14–17]. p97 plays critical roles in a broad range of diverse cellular processes, including Golgi, endoplasmic reticulum (ER), and nuclear membrane reassembly [18–22], ER associated degradation (ERAD) [23–25], the ubiquitin-proteasome system [14,26–29], cell cycle regulation [22,30–33], DNA repair [34,35], preventing polyglutamine aggregation [3,36–39], autophagosome maturation [40–43], and mitophagy [44] (Fig. 1). Functional diversity of p97 is mainly determined by differential binding of distinct cofactors/adaptor proteins [45–47].

p97 has been linked to a severe degenerative disorder identified as hereditary inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (IBMPFD), which mostly affects muscles and brain [48]. The pathogenesis of IBMPFD is attributed to autosomal-dominant single-amino-acid substitutions in highly conserved residues within the p97 N-terminal domain and D1 domain [48,49]. Recently, p97 mutations have also been identified as a cause of familial amyotrophic lateral sclerosis (ALS), which links p97 defects to motor neuron degeneration [50].

p97 is a multifunctional protein and its functions are required for cell viability. However, its precise role and mechanism for each cellular function remain elusive. In this review, we will focus on recent progress in ubiquitin-mediated protein degradation, autophagy and cell cycle, and discuss p97 functions and the relationship to p97-

Abbreviations: AAA, ATPases associated with diverse cellular activities; VCP, valosin-containing protein; ER, endoplasmic reticulum; ERAD, ER associated degradation; IBMPFD, inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia; ALS, amyotrophic lateral sclerosis; OMM, outer mitochondrial membrane; Vms1, VCP/Cdc48-associated mitochondrial stress-responsive 1; TDP-43, TAR (transactive response) DNA-binding protein 43; FTLD, frontotemporal lobar degeneration

☆ This article is part of a Special Issue entitled: AAA ATPases: structure and function.

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