Structures of closed and open conformations of dimeric human ATM

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ATM (ataxia-telangiectasia mutated) is a phosphatidylinositol 3-kinase–related protein kinase (PIKK) best known for its role in DNA damage response. ATM also functions in oxidative stress response, insulin signaling, and neurogenesis. Our electron cryomicroscopy (cryo-EM) suggests that human ATM is in a dynamic equilibrium between closed and open dimers. In the closed state, the PIKK regulatory domain blocks the peptide substrate–binding site, suggesting that this conformation may represent an inactive or basally active enzyme. The active site is held in this closed conformation by interaction with a long helical hairpin in the TRD3 (tetratricopeptide repeats domain 3) domain of the symmetry-related molecule. The open dimer has two protomers with only a limited contact interface, and it lacks the intermolecular interactions that block the peptide-binding site in the closed dimer. This suggests that the open conformation may be more active. The ATM structure shows the detailed topology of the regulator-interacting N-terminal helical solenoid. The ATM conformational dynamics shown by the structures represent an important step in understanding the enzyme regulation.

INTRODUCTION

ATM (ataxia-telangiectasia mutated) is a key regulator of the DNA damage response with a broad role in stress response cellular homeostasis. Double-strand DNA breaks in conjunction with the damage-sensing Mre11, Rad50, and Nbs1 (MRN) complex activate ATM, which phosphorylates hundreds of targets (1, 2). ATM mutations are associated with the pleiotropic neurodegeneration disease ataxia telangiectasia (A-T), whose symptoms include malignancy and genome instability (3, 4). ATM mutations are often accompanied by immunodeficiencies, premature aging, insulin resistance, and sterility (5, 6). ATM is also activated by oxidation in an MRN-independent manner (7). ATM directly binds DNA (8, 9), but the MRN complex facilitates its recruitment to sites of DNA damage (10).

ATM is a member of the phosphatidylinositol 3-kinase–related protein kinase (PIKK) family that also includes protein kinases mTOR (mechanistic target of rapamycin) and DNA-PKcs (DNA-dependent protein kinase catalytic subunit) (11, 12). The C-terminal PIKK-conserved unit, which we refer to as the FATKIN, consists of a unique region known as the FAT [FRAP (FKBP12-rapamycin–associated protein), ATM, and TRRAP (transformation/ transcription domain-associated protein)] and a C-terminal phosphatidylinositol 3-kinase–like kinase domain (here abbreviated as KIN) but makes up no more than one-half of any PIKK. All PIKKs have about 1400 to 3000 residues at their N termini consisting predominately of helical repeats known as HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, and TOR1) repeats, which we will refer to as the N-solenosid.

Several reconstructions of yeast and human ATM at resolutions from 14 to 30 Å have been reported using electron microscopy (9, 13, 14). Most recently, an 8.7 Å resolution electron cryomicroscopy (cryo-EM) single-particle reconstruction was reported for the Schizosaccharomyces pombe Tel1 (15), where it was not possible to trace the course of the polypeptide. To gain insight into the structural basis for ATM activation, we have determined cryo-EM structures of human ATM at a highest resolution of 4.4 Å.

RESULTS

Our cryo-EM analysis and multiangle light scattering (MALS) and biochemical assays indicate that purified human ATM is a dimer and is enzymatically active (Fig. 1A, fig. S1 to S5, and table S1). Sub-classification of ATM particles indicated the presence of five three-dimensional (3D) classes. We have structurally interpreted four of these, classes A to D (fig. S5). Two types of dimers are present in the sample. One type of dimer has symmetrically related FATKINs (Fig. 1B), and we will refer to these as “closed” dimers (fig. S5, classes A and B). A second type of dimer is asymmetric (Fig. 1C), and we will call these “open” dimers (fig. S5, classes C and D). Our structures suggest that the open dimer might be a more active form of ATM.

Structure of the closed ATM dimer

The 3D class A (fig. S5) constitutes the closed dimer in which density is visible for both protomers. The closed dimer has a butterfly shape (Fig. 1B), where the N-solenoids form the wings and the FATKINs constitute the head. The path of the polypeptide in the closed dimer is clear, which allowed us to build an atomic model for the entire molecule into a density with an overall resolution of 4.7 Å (Fig. 1B, figs. S6 to S8, and table S2). In this model, we have not included side-chain atoms beyond CB because side-chain density was only rarely evident at this resolution. The N-solenoid has an N-terminal Spiral followed by a second region that we divide into the N-pillar, Bridge, C-pillar, Railing, and Cap (Figs. 1, B and E, and 2A). This second region was noted as the “C-Pincer” in the structurally unresolved density of the yeast Tel1 (15). The Pincer is then followed by the FATKIN.

The local resolution is highest in the C-terminal half of ATM (Pincer-FATKIN), which was reconstructed to a resolution of 4.4 Å (Fig. 1B and fig. S5). At this resolution, most helices appear as spirals (fig. S9), and the loops connecting secondary structure elements are mainly ordered. The ATM FATKIN can be divided into five domains: tetratricopeptide repeat domains TRD1 (a0 to a3, residues 1903 to 2025), TRD2 (a4 to a12, residues 2032 to 2190), and TRD3 (a13 to a22, residues 2195 to 2476); HRD (HEAT-repeat domain) (a23 to a28, residues 2484 to 2612); and a kinase domain (residues 2618 to 3056) (figs. S10 and S11). These regions assemble into a structure that is broadly
The upper interface includes portions of the kinase domain and TRD3. (Fig. 1. Structures and activity of human ATM dimers.)

similar to the structure of the mTOR FATKIN (fig. S10) (16); however, the ATM FATKIN mediates ATM dimerization, and this is important for the ATM activation mechanism.

The interface in the closed dimer
The closed dimer interface can be divided into the “upper” and the “lower” interface (Fig. 2, B and C). The upper interface involves TRD3 in one molecule forming compelling interactions with the kinase domain of its dimeric partner. The lower interface involves TRD2 from one molecule interacting with TRD2 and TRD3 of the other molecule.

Several kinase domain C-lobe elements are important for the transition between closed and open dimers. The FAT C-terminal (FATC), LST8-binding element (LBE), activation loop, and PIKK regulatory domain (PRD; helices kr9b, kr9c, and kr9d) form a compact arrangement that we will refer to as the FLAP [FATC, LBE, activation loop, and PRD] (Fig. 3A), which is key to the regulation of ATM. Because the TRD3 helices α21 and α22 (residues 2378 to 2476) interact with all of the elements of the FLAP, we will refer to this helical hairpin as the FLAP-binding element (FLAP-BE). Much of the upper interface consists of this FLAP/FLAP-BE interaction (Fig. 2B). The FLAP-BE of one molecule pushes on helices kr9c and kr9d (residues 2966 to 2979) in the PRD of the other molecule, causing kr9c and kr9d to enter the active site (Fig. 3A and fig. S12). We modeled a peptide substrate bound to ATM based on the structure of CDK bound to its substrate (Fig. 3A). In this model, a peptide substrate would completely clash with the helices kr9c/kr9d and FLAP-BE, suggesting that the closed dimer might be less active, because the peptide substrate would have to compete with the PRD for binding to the active site (movie S1).

Structure of the N-terminal solenoid
The N-solenoid has six domains (Fig. 1, B and E, and fig. S13). The ATM Spiral (residues 1 to 1161) has roles in binding substrates, regulators, and adaptors. Two corkscrew-like turns of the Spiral wrap around a 30 Å open cavity (figs. S13A and S14C). The first turn of the Spiral contacts the second turn where several short helices, which may be part of an extension from the N-protomer, are visible and may function to rigidify the Spiral (fig. S14).

The exposed N-terminal tip of the Spiral bears the Tel1/ATM N-terminal (TAN) motif (residues 18 to 40 ) (Fig. 4A) (17), which is essential for the ATM function in yeast. Spiral residues 90 to 97 interact with some ATM substrates, including p53, BRC1a, and LKB1 (Fig. 4A) (18). The binding site for Nbs1 of the MRN complex (19) maps approximately to the Spiral/N-terminal interface (Fig. 4A). ATM phosphorylates p53 in response to DNA damage (20), but p53 binding was only detected after ATM activation by DNA damage, which led to the proposal that this N-terminal region becomes exposed in a dimer-to-monomer transition (18). The structure shows that the p53-binding region is not involved in the dimer interface (Fig. 4A) and is exposed in all states that we observe. It may be that, in cells, an ATM binding partner could stabilize the closed dimer by binding to this site. If this blocking partner were removed in the activation process, it would explain the current observations. Similarly, activating partners such as Nbs1 or ATMIN (19, 21) might bind to this N-terminal region to lock the enzyme in the open conformation.

The ATM and TOR N-solenoids share four structural modules: the Spiral, Bridge, Railing, and Cap (fig. S13); however, these elements are arranged differently in space (fig. S16). The two pillars (Fig. 2A) are ATM N-solenoid modules absent in TOR. The N-terminal end of the Spiral in TOR/mTOR is freely accessible (22, 23), thereby correcting an earlier proposed topology that traced this region in the reverse direction (24). This corrected topology is consistent with the topology recently reported for DNA-PKcs (25) and the topology we observe for the ATM Spiral.

Structure of the ATM FAT domain
The FAT domain of ATM wraps around the kinase domain (fig. S10A). We have mapped three PIKK-specific polar interactions between the FAT and the kinase domains (16) onto the ATM FATKIN: Glu1959, Arg2849, Arg2466, Glu2950, and Gln2522-Gln2730. These residues are highly conserved in ATMs (fig. S11). Mutation R2849P at this interface is associated with A-T disease (26), whereas an R2486P mutation is associated with T-cell prolymphocytic leukemia (27), suggesting that these interactions are important to stabilize ATM.

Ser1981 in a loop between α1c/α2 helices of TRD1 (Fig. 4A) is a site of autophosphorylation and is part of a metazoan-specific insertion in ATM (15). In vitro, the S1981A mutation has no effect on kinase activity (2). However, in cells, Ser1981 phosphorylation enables ATM to remain associated with sites of double-strand DNA breaks by binding to the MDC1 FHA domain (28). The Ser1981 loop is not ordered in either the open or closed dimers, so it is unlikely that it controls the intrinsic activation state of the enzyme.

Structure of the open ATM dimer
We observed one class of the open dimer (asymmetric) in which both full protomers are visible (fig. S5, 3D class C). We have refined this class as the open dimer at a resolution of 11.5 Å (fig. S5). There is a second class of open dimers in which only one protomer is fully visible, which we will refer to as the open multi-conformer dimer (fig. S5, 3D class D). The full protomer in this class is associated with a second protomer that has only weak density for the Pincer-FATKIN and no density for the Spiral. The weak density probably arises from the protomer being averaged in an ensemble of orientations/conformations, as the dimer transitions between closed and open states (Fig. 4B and fig. S5). Because both classes C and D have one full protomer, we combined these and carried out masked refinement to obtain the structures of the open protomer at a resolution of 5.7 Å and its Pincer-FATKIN region at a resolution of 4.8 Å (Fig. 1D and fig. S5).

In the 11.5 Å resolution density for the open dimer with two full protomers (3D class C), we could readily fit the model for the open protomer (see above) and a second FATKIN molecule (Fig. 1C and fig. S5). There is a ratcheted rotation of about 24° for one FATKIN with respect to the other, compared with the FATKINS in the closed dimer (Fig. 1, B and C).

In the closed conformation, the FLAP/FLAP-BE interaction restricts access to the ATM substrate–binding site (Fig. 3A). However, the open dimer does not make the full set of contacts observed in the closed dimer. Notably, there is no intermolecular contact between the FLAP and the TRD3 in the upper interface of the open dimer (Figs. 1C and 3B and fig. S12). Moreover, PRD helices kr9d and most of kr9c are disordered, leaving sufficient space for a peptide substrate to bind (Fig. 3B). Release of constraints on the PRD in the open dimer arises from loss of contacts between the FLAP and the dimer-related FLAP-BE.

In the lower interface of the open dimer, density for only part of TRD2 is visible (Fig. 1D). However, contacts with α7 of TRD2 and α16 and α18 of TRD3 are still present (fig. S17). Therefore, in the open dimer, only the lower interface is partially retained with no preserved contacts in the upper interface.

DISCUSSION

Our structural work shows that ATM can form an open dimer with a limited intermolecular interface and a tightly packed closed dimer with a larger interface. The active site of the open dimer is compatible with substrate binding, whereas the PRD acts as a pseudosubstrate to block the active site in the closed dimer. This provides structural insight into several previous observations regarding the PRD. The PRD was named on the basis of findings that mutations in this region prevent ATR activation and that the analogous region in DNA-PKcs is important for its regulation (29). An earlier study showed that partial deletion of the PRD in mTOR led to a hyperactive enzyme (30). The shift of the PRD between the closed and the open dimers suggests

Fig. 3. Open and closed ATM dimer conformations of the active site. (A) The side view on the left shows the FATKIN arrangement in the closed dimer. The FLAP-BE' (purple) from the symmetry-related molecule restricts the PRD to a conformation that blocks the substrate peptide from entering the active site (right panel). The location of ADP-F3Mg was modeled on the basis of the crystal structure of the mTOR FATKIN [Protein Data Bank (PDB) ID 4JSV]. The location of the substrate peptide (yellow spheres) was modeled by superimposing a peptide-bound Cdk2 [PDB ID 3QHW (50)] onto the ATM active site. (B) A model of the active site of the 4.8 Å resolution open protomer (right panel). The side view on the left is the arrangement of the two FATKIN moieties in the 11.5 Å resolution open dimer. The green dashed lines in each panel represent the disordered portion of the PRD.

ATM is posttranslationally modified in a variety of different ways (Fig. 4A). In addition to the autophosphorylation of Ser1981, which is widely used as a marker of ATM activation, ATM is also acetylated on Lys3016 just before the FATC domain upon DNA damage (31, 32). The helix and the loop just preceding the FATC region form an elbow that could control the position of the FATC. The elbows from the two subunits in the closed dimer interact with each other at the twofold axis of the dimer. Lys3016 is sufficiently close to Glu3021 or Tyr2864 that their side chains may interact. Acetylation of Lys3016 could break these interactions, resulting in a conformational change of FATC that would help break the upper contacts in the dimer, thereby favoring an open dimer. This would be consistent with the observation that mutation of Lys3016 prevents dissociation of ATM dimers (32).

The open dimer has a different and less extensive intermolecular interface compared to the closed dimer, yet both closed and open states coexist, at least under the cryo-EM conditions. It is possible that these two states are in equilibrium in solution (Fig. 4B). An early work proposed that ATM is an inactive dimer and that DNA damage induces Ser1981 autophosphorylation, leading to dimer dissociation and activation (2). We find that the purified dimeric ATM used in this study is enzymatically active (Fig. 1A and fig. S2). Our cryo-EM structures show that about half of the particles are in an open conformation (fig. S5). It is possible that these open dimers account for the activity of the recombinant protein that we see in solution; however, we have no direct way to demonstrate what the active conformation in solution is. Although we see no evidence for free monomers, it may be possible for ATM monomers to form under some conditions. The open dimer that has a more limited intermolecular interface than the closed dimer may be more readily dissociated by binding other partners such as the MRN and DNA (10) or by harsher ATM purification protocols that include high salt concentration (10). It is also possible that the presence of substrate would affect the oligomeric state of the enzyme. Our structures represent a framework that will help guide future work aimed at understanding regulation of ATM in both DNA damage response and other contexts.

**MATERIALS AND METHODS**

**Recombinant human ATM expression**

Full-length ATM (residues 1 to 3056, Q13315) with an N-terminal FLAG tag (MDYKDDDDKH) was synthesized and codon-optimized for expression in mammalian cells. The FLAG-ATM gene was cloned into pDEST12.2-OriP to generate pDEST12.2-OriP–ATM, as described previously (33). All DNA solutions used for transfection were prepared under sterile conditions at 1 mg/ml in TE buffer [10 mM tris-HCl and 1 mM EDTA (pH 7.4)].

Small-scale transfections were carried out in Expi293F (Thermo Fisher Scientific, catalog no. A14528) and CHO (Chinese hamster ovary)–EBNA (Epstein-Barr virus nuclear antigen)–GS (glutamine synthase) cells (34) to determine the highest-yielding system (data not shown). For protein supply, Expi293F cells were transfected on a 1-liter scale. A 450-ml culture was seeded at 2 × 10⁶ cells/ml 24 hours before transfection. For protein supply, Expi293F cells were transfected on a 1-liter scale. A 450-ml culture was seeded at 2 × 10⁶ cells/ml 24 hours before transfection. To generate the transfection complexes, 3 mg of 40-kDa polyethylenimine hydrochloride (Polysciences, catalog no. 24765) was mixed with 750 μg of pDEST12.2-OriP–ATM in a total volume of 50-ml Expi293 Expression Medium (Thermo Fisher Scientific, catalog no. A1435101) and incubated for 15 min at room temperature. After

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incubation, the transfection complexes were added to the culture. The cells were incubated at 37°C, 5% CO₂, and 140 rpm in an orbital incubator (25-mm orbit). At 24 hours after transfection, the culture was diluted with 500 ml of Expi293F Expression Medium. At 4 days after transfection, cells were harvested by centrifugation at 10,000g for 20 min.

**Human ATM purification for cryo-EM**

The cell pellet from 1 liter of culture was resuspended in 100 ml of cold lysis buffer [50 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 100 mM arginine (Sigma-Aldrich, catalog no. A-5006), 10% (v/v) glycerol, two tablets of complete EDTA-free protease cocktail inhibitors (Roche) per 0.1 liter, and 1000 U of Pierce Universal Nuclease (Thermo Fisher Scientific, catalog no. 88702).] per 0.1 liter, and 1000 U of Pierce Universal Nuclease (Thermo Fisher Scientific, catalog no. 88702) per 0.1 liter. The following steps were performed in a cold room, and a roller was used for the incubation steps, unless mentioned otherwise. A 3-ml slurry of anti-FLAG resin (M2 agarose affinity gel; Sigma-Aldrich, cat-

**Human ATM purification for kinase assay**

The catalytic activity of human ATM (stored at −80°C and then thawed for the assay) prepared in the purification buffer C [25 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 10% (v/v) glycerol, and 2 mM TCEP] was determined using the full-length human p53 substrate at a concentration of 750 nM. The reactions were carried out in a final volume of 14 μl in two different reaction buffers prepared at room temperature (23°C): (i) the purification buffer [25 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 10% (v/v) glycerol, and 2 mM TCEP], supplemented with 5 mM MgCl₂ and (ii) the cryo-EM buffer [25 mM Hepes (pH 7.5, 23°C), 25 mM tris (pH 8.8, 23°C), 150 mM NaCl, 0.01% (v/v) Tween 20, and 2 mM TCEP, with the addition of 5 mM MgCl₂]. The ATM-specific inhibitor KU55933/AZ12622702 (AstraZeneca) was dissolved in dimethyl sulfoxide and then added to the reaction at a concentration of 770 nM. The reactions were started with the addition of 100 μM adenosine 5'-triphosphate (ATP) and 0.3 μCi [γ-³²P]ATP, following the incubation for 15 min at 30°C. The reactions were stopped with the addition of SDS–polyacrylamide gel electrophoresis (PAGE) loading buffer. An aliquot of 11 μl of each reaction mixture was resolved by SDS-PAGE using 4 to 12% NuPAGE bis-tris gel (Invitrogen, Thermo Fisher Scientific, catalog no. 36-6892). The supernatant was removed by pipetting, and the resin was resuspended in buffer A before it was transferred to a 2.5 cm × 10 cm glass Econo-Column (Bio-Rad). The resin was then washed with 2× 50 ml of buffer A followed by a 2× 50-ml wash with buffer B [50 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 10% (v/v) glycerol, and 1% (v/v) imidazole]. Elution buffer was prepared by diluting 3X FLAG peptide stock solution (peptide (200 mg/ml), 10 mM tris (pH 7.4, 23°C), and 250 mM NaCl) to the peptide (200 μg/ml) using buffer B. The resin was gently resuspended in 5 ml of the elution buffer and incubated for 10 min while sitting at 4°C. A total of 5× 5 ml of elution fractions were collected in this way. The combined elution fractions (25 ml) were transferred to dialysis tubing [SnakeSkin, 10,000 molecular weight cutoff (MWCO), Thermo Fisher Scientific] and dialyzed overnight in 5 liters of the dialysis buffer [50 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 0.01% (v/v) Tween 20, and 2 mM tris(2-carboxyethyl) phosphine (TCEP)]. The dialyzed protein was then concentrated (50,000 MWCO, Amicon Ultrafree concentrator) to 50 μl, spun for 1 min at 20,283g in a tabletop centrifuge to remove large aggregates, and finally purified by size exclusion chromatography (SEC) (at a flow rate of 250 μl/min, TSKgel G4000SWXL, Tosoh Corporation Bio-science) in 50 mM Hepes (pH 7.5, 23°C), 150 mM NaCl, 0.01% (v/v) Tween 20, and 2 mM TCEP. Purified protein was concentrated to 1.2 mg/ml (3.4 μM), plunged-frozen in liquid nitrogen in 10-μl aliquots, and stored at −80°C until it was used for the cryo-EM grid preparation.

**Human p53 expression and purification for kinase assay**

A full-length variant of human p53 carrying four stabilizing mutations (M133L, V203A, N239Y, and N268D) (35, 36) in the DNA binding domain was expressed as an N-terminal Hist₄₅-lipoyl domain fusion in *Escherichia coli* C41 (DE3) cells (Lucigen) and purified at 4°C by a combination of Ni–nitrotoltriacetic acid, heparin affinity, and gel filtration chromatography, as described previously (37, 38). Briefly, expression cultures were incubated at 37°C and shaken at 250 rpm until an optical density at 600 nm of ~0.8 was reached. Zinc sulfate was added to a final concentration of 50 μM, and protein expression was induced with 500 μM isopropyl β-D-1-thiogalactopyranoside at 22°C. Cells were harvested 16 hours later by centrifugation and lysed using a high-pressure homogenizer (Avestin, Biopharma) in a lysis buffer containing 50 mM Hepes (pH 7.4, 4°C), 500 mM NaCl, 5% (v/v) glycerol, 20 mM imidazole, 0.05% (v/v) Tween 20, 8 mM β-mercaptoethanol, EDTA-free protease inhibitor cocktail (Roche), ribonuclease A (Sigma-Aldrich), deoxyribonuclease I (Sigma-Aldrich), and lysozyme (Sigma-Aldrich). Clarified lysate was loaded onto a HisTrap FF Crude column (GE Healthcare) and step-eluted with 50 mM Hepes (pH 7.4, 4°C), 500 mM NaCl, 5% (v/v) glycerol, 500 mM imidazole, and 8 mM β-mercaptoethanol. Fractions containing recombinant protein were pooled, and the N-terminal Hist₄₅-lipoyl domain was cleaved off by a tobacco etch virus protease (a Gly-Gly-Ser sequence remains to precede the initial Met of p53) full-length sequence in the final cleavage product). The protein was subsequently dialyzed for 20 hours against the dialysis buffer containing 20 mM Hepes (pH 7.4, 4°C), 150 mM NaCl, 200 mM arginine, 10% (v/v) glycerol, and 4 mM DTT and then loaded onto a HiTrap Heparin HP column (GE Healthcare) followed by column equilibration in the dialysis buffer containing 210 mM NaCl. The protein was then step-eluted with the dialysis buffer containing 450 mM NaCl. Finally, the elution fractions were directly subjected to SEC using an XK 26/70 Superose 6 prep grade column (GE Healthcare) equilibrated in 20 mM Hepes (pH 7.3, 4°C), 500 mM NaCl, 200 mM arginine, 10% (v/v) glycerol, and 4 mM DTT. The protein was eluted at 3 mg/ml (67 μM), and the aliquots were flash-frozen in liquid nitrogen for storage at ~80°C before p53 was used for the kinase assay.
Fisher Scientific). The gel was stained in Coomassie (InstantBlue, Expe-
dion), destained in water, dried for 1 hour at 80°C (electrophoresis slab
gel dryer), and exposed for up to 24 hours to a storage phosphor screen
(GE Healthcare Molecular Dynamics). The 32P incorporation was
analyzed using a Typhoon FLA 7000 PhosphorImager. In addition,
the p53 phosphorylation was detected by mass spectrometry analysis
of the above catalytic reactions containing nonradioactive ATP.

Cryo-EM grid preparation and microscopy
Several freezing conditions were tested to obtain single particles of hu-
man ATM distributed in thin ice (we found that a thin carbon support
gave rise to a strong preferential orientation of ATM). ATM was de-
frosted on ice and mixed with 1 volume of the cryo-EM freezing so-

olution [50 mM tris (pH 8.8, 23°C), 150 mM NaCl, 0.01% (v/v) Tween
20, and 2 mM TCEP]. An aliquot of 3 μl (final protein concentration
of ~0.6 mg/ml) was applied to a plasma-cleaned (NanoClean model
1070, vacuum of 10−5 mbar, argon/oxygen ratio of 9:1, 70% power,
0.331 cubic centimeter per minute flow, and 20-s exposure) grid with
no support [QUANTIFOIL R1.2/1.3 Au (300 mesh) or UltrAuFoil
R1.2/1.3 (300 mesh)] and then blotted for 12 s and plunge-frozen in
liquid ethane using a custom-built manual plunger [Medical Re-
search Council Laboratory of Molecular Biology (MRC LMB),
Cambridge, UK] at 4°C. An FEI Titan Krios operated at 300 kV
was used for data collection. The micrographs were recorded either
manually or in a semiautomated fashion [University of California,
San Francisco Image4 (39)]. We used a Gatan K2 Summit direct elec-
tron detector in a super-resolution counting mode at a calibrated magni-
fication of 35,714× (nominal magnification of 97,902×), which
resulted in a physical pixel size of 1.43 Å. A GIF Quantum Energy Fil-
ter with a slit width of 20 e− was used. A total exposure of about 42 e−/Å2
over 16 s (−2.6 e−/Å2 per second or −5.3 e−/pixel per second) was
dose-fractionated in 20 movie frames. A defocusing range of −2.5 to
−4 μm in 0.25-μm steps was applied during the data collection.

Cryo-EM image processing
We collected a total of 2720 good 20-frame movie stacks (see example
in fig. S3) that were corrected for whole-image drift using the MotionCorr
program (40). The graphics processing unit (GPU)–accelerated pro-
gram Gctf (41) was used for determining the contrast transfer
function parameters from the drift-corrected averaged images. The
subsequent data processing steps were done in RELION 1.4 (42, 43),
if not specifically mentioned.

At first, about 2500 particles were manually picked with a particle
mask of 260 Å in diameter, extracted with a 300 pixel x 300 pixel box
size to generate initial reference-free 2D class averages. Reasonable 2D
class averages were low-pass–filtered to 20 Å and used as a template
for a reference-based particle autopicking (44) of the first data set
containing 649 micrographs.

The automated particle selection was manually checked for each
micrograph to remove obvious protein aggregates and contaminants
to finally extract 67,756 particles. Further reference-free 2D classifica-
gion gave class averages with prominent features altogether containing
21,135 particles (fig. S3B). An initial 3D model (fig. S4A) was recon-
structed in EMAN2 (45) by imposing C2 symmetry and using nine 2D
class averages. Projections of the initial model were in agreement with
the experimentally obtained 2D class averages. A 3D classification
restricted to five classes was performed without imposing symmetry
(C1), using the 60 Å low-pass–filtered initial EMAN2 model, an
angular sampling of 7.5°, and a regularization parameter T of 4, and
without doing local angular searches around the refined orientations
during 40 iterations. A single 3D class that matched well with the ex-
perimental 2D projections had clear C2 symmetry and the highest ac-
curacy of rotations (1.98°) (fig. S4A), and this was used as a new initial
3D model for further reconstructions. A second round of the 3D clas-
sification (following the steps from above) using the new initial 3D
model resulted in three prominent classes that indicated conformation-
ally distinct species (fig. S4B). To increase the number of good par-
icles, we combined the best three classes (a total of 16,873 particles) to
obtain an interpretable cryo-EM map of human ATM. Auto-refinement
of the combined classes resulted in a conformationally averaged re-
construction at an overall resolution of 7.5 Å (accuracy of the rotational
alignment was 2.79°, and the translational alignment accuracy was
1.17 pixels) (fig. S4B). We used this cryo-EM map to determine the
diagrammability by manually fitting in a model of the mTOR FATKIN
(PDB ID 4JSP was manually modified by deleting the FRB domain,
using Coot) (fig. S4B).

To select particles from the entire data set, we used the GPU-
accelerated program Gautomatic [K. Zhang, MRC LMB (www.mrc-
lmb.cam.ac.uk/kzhang/)]. The 2D classes obtained earlier were used as
a new template for the automated particle selection. After refiltering
the selected particle coordinates [using the script box_filter2rl.com (www.
mrc-lmb.cam.ac.uk/kzhang/Gautomatch/)] and manually examining
each micrograph, a total of 371,671 particles were subsequently
retracted from bad particles during the two cycles of 2D classification.
A total of 139,086 particles were included in further data processing.

The 3D classification was performed as already described above,
resulting in four distinct 3D class averages (fig. S5): (A) the closed dimer
(consisting of two full protomers related by a C2 symmetry), (B) the
closed multi-conformer dimer (consisting of one full protomer asso-
ciated with a second protomer having FATKIN, Pincer, and a flexible
Spiral; the two FATKINs in the dimer have C2 symmetry), (C) the open
dimer (consisting of one ordered protomer associated with a density
corresponding to a second protomer with a conformational change in Pincer),
and (D) the open multi-conformer dimer (consisting of a full protomer
associated with a second protomer having an ordered FATKIN with
Pincer and Spiral not visible). We used statistical movie processing in
RELION (46) to estimate per-particle beam-induced movement for all
20 movie frames with running averages of 5 movie frames and an SD
of 1 pixel for the translational alignments. For each class, we independently
used a particle-polishing step (47) that fits linear tracks through the es-
timated particle movement to complete modeling of the beam-induced
particle motion. This step included the B-factor weighting to estimate the
dose- and resolution-dependent radiation damage with running
averages of three movie frames. Below, we describe individual process-
ing of all four 3D classes (fig. S5).

Model building and refinement
We used I-TASSER (48) to build an initial homology model of the
human ATM FATKIN based on the mTOR FATKIN crystal struc-
ture. This model was first rigidly fit to the density for the head in
the highest-resolution reconstruction for the closed dimer. We re-
moved all side chains from the model and then used Coot to manually
fit the homology model into the density. The model of the N-solenoid
was manually built by first fitting a polyalanine model into the largely
helical density. An approximate sequence was assigned to this region
based on the locations of predicted helices in the sequence. However,
no side chains were visible in the density. All atomic models consisting
of only backbone atoms and β-carbons (for non-glycine residues)
were refined with PHENIX (phenix_real_space) (49). For the refinement, secondary structure elements were restrained. The atomic displacement parameter (ADP) or B-factor refinement was performed in the last macrocycle. The starting model for each of the open conformations was the model of the closed conformation broken into pieces and fit to the density by rigid-body refinement. These models were then manually adjusted using Coot and refined using PHENIX. In the final model for the closed dimer, we have built and refined 4966 of the 6112 residues in the ATM dimer.

Closed dimer (two full protomers visible, 3D class A)
A total of 25,315 polished particles from 3D class A (fig. S5) were auto-refined using the C2 symmetry and a soft spherical mask around the entire volume, which resulted in a final map with an overall resolution of 4.7 Å (rotational and translational alignment accuracies of 1.25° and 0.64 pixel, respectively). The auto-refinement was repeated with a soft mask (5-pixel falloff) around only the Pincer-FATKIN region of the map, excluding the Spiral (the most flexible part of the molecule). This procedure was helpful in obtaining a map of the Pincer-FATKIN region of the closed dimer at a resolution of 4.4 Å (accuracies of 1.65° and 0.65 pixel). This Pincer-FATKIN map was used for initially building the model of this region (see the “Model building and refinement” section). To better resolve the secondary structure elements of the Spiral domain, we carried out focused 3D classification (with fixed particle orientation and a T value of 10) of the auto-refined closed dimer particles with the signal from the Pincer-FATKIN subtracted. The auto-refinement (imposed C2 symmetry) of the best 3D class resulted in a map for the Spirals with an overall resolution of 7.9 Å (accuracies of 2.22° and 1.3 pixels). Finally, a model for the Pincer-FATKIN and a model for the Spirals were fitted into the 5.7 Å map that was obtained after the auto-refinement with a soft spherical mask around the entire dimer with no imposed symmetry (accuracies of 1.42° and 0.71 pixel).

Plots of the Fourier shell correlation (FSC) between two independently refined half-maps for all four described maps are shown in fig. S6A. The Euler angle distribution for the particles representing a closed dimer is shown in fig. S7A. The schematic overview of the above processing steps is shown in fig. S5.

Closed multi-conformer dimer (only one full protomer visible, 3D class B)
A total of 34,155 polished particles of 3D class B (fig. S5) were auto-refined using no symmetry and a soft solvent mask (5-pixel falloff) around the ordered region of the dimer, which converged to a final map with an overall resolution of 8.8 Å (rotational and translational accuracies of 2.64° and 1.24 pixels). The plot of the FSC between two independently refined half-maps is shown in fig. S6B. The Euler angle distribution for the particles representing the closed multi-conformer dimer is shown in fig. S7B. A focused 3D classification (with the orientation of particles fixed and a T value of 10) revealed subclasses with a more apparent density for what resembles a flexible N-solenoid. The schematic overview of these processing steps is shown in fig. S5.

Open dimer (two full protomers visible, 3D class C)
The particles representing 3D class averages of the open dimer, 3D class C (15,837) and the open multi-conformer dimer, 3D class D (44,719) were combined, and auto-refinement was performed using a spherical mask around the entire dimer volume. We have performed seven cycles of the focused classification (without particle realignment, using a T value of 10) and 3D refinement (less extensive searches with the initial angular sampling of 3.7° and local searches of 7.5°) to finally obtain the open dimer map from 17,816 particles at a resolution of 11.5 Å (accuracies of 2.3° and 1.28 pixels). The plot of the FSC between two independently refined half-maps is shown in fig. S6C. The Euler angle distribution for the particles representing the open dimer is shown in fig. S7C. The schematic overview of this processing is shown in fig. S5. This dimer has approximately the same dimensions as the closed dimer (Fig. 1), but the relationship of the two molecules to each other is different. One molecule could be readily interpreted by rigidly fitting the ATM protomer into the density. A FATKIN was rigidly fit into the other molecule.

Open multi-conformer dimer (only one full protomer visible, 3D class D)
A total of 44,719 polished particles from 3D class D (fig. S5) were auto-refined with no imposed symmetry and a soft mask (5-pixel falloff) around the ordered region of the dimer, which converged to a map (not shown) with an overall resolution of 7.8 Å (rotational and translational accuracies of 1.76° and 0.83 pixel). A focused 3D classification (T value of 10) revealed several good classes containing 19,887 particles altogether. The final auto-refinement converged to a map with an overall resolution of 8.4 Å (accuracies of 2.03° and 0.92 pixel). This map indicated two FATKINS related by approximate C2 symmetry. However, one FATKIN is better ordered than the other. The better-ordered FATKIN has a clearly visible FLAP-BE and a well-defined dimer interface, whereas the other FATKIN does not have a visible FLAP-BE and has a poorly ordered density for its TRD3. The FSC between two independently refined half-maps is plotted in fig. S6D. The Euler angle distribution of the particles representing the open multi-conformer dimer is shown in fig. S7D. A schematic overview of these processing steps is shown in fig. S5.

Open protomer (derived from 3D classes C and D)
The 44,719 particles representing 3D class D, the multi-conformer open dimer (only one full protomer visible), and the 15,837 particles representing 3D class C, the open dimer (two full protomers visible), were combined, and auto-refinement was performed using a soft mask (5-pixel falloff) around the well-ordered protomer. In this way, we obtained a map of the open protomer at an overall resolution of 5.7 Å (accuracies of 1.80° and 0.81 pixel). A further masked auto-refinement around the Pincer-FATKIN region converged to a map with an overall resolution of 4.8 Å (accuracies of 1.79° and 0.82 pixel). Both maps were used to build a complete model of the open ATM protomer (see the “Model building and refinement” section). The plot of the FSC between two independently refined half-maps for both maps is shown in fig. S6E. The Euler angle distribution for the particles representing the open protomer is shown in fig. S7E. The schematic overview of these processing steps is shown in fig. S5. The model of the closed dimer (3D class A) was broken into segments that were fit as rigid bodies into the 4.8 and 5.7 Å resolution densities for the open protomer.

MALS analysis
FLAG-ATM protein for MALS analysis was expressed and purified similarly as described above, except that after elution from the anti-FLAG resin protein, TCEP was added to the eluate to a final concentration of 2 mM. The protein was concentrated in an Ultra-15 Ultracel 100K Concentrator (Millipore, UFC910024) and purified by SEC on a Superose 6 10/300 (GE Healthcare). The entire non-aggregated peak was pooled and concentrated to 1 mg/mL, and 0.1 mL of the sample was further characterized by SEC-MALS using a Wyatt Heleos II 18-angle

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light scattering instrument coupled to a Wyatt Optilab rEX online refractive index detector (Wyatt Technology Corporation). Detector 12 in the Heleos instrument was replaced with a WyattQELS detector for dynamic light scattering measurement. The ATM sample was analyzed at room temperature using a Superose 6 10/300 SEC column (GE Healthcare) run at 0.5 ml/min in 25 mM Hepes (pH 7.5, 23°C), 25 mM tris (pH 8.8, 23°C), 150 mM NaCl, 2 mM TCEP, and 0.01% (v/v) Tween 20 buffer before passing through the light scattering and refractive index detectors in a standard SEC-MALES format (fig. S1C). Protein concentration was determined from the excess differential refractive index based on a 0.186 refractive index increment for a protein solution (1 g/ml) or the ultraviolet signal using 1.004 absorbance units/cm for 1 mg/ml. The concentration and the observed scattered intensity at each point in the chromatogram were used to calculate the absolute molecular mass from the intercept of the Debye plot using the Zimm model as implemented in Wyatt’s ASTRA software. Autocorrelation analysis of data from the dynamic light scattering detector was also performed using Wyatt’s ASTRA software, and the translational diffusion coefficients determined were used to calculate the hydrodynamic radius using the Stokes-Einstein equation and the measured solvent viscosity of 9.3 x 10^{-3} poise.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/e1700933/DC1

fig. S1. Purification of recombinant human ATM.
fig. S2. Recombinant human ATM is catalytically active.
fig. S3. Purification of recombinant human ATM.
fig. S4. Reference models and 3D classes of human ATM.
fig. S5. Summary of the cryo-EM 3D classification and focused refinement of ATM structures.
fig. S6. FSC curves for the observed reconstructions.
fig. S7. Euler angle distribution of particles for each 3D class (A to E).
fig. S8. FSC between the ATM models and EM density maps.
fig. S9. Quality of the density for the highest-resolution human ATM map.
fig. S10. Comparison of the ATM FATKIN with mTOR.
fig. S11. Multiple sequence alignment of the FATKIN regions of human ATM and its representative orthologs, along with that of human mTOR.
fig. S12. Open and closed conformations of the ATM active site.
fig. S13. Domains of the N-solenoid of ATM.
fig. S14. Density bridging the two turns of the Spiral.
fig. S15. Comparison of structurally conserved domains in the N-solenoids of ATM and TORK.
fig. S16. Domain organization of ATM compared with TORK.
fig. S17. Highest-resolution subclass (8.4 Å resolution) of the open multi-conformer dimer (from fig. S5, 3D class D) has one ordered protomer associated with a weakly ordered second FATKIN.

table S1. Data collection and refinement statistics.

table S2. Statistics for refined atomic models.

movie S1. Open and closed conformations of ATM.

References (51–58)

REFERENCES AND NOTES


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Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors. Coordinates (closed dimer PDB ID SNP0 and open protomer PDB ID SNP1) and densities (closed dimer (C2) EMD-3668, closed dimer (C1) EMD-3669, closed dimer Pincer-FATKIN EMD-3670, open dimer EMD-3671, open protomer EMD-3672, and open protomer Pincer-FATKIN EMD-3673) are deposited with the wwPDB OneDep System.

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