MFN1 structures reveal nucleotide-triggered dimerization critical for mitochondrial fusion

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Mitochondria are double-membraned organelles with variable shapes influenced by metabolic conditions, developmental stage, and environmental stimuli1–4. Their dynamic morphology is a result of regulated and balanced fusion and fission processes5,6. Fusion is crucial for the health and physiological functions of mitochondria, including complementation of damaged mitochondrial DNAs and the maintenance of membrane potential6–8. Mitofusins are dynamin-related GTPases that are essential for mitochondrial fusion9,10. They are embedded in the mitochondrial outer membrane and thought to fuse adjacent mitochondria via combined oligomerization and GTP hydrolysis11–13. However, the molecular mechanisms of this process remain unknown. Here we present crystal structures of engineered human MFN1 containing the GTPase domain and a helical domain during different stages of GTP hydrolysis. The helical domain is composed of elements from widely dispersed sequence regions of MFN1 and resembles the ‘neck’ of the bacterial dynamin-like protein. The structures reveal unique features of its catalytic machinery and explain how GTP binding induces conformational changes to promote GTPase domain dimerization in the transition state. Disruption of GTPase domain dimerization abolishes the fusogenic activity of MFN1. Moreover, a conserved aspartate residue trigger was found to affect mitochondrial elongation in MFN1, probably through a GTP-loading-dependent domain rearrangement. Thus, we propose a mechanistic model for MFN1-mediated mitochondrial tethering, and our results shed light on the molecular basis of mitochondrial fusion and mitofusin-related human neuromuscular disorders14.

We constructed an internally modified human MFN1 (MFN1IM) composed of the GTPase (G) domain (residues 75–336) and a four-helix-bundle that we term helical domain 1 (HD1; Fig. 1a, b, Extended Data Fig. 1a–g and Extended Data Table 1). The G domain contains a central eight-stranded β-sheet surrounded by eight α-helices. Compared to the canonical GTPase Ras, the G domain of MFN1 has two extra lobes that shield the nucleotide-binding pocket, and a specific short α-helix (α2G) sitting between α4G and β6G (Fig. 1c). Lobe 1, containing two β-strands (β1G and β2G) and an α-helix (α1I), is located between β2G and β3G, whereas lobe 2, consisting of an α-helix (α3G) and a loop, is located between β6G and α5G (Extended Data Fig. 2a). The four α-helices of HD1, derived from widely dispersed sequence regions, form a vast and conserved hydrophobic network (Fig. 1a, d and Extended Data Fig. 2b). HD1 is connected to the G domain via Arg74 at the C-terminal end of α2H and Lys336 between α5G and α3H (Fig. 1b). The N terminus of α1H substantially contacts the G domain (Extended Data Fig. 2c–e). On the other side of HD1, part of the artificial linker folds into an α-helix extending α3H (Fig. 1b). This is in agreement with the secondary structure prediction for the replaced residues (Extended Data Fig. 3), suggesting that α3H may be longer in full-length MFN1.

The overall topology of MFN1IM is typical of the dynamin superfamily15–20 (Extended Data Fig. 4). Apart from the G domain, the MFN1IM HD1 is particularly consistent with the neck of the *Nostoc punctiforme* bacterial dynamin-like protein (BDLP), which was suggested to mediate membrane fusion in bacteria21 (Extended Data Figs 2a, 4, 5a). Given the compact organization of HD1 and the predicted secondary structure (Extended Data Fig. 3), the missing portion of MFN1 (excluding the transmembrane domain) from MFN1IM is likely to fold into a helix-rich domain resembling the ‘trunk’ and ‘paddle’ of BDLP21. We term this putative region helical domain 2 (HD2).

**Figure 1** | **Overall structure of MFN1IM.**

a, Schematic representation showing the organization of MFN1IM based on full-length MFN1 with the conventional terminology. G domain, GTPase domain; HR1, heptad repeat region 1; T, transmembrane region. Elements for MFN1IM are assigned according to the structure. L denotes the artificial linker. Borders of each element are indicated by residue numbers. b, Structure of MFN1IM. α-helices of HD1 are differentially coloured to specify their distribution on the primary structure as in a. The artificial linker is in grey. Disordered loop is shown as dashed lines. The Cα atoms of Arg74 and Lys336 that link the G domain and HD1 are shown as grey spheres. c, The G domain of MFN1IM. Lobs 1, 2 and α2G are colour-specified. The core region corresponding to Ras is in orange and the GTPase active site is indicated by an ellipsoid. d, Hydrophobic network within HD1. Side chains of the residues involved in the network are shown in the same colour as the helices they belong to. e, Comparison between the putative hinge 2 of MFN1IM and BDLP.
When bound to tubulated liposomes in the presence of GMPPNP, BDLP bears G domain–neck and neck–trunk rearrangements via so-called hinge 2 and hinge 1 (ref. 22). Notably, Lys336 of MFN1IM exactly overlaps with BDLP Arg327 at hinge 2b, whereas MFN1IM Gly309 and Arg74 also have counterparts in BDLP (Gly309 in hinge 2b and Gly68 in hinge 2a) at equivalent positions (Fig. 1e). Mutation of these hinge-2-related residues diminished GTPase activity and mitochondrial elongation, although the G domain and HD1 exhibited only limited relative movement in different nucleotide-loading states (Extended Data Fig. 5b–e). Similar to dynamins, the potential hinge 1 between HD1 and predicted HD2 (368–YSVEER–373 and 692–EELIAR–697) lacks overall conservation among mitofusins (Extended Data Fig. 3). A proline insertion in 692–EELIAR–697 abolished mitochondrial elongation activity (Extended Data Fig. 5e, f). Altogether, full-length MFN1 may undergo conformational changes similar to BDLP when mediating outer mitochondrial membrane (OMM) fusion via aforementioned hinges.

G1–G4 elements of GTPases are essential for binding and hydrolysis of GTP (Fig. 2a). Notably, in the nucleotide-free (apo) MFN1IM structure, the nucleotide-binding pocket is occupied by the bulky side chain of Trp239 from G4, a residue conserved only in mitofusins and BDLP (Fig. 2b). Loading of GTP drives Trp239 away, causing it to wedge into a wide hydrophobic groove formed by Met249 from α3G and Phe282 from β6G. This rearrangement allows the suitable positioning of Asn237 and Asp240 to dock the guanine base (Fig. 2b). Mutation of Trp239 to alanine abolished nucleotide-binding and GTPase activity of MFN1IM (Fig. 2c, d). Both MFN1(W239A) and the corresponding MFN2 mutant MFN2(W260A) were nonfunctional for mitochondrial elongation (Fig. 2e), manifesting the importance of this tryptophan switch. When accommodating a nucleotide, MFN1IM uses α2–G to loosely buttress the guanine from a vertical orientation, and a large area of the nucleotide is thus exposed. This feature is shared by BDLP but not with dynamin-1 (refs 21,23), in which the nucleotide is tightly wrapped (Extended Data Fig. 5g).

Whereas the apo and GTP structures are monomeric, MFN1IM forms a homodimer in the presence of the transition state mimic GDP•AlF4− (Extended Data Fig. 6a). Dimerization is mediated by association of the G domains across the nucleotide-binding pocket, and the HD1s protrude in opposite directions from the dimer axis (Fig. 3a). Major interactions of this 995 Å2 “G interface” include a pair of symmetrical, parallel aligned salt bridges between Arg238 in the G4 element and Glu209 in the loop between 33α and 33β (Fig. 3b). Flanking this central salt-bridge pair, close in trans contacts are also observed between Lys99–Glu245, His144–Glu247, and His147–Asp251. In addition, the side chain of Tyr248 inserts into the groove between switch I and α1–G of the other molecule (Fig. 3b). G domain dimerization has been found in several dyanmin superfamily members in the transition state of GTP hydrolysis23–25. Compared to the approximately 2,500 Å2 G interface of dynamin-1 (ref. 23) involving extensive hydrogen bonds and hydrophobic associations, the substantially smaller G interface of MFN1IM is dictated by charged interactions, and no in trans stabilization of the nucleotides is observed (Fig. 3c).

To verify the functional relevance of G-domain-mediated dimerization, we performed mutagenesis studies on residues Glu209 and Arg238. Whereas GDP•AlF4− induced the formation of MFN1IM dimers in analytical gel filtration coupled to right angle light scattering (RALS) assays, MFN1IM(E209A) and MFN1IM(R238A) failed to dimerize (Fig. 3d). Moreover, the two mutants lacked stimulated protein-concentration-dependent GTPase activity (Fig. 3e), even though they bound guanine nucleotides with wild-type affinity (Fig. 3f). These results suggest that GTPase activation is mediated by dimerization via the G interface. In addition, MFN1IM(E209A) and MFN1IM(R238A) showed suppressed liposome tethering activity in vitro (Extended Data Fig. 6b). Both MFN1(E209A) and MFN1(R238A), as well as the corresponding MFN2 mutants MFN2(E230A) and MFN2(R259A), failed to elongate mitochondria (Fig. 3g). Thus, G domain association of mitofusins during the transition state of GTP hydrolysis is an indispensable step for OMM fusion. In addition, mutations of most other residues involved in the G interface also impinge dimerization, GTP hydrolysis and mitochondrial elongation to various extents (Extended Data Fig. 6c–e).

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Nucleotide binding induces conformational changes in the residues defining the G dimer interface. In the nucleotide-free state, the conformation of MFN1IM disfavours dimerization: Arg238 is blocked by the carbonyl oxygens of Ala241 and Ala243, whereas Glu209 and Glu245 are attracted by Arg253 (Extended Data Fig. 6f). GTP-loading-induced rearrangement of the G4 element translocates Trp239 to push Arg253 aside towards Glu316. As a result, Glu209, Glu245 and Arg238 are released and become solvent-facing, allowing for G domain dimerization.

During GTP hydrolysis, the conformationally flexible switch regions must be stabilized to favour catalysis. In our MFN1IM structure solved from co-crystallization with GDP•AlF$_4^-$, although the AlF$_4^-$ moiety is absent, the switch I holds a catalysis-compatible conformation analogous to those of other dynamin superfamily members in the transition state (Fig. 4a and Extended Data Fig. 7a–d). Notably, unlike many dynamin-related proteins, switch I of nucleotide-free MFN1IM is fixed in a conformation distinct from that in the transition-like state by a hydrophobic network involving G2 element and Gly114 of atlastin-1 (Figs 2a, 4a). Mutation of His107 to alanine did not perturb the binding of guanine nucleotides but eliminated the oxygen of Gly104, which corresponds to Gly60 of dynamin-1 and the corresponding mutant MFN2(H128A) were defective in mitochondrial elongation (Fig. 4d). Thus, we propose this His107 finger as a charge-compensating factor during catalysis. The catalytic water moiety is not present in MFN1. Instead, a mitofusin-specific His107 in mitochondrial fibroblasts (MEFs) transfected with either MFN1(H107A) or MFN2(E230A) and MFN2(R259A). For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10 μm.

Compared to other scission-related dynamin family members, MFN1IM shows extraordinarily weak GTPase activity (Fig. 2d). The stable conformation of switch I and the shielded GTP-binding pocket in the nucleotide-free state may account for this feature (Fig. 4e). Slow GTP turnover, exemplified by BDLP22 and EHD2 (ref. 15), has been suggested to favour self-assembly over disassembly, and thus promote membrane fusion26.
MFN1IM(H107A). Results from two separated experiments are presented.

We also purified a near-full-length construct termed MFN1ΔTM that contains the G domain, HD1 and HD2, but lacks the transmembrane domain (Extended Data Fig. 8a, b). MFN1ΔTM elutes as a stable dimer in RALS assays without nucleotide (Extended Data Fig. 8c). This dimer, possibly mediated by HD2, may relate to the 180-kDa stable complex of rat MFN1 found in sedimentation studies. Moreover, MFN1ΔTM oligomerizes only when GDP$\cdot$AlF$_4^-$ is present (Extended Data Fig. 8d), consistent with the previous observation that the 180-kDa MFN1 complex can form higher-order oligomers when incubated with GTP but not with GTP$^\gamma$S. MFN1ΔTM with either Glu209Ala or Arg238Ala mutations failed to oligomerize (Extended Data Fig. 8e), indicating that oligomerization is dependent on the G interface.

In the apo and GMPPNP-bound states, the neck and trunk of BDLP take either a ‘closed’ or ‘open’ conformation, and the contact between the G domain and the trunk may stabilize the former. Given the structural congruence with BDLP, MFN1 may also adopt this feature. A conserved Asp189 potentially participates in the plausible G-domain–HD2 contact (Fig. 4f).

Our data highlight the role of G domain dimerization, regulated by guanine nucleotide, in membrane fusion mediated by mitofusin. GTP loading may induce a conformational change from the closed tethering-constrained state to the open tethering-permissive state, possibly involving the Asp189 trigger. In the open state, stretched MFN1 molecules allow efficient tethering of two OMMs with a distance of as far as 30 nm. During GTP hydrolysis, HD2 may fold back to the closed state to bring opposing membranes in close proximity (Extended Data Fig. 9a–c). An analogous model based on the BDLP1 structure has been proposed. This process may be reversible and controllable by local GTP concentration and MFN1 density, so that excessive tethering can be avoided.

A key issue to be resolved is the reconciliation of this model with the previous MFN1 tethering model, in which an antiparallel coiled coil is differently oriented between nucleotide-free and GTP-bound states, and in latter case its side chain swings away from the HD2 (Fig. 4g, Extended Data Fig. 8f, g). MFN1(D189A) led to mitochondrial clumping (Extended Data Fig. 8h).

Figure 4 | Catalytic machinery of MFN1. a, Comparison of the catalytic centres of MFN1 (transition-like state), dynamin-1 (PDB code 2X2E) and atlastin-1 (PDB code 4JDO). b, Binding affinities of MFN1IM(H107A) to GTP$^\gamma$S and GDP. c, GTP turnover rates of wild-type MFN1 and MFN1IM(H107A). Results from two separated experiments are presented for each protein. d, Mitochondrial elongation assay with quantification for MFN1(H107A) and related mutant MFN2(H218A). For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10 μm. e, Schematic drawing summarizing the rearrangements in the G domain during GTP hydrolysis. G domains are coloured grey. Residues and nucleotides are colour/shape-specified. Salt bridges are specified by brown dots between involved residues. P$_i$ denotes phosphate ion. f, Superposition of nucleotide-free MFN1IMB construct and BDLP (PDB code 2J69) with the predicted-HD2-facing Asp189 of MFN1 specified. g, Structural comparison of MFN1IM in different nucleotide-loading states at α2° reveals distinct orientation of Asp189 in the GTP-bound state from other states.
at the C terminus had a central role. One possibility is that MFN1 operates through sequential tethering events, an initial one using nucleotide-regulated, G domain dimerization followed by closer apposition via the coiled coil. For this sequence to happen, a large energy barrier must be overcome to detach α4H from the large hydrophobic network in HD1. It should also be noted that the G domain association of MFN1 may also occur in cis, as both forms have been proposed to have functions in BDLP and atlastin-1 (ref. 30).

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions S.G. and D.C.C. conceived the project. Y.-L.C. made the constructs, purified proteins, and performed crystallographic and biochemical experiments. S.M. carried out mitochondrial elongation assays. Y.C. performed ITC measurements and helped with collection of X-ray diffraction data. J.-X.F. and S.L. helped with collection of X-ray diffraction data. B.Y. and Y.-J.L. performed cloning and purification for some of the MFN1IM mutants. D.-D.G. performed some of the SEC-RALS experiments, D.-D.G., J.-Y.Y. and O. Daumke for comments on the manuscript. This work was supported by grants of National Basic Research Program (China) (2013CB910500), National Natural Science Foundation of China (31200553), Natural Science Foundation of Guangdong Province (2014ATQ01RS4 and 2014A03031015), New Century Excellent Talents in University (NCET-12-0567) and the Recruitment Program of Global Youth Experts to S.G., and the National Institutes of Health (GM110039 and GM119388) to D.C.C.

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment.

Protein expression and purification. cDNAs of all truncated human MFN1 constructs, including those for crystallization and indicated mutants for biochemical assays were individually cloned into a modified PET28 vector. For constructs used in co-crystallization with GTP, an extra Thr109 Ala mutation was introduced to the MFN1 construct to suppress the GTPase activity. Details of these constructs are illustrated in Extended Data Fig. 1a. For MFN1Δ25, residues 580–631 were replaced by an (SAA)₆ linker. For MFN1Δ25 and corresponding mutants, recombinant proteins containing a N-terminal His₆ tag followed by a cleavage site for PreScission protease (PSP) were expressed in Escherichia coli Rosetta (DE3) cells. Transformed bacteria were cultured at 37 °C before induced with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) at an OD₆₀₀nm of 0.6, and grown overnight at approximately 17–18 °C. Cells expressing MFN1Δ25 were lysed in 50 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂, 30 mM imidazole, 1 mM DNTase I, 1 mM phenylmethanesulfonylfluoride (PMSF) and 2 mM β-mercaptoethanol (β-ME) using a cell disruptor (INBIO) and subjected to centrifugation at 40,000g for 1 h. The supernatant was filtered and applied to a Ni-NTA (first Ni-NTA column) (GE Healthcare) equilibrated with binding buffer 1 containing 20 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂, 30 mM imidazole and 2.5 mM β-ME. After washing with binding buffer 1, proteins were eluted with elution buffer containing 20 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂, 300 mM imidazole and 2.5 mM β-ME. Eluted proteins were incubated with 20 μg glutathione S-transferase (GST)-fused PSP to remove the His₆-tag and dialysed overnight against binding buffer 2 containing 20 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂ and 2.5 mM β-ME. After dialysis, PSP was removed using a GST column. The protein was re-applied to a Ni-NTA column equilibrated with binding buffer 2. Binding buffer 1 was used to elute the proteins which were subsequently loaded onto a Superdex200 16/60 column (GE Healthcare) equilibrated with gel filtration buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM MgCl₂ and 1 mM dithiothreitol (DTT). The proteins eluted in a discrete peak corresponding to a molecular mass of approximately 50 kDa. Cell lysis and protein purification were both performed at 4 °C. The selenomethionine (SeMet) derivative of MFN1Δ25 was expressed in E. coli Rosetta (DE3) in M9 minimal media. For 11 bacteria culture, 100 mg lysine, 100 mg phenylalanine, 100 mg threonine, 50 mg isoleucine, 50 mg leucine, 50 mg valine and 60 mg SeMet were added when OD₆₀₀nm reached 0.5. The cells were then induced with 100 μM IPTG and cultured at 18 °C for 24 h. Purification protocols for the SeMet derivative were the same as those of native protein. Mutants used in RALS, ITC and GTP hydrolysis assays were all based on MFN1Δ25C, unless specified. Structure of MFN1Δ25B in the nucleotide-free state (assigned PDB code 5GO4) was used in Fig. 1a. Representative constructs based on MFN1Δ25TM each possess an extra Streptag that was inserted between the His₆-tag and PSP cleavage site. Cells expressing MFN1Δ25TM (wild type or mutants) were collected and lysed in 50 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂, 30 mM imidazole and 1% Tween-20, 1 mM DNTase I, 1 mM PMSF and 2.5 mM β-ME with a cell disruptor (INBIO). After centrifugation at 40,000g for 1 h, the supernatant was filtered and applied to a Ni-NTA column equilibrated with binding buffer 3 containing 20 mM HEPES, pH 7.5, 400 mM NaCl, 5 mM MgCl₂, 30 mM imidazole, 1% Tween-20 and 2.5 mM β-ME. Proteins were eluted with elution buffer after washing with binding buffer 1 and reapplied to a Streptactin column (GE Healthcare) equilibrated with binding buffer 2. Binding buffer 2 containing extra 2.5 mM dethiobiotin was used to elute the proteins. After the tags and PSP were removed in the same way as for MFN1Δ25, proteins were applied to gel filtration using a Superdex200 16/60 column equilibrated with gel filtration buffer for GTPase assays or buffer containing 20 mM HEPES, pH 7.2, 30 mM NaCl, 5 mM MgCl₂, 300 mM imidazole, 1% β-ME, 0.5 mM DNTase I, 0.5 mM PMSF and 30 mM imidazole and 2.5 mM β-ME. Resulting protein samples were dialysed against 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM MgCl₂ and 1 mM DTT. Resulting protein samples were dialysed against 20 mM HEPES, pH 7.2, 30 mM NaCl, 5 mM MgCl₂, and 1 mM DTT. Data were analysed with the provided OMNISEC software. All experiments were repeated at least twice and the data showed satisfying consistency.

GTP hydrolysis assay. GTP hydrolysis assays for MFN1Δ25C, MFN1Δ25TM and corresponding mutants were carried out at 37 °C in 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM MgCl₂ and 1 mM DTT as described earlier42. For measuring stimulated GTP turnover of wild-type MFN1Δ25C, MFN1Δ25C(Δ283A), protein at concentrations of 0.5, 1, 2.5, 5, 10, 20 and 40 μM were individually mixed with 2–1 μM GTP and hydrolysis rates were determined from a linear fit to the initial rate of the reaction (∼40% GTP hydrolysed). For other experiments, 20 μM protein and 1 mM GTP were used. Nucleotide binding study. The equilibrium dissociation constants for MFN1Δ25C and indicated mutants to guanine nucleotides were determined by isothermal titration calorimetry (ITC) using MicroCal ITC200 (Madison) at 25 °C. 2 mM nucleotide was titrated at 2 μL step against 60–80 μM protein in the buffer containing 20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 2.5 mM β-ME. Resulting heat changes upon each injection was integrated and the values were fitted to a standard single-site binding model using Origin7. All experiments were repeated at least twice and the data showed satisfying consistency.

Mitochondrial elongation assay. To examine the effect of point mutations, MFN1–Myc and MFN2–Myc variants were expressed in Mfn2–null MEFs from Mfn2 Δ/Δ mice. MEFs were generated in-house. The cell line is free of mycoplasma and has been authenticated by genotyping with PCR to confirm deletion of the Mfn2 gene. Point mutants in mouse Mfn1 and Mfn2 were constructed by overlapping PCR with primers encoding the point mutation. All mutations were confirmed by DNA sequencing. The types and positions of the residues mutated in this study are all consistent between human and mouse mitochondria. Mitochondrial supernatants were produced from 293T cells transfected with the retroviral vector and the packaging plasmid pCLeo. Mfn1 Δ/2 null MEFs were maintained in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37 °C and 5% CO₂. After retroviral transduction of Mfn1 Δ/2 null MEFs, puromycin selection was applied for 2 days. Cells were plated onto 8-well chambered slides for analysis. In the case of nuclease-dead and nuclease-dead mutants in mouse Mfn1 and Mfn2 were constructed by overlapping PCR with primers encoding the point mutation. All mutations were confirmed by DNA sequencing. The types and positions of the residues mutated in this study are all consistent between human and mouse mitochondria. Mitochondrial supernatants were produced from 293T cells transfected with the retroviral vector and the packaging plasmid pCLeo. Mfn1 Δ/2 null MEFs were maintained in DMEM supplemented with 10% fetal bovine serum and penicillin/streptomycin at 37 °C and 5% CO₂. After retroviral transduction of Mfn1 Δ/2 null MEFs, puromycin selection was applied for 2 days. Cells were plated onto 8-well chambered slides for analysis. In the case of Mfn1 constructs, Mfn1 Δ/2 null cells stably expressing Mfn1 WT and Mfn1 Δ283A were transfected with pCMV- ΔsRedexpressing-mito–eGFP were used. Immunostaining and western blot analysis with the 9E10 antibody against Myc was performed to confirm localization and proper expression of the mito-fusin variant. Mitochondrial morphology was scored by analysis of ΔsRed or mito-eGFP as described previously12.
Liposome tethering assay. POPC (1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine), DOPS (1,2-dioleoyl-sn-glycero-3-phospho-l-serine), DOGS-NTA-Ni²⁺ (1,2-dioleoyl-sn-glycero-3-[(N-5-amino-1-carboxypentyl)iminodiacetic acid] succinyl) (nickel salt)) and Rho-DPPE (1,2-dipalmitoyl-sn-glycero-3-phosphothanolamine-N-(lissamine rhodamine B sulfonyl)) (Avanti Polar Lipids) were mixed in a molar ratio of 78.5:15:5:1.5. Lipid film formed by evaporating chloroform under mild nitrogen stream was dehydrated in a vacuum drier before being re-suspended in buffer L (20 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM β-ME) to a final concentration of 10 mM. Liposomes were prepared as previously described30. An internal His₁₂ tag was inserted between α3β and α4δ of MFN1₂C and corresponding mutants. For each reaction, 0.5 μM protein was mixed with 1 mM prepared liposomes in buffer L for 30 min at 4 °C before 5 mM GTP or GTP-S was subsequently added. After incubation for another 40 min at 37 °C, the proteoliposomes were imaged by a fluorescent microscope. Otherwise, the proteoliposomes were incubated with 300 mM imidazole, pH 7.5, for another 20 min to release the protein and then imaged. All experiments were repeated at least twice and the data showed satisfying consistency.

Data availability. The X-ray crystallographic coordinates and structure factor files for MFN1₃₂ structures have been deposited in the Protein Data Bank (PDB) under the following accession numbers: 5GO4 (apo MFN3₃B), 5GOF (GTP-bound MFN1₃₂C(T109A)), 5GOM (transition-like state MFN1₃₂C), and 5GOE (GDP-bound MFN1₃₂C(T109A)). All other data generated or analysed during this study are included in this published article, and are available from the corresponding author upon reasonable request.

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Extended Data Figure 1 | MFN1 constructs and their biochemical properties. a, Schematic representation showing the strategy of generating human MFN1 constructs for crystallization. Indication of the labels and numbers are the same as in Fig. 1a. HR1\(^T\) and HR2\(^T\) denote truncated HR1 and HR2, respectively. We removed the transmembrane (TM) region and flanking residues from human MFN1 and inserted artificial linkers as illustrated. Three different constructs used for crystallization are named MFN1\(^{MA}\), MFN1\(^{MB}\) and MFN1\(^{MC}\), respectively (collectively termed MFN1\(^{IM}\)). b, Summary of the crystal structures. 'Initial ligands' denotes ligands added to the protein solution before crystallization, whereas 'final state' denotes the contents from the refined structure. Resolutions for the structure are specified. c, ITC results showing that MFN1\(^{IM}\) constructs have no binding affinity to GMPPNP or GMPPCP. Only the result of the MFN1\(^{IM}\)C construct is shown here as representative. d, Electron density of the guanine nucleotides in corresponding structures. The electron density maps are all shown at a contour level of 1.2\(\sigma\). The residues involved in ligand coordination are shown as ball-and-stick models. e,Details of the MFN1\(^{IM}\) active site in the GTP-bound state. Key hydrogen bonds for coordinating the GTP were indicated by dotted lines. In the bottom panel, details of the Mg\(^{2+}\) coordination is depicted. The electron density for Mg\(^{2+}\) ion, water and GTP was shown as grey mesh at a contour level of 1.2\(\sigma\). f, GTP turnover rates of wild-type MFN1\(^{IM}\) and MFN1\(^{IM}\)(T109A). MFN1\(^{IM}\)(T109A) shows greatly impaired GTPase activity that facilitates the co-crystallization with GTP. Results from two separated experiments are presented for each protein. g, ITC results showing that MFN1\(^{IM}\)(T109A) binds both GTP and GDP.
Extended Data Figure 2 | Overall structure of MFN1IM. a, The topology diagrams of the G domains of Ras, MFN1 and BDLP. Secondary structural elements were not drawn to scale and positions of G1–G4 motifs are indicated. Elements of MFN1 are named and coloured as in Fig. 1c. For BDLP, elements extra than Ras in light blue. The helices of BDLP are named as in ref. 21. b, Helical wheel diagrams of HD1. Hydrophobic residues are coloured yellow and other residues are coloured with the corresponding helices as in Fig. 1d. The plots are arranged according to the positions of the four helices of HD1 in the crystal structure, showing a massive hydrophobic core of HD1. c, Intramolecular association of MFN1IM. For the G-domain–HD1 interaction, Leu8, Met76, Val333 and Phe337 embrace Phe11, whereas Lys15 forms a salt bridge with Asp173 and a hydrogen bond with the main chain oxygen of Arg74. The MFN1(L705P) mutant was previously found to be non-functional in mediating mitochondrial fusion13. Leu705 is surrounded by several hydrophobic residues including Ile45, Ile48, Ala362 and Ile708, as well as a salt bridge formed by Arg365 and Glu701. The proline mutation of Leu705 may disrupt α4H and the local hydrophobic interactions, thereby impeding the folding of the protein. d, GTP turnover rates of wild-type MFN1IM and MFN1IM(K15A) and MFN1IM(L705P). Results from two separated experiments are presented for each protein. e, Mitochondria elongation assays of wild-type MFN1 and MFN1(K15A). The Myc-tagged MFN1 constructs were assayed for mitochondrial elongation activity by expression in Mfn1/2-null MEFs, which have completely fragmented mitochondria. Overexpression of wild-type MFN1 in Mfn1/2-null MEFs induces the formation of mostly tubular mitochondria, indicating normal elongation activity, whereas MFN1(K15A) induces substantially less mitochondrial tubulation. Green fluorescence is from immunostaining against the Myc epitope; red fluorescence is from mito-DsRed. The data are quantified on the right. For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10 μm.
Extended Data Figure 3 | Sequence alignment of mitofusins and BDLP. Sequence alignment of mitofusins and BDLP. Amino acid sequences of human (hs) MFN1 (UniProt accession Q8WA4) and MFN2 (Q95140), mouse (mm) MFN1 (Q8114U) and MFN2 (Q806U3), fruitfly (Drosophila melanogaster, dm) Marf (Q7YU24), fruitfly Fzo (O18412) and BDLP from Drosophila (BDLP) were aligned using Clustal W. Residues with a conservation of 100% are in red shades, greater than 80% in green shades.
Extended Data Figure 4 | Structural comparison of MFN1 IM with other dynamin family members. Structural comparison of nucleotide-free MFN1 IMB with nucleotide-free BDLP (PDB code 2J69)\(^{21}\), GDP-bound atlastin-1 (3Q5D)\(^{19}\), nucleotide-free GBP1 (1DG3)\(^{45}\), nucleotide-free dynamin-1 (3SNH)\(^{17}\), nucleotide-free DNM1L (4BEJ)\(^{46}\), nucleotide-free MxA (3SZR)\(^{16}\), and AMPPNP-bound EHD2 (2QPT)\(^{15}\). For these molecules, the region N-terminal to the G domain is in red, the G domain itself in orange, the conventional middle domain is in green, the conventional GTPase effector domain (GED) in marine, the paddle region of BDLP and the pleckstrin homology (PH) domain of dynamin-1 is in cyan, and the Eps15 homology (EH) domain of EHD2 is in magenta. The hinges between the G domains and middle domains are depicted by grey spheres. Nucleotides are shown as ball-and-stick models.
Extended Data Figure 5 | Structural comparison of MFN1IM with BDLP and dynamin-1. a, Structural comparison of the G domains between MFN1IM and BDLP (left) or dynamin-1 (right) in the nucleotide-free state. The MFN1 G domain (coloured as in Fig. 1b) is separately superimposed with G domains of BDLP (PDB code 2J69, light blue) and rat dynamin-1 (2AKA, wheat). The root mean standard deviation (r.m.s.d.) values of aligned Cα atoms are shown. α-helices on the two lobes are labelled. The G domain of MFN1IM resembles the BDLP G domain, except that at lobe 1, dynamin-1 is similar to MFN1IM in lobe 1, but at lobe 2 the αC tilts 60° from its counterpart α2'G in MFN1IM.
b, Structural comparison of MFN1IM in different nucleotide-loading states. Structures of nucleotide-free MFN1IM, GTP-bound MFN1IMC(T109A), transition-like state MFN1IMC(T109A), and GDP-bound MFN1IMC(T109A) are colour-specified and superimposed on their G domains. c, Architectures of MFN1IM-A•GTP and MFN1IM-A•GDP. Shown are the corresponding Cα traces and electron density maps (contoured at 2.8σ), by which molecule outlines are clearly discernible. These two structures are presented to exclude possible influence of the Thr109Ala mutation in the GTP- and GDP-bound structures shown in b. d, GTP turnover rates of wild-type MFN1IM and the hinge mutants. Results from two experiments are shown for each protein. e, Mitochondrial elongation assay for wild-type MFN1 and the hinge mutants. For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10μm. f, Full-length MFN1 models showing the plausible hinge 1 between HD1 and HD2. Models were based on nucleotide-free (PDB code 2J69, top) and GMPPNP-bound (2W6D, bottom) BDLP. G domain and HD1 are coloured as in Fig. 1b; HD2 is in light blue. Hinge 1 is shown as dashed lines. Yellow triangles indicate approximate position for the Pro695 insert. g, Extra support of the guanine base in MFN1 (GDP-bound MFN1IMC(T109A), coloured as in Fig. 1c), BDLP (PDB code 2J68, light blue) and dynamin-1 (5D3Q, wheat). The α-helices that support the guanine base are specified. Parts of the G domains are removed for clarity. Note the similarity between MFN1IM and BDLP, as well as the difference between MFN1IM and dynamin-1 in nucleotide coordination.
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Dimerization of MFN1IM G domains in the transition-like state. a, Oligomerization states of MFN1IM in different nucleotide-loading conditions by RALS. MFN1IM is monomeric in nucleotide-free, GTPγS-bound and GDP-bound states, and forms dimers in the presence of GDP•AlF₄⁻. Data are as in Fig. 3d. b, Liposome tethering assay for wild-type MFN1IM and corresponding mutants. Representative images from five separate experiments are shown. Wild-type MFN1IM tethered liposomes carrying fluorescence in the presence of GTP hydrolysis-dependent manner as large aggregated liposomes were observed (first left). In GTPγS-present condition the liposome aggregation was largely attenuated, suggesting that tethering is dependent on GTP hydrolysis (second left). When proteins were washed off the liposome by imidazole, the liposomes became homogeneously scattered (middle), indicating that the liposomes were tethered but did not merger. MFN1IM(E209A) and MFN1IM(R238A) displayed suppressed tethering activity (right two). Scale bars, 50μM. c, Dimerization test of the G interface mutants in the presence of GDP•AlF₄⁻. d, GTP turnover rates of the G interface mutants compared with wild-type MFN1IM. Results from two separated experiments are presented for each protein. e, Mitochondrial elongation assay for MFN1(E245A) and related MFN2(E266A). For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10μm. Both mutants lost fusogenic activity. f, Rearrangement of residues in the G interface upon nucleotide binding. Structures shown from left to right are: nucleotide-free MFN1IMB; GTP-bound MFN1IMC(T109A); transition-like state MFN1IMC; and GDP-bound MFN1IMC(T109A). Key residues involved in the structural rearrangement of the G interface are shown as ball-and-stick models. Yellow surface representation is used for GTP and GDP.
Extended Data Figure 7 | Analysis of the switch I conformations.

**a**, Configuration of switch I of MFN1IM in nucleotide-free and the transition-like states (molecule A of the dimer is used). Switch I is coloured yellow. Residues involved in the hydrophobic networks are shown as ball-and-stick models. Note the rearrangements of this region between the two states. **b**, Stability of switch I region of MFN1IM at different states. The stability of switch I is reflected by the mean B factor of the main-chain atoms of switch I compared to that of the whole peptide chain. TransA and TransB denote molecules A and B of the MFN1IM dimer in the transition-like state, respectively. The switch I regions in both nucleotide-free and transition-like (TransA) states have relatively stable conformations with regard to the whole molecule. **c**, Superposition of the GTPase catalysis centres of two molecules of the MFN1IM dimer in the transition-like state. The G1–G4 elements are as in Fig. 2b, except that the G2 element of the molecule B in pale green. His107 and is shown as ball-and-stick models. **d**, The electron density of the switch I regions in the two molecules of the MFN1IM dimer. The density is shown as blue mesh at a contour level of 1.2σ for both molecules A (left) and B (right). His107 is shown as ball-and-stick models.
Extended Data Figure 8 | Characterization of MFN1ΔTM and the Asp189 trigger. 
a, Schematic representation showing the strategy of generating the MFN1ΔTM construct. Colour as in Fig. 1a, and HD2 is in purple. 
b, Comparison of GTPase activity between MFN1IM and MFN1ΔTM. Results from two separated experiments are presented for each protein. 
c, RALS analysis of MFN1ΔTM showing that it is a stable dimer in nucleotide-free state. 
d, Analytical gel filtration results of MFN1ΔTM in the GTPγS, GDP•AlF₄⁻ and GDP-bound states. 
e, Analytical gel filtration results of MFN1ΔTM(E209A) and MFN1ΔTM(R238A) in nucleotide-free and GDP•AlF₄⁻-bound states. Note that in the GDP•AlF₄⁻-bound state, no peak at the exclusion volume is observed, indicating that both mutants do not oligomerize. 
f, Structural comparison of MFN1IM in different nucleotide-loading states at α2G. Note the distinct orientation of Asp189 in the GTP-bound state, and the uniformly oriented Asp193. Asp193 is a conserved residue that also faces the predicted HD2. Colour as in Fig. 4g. 
g, Electron density of Asp189 and Asp193 on α2G in MFN1IM structures contoured at 1.0σ. Note the difference in orientations of α2G in these structures as revealed by the density maps. Although the side chain of Asp189 is not fully traceable in some non-GTP-bound cases, their locations would differ from that in the GTP-bound form. 
h, Mitochondrial elongation assay for the mutants in the plausible G-domain–HD2 contact. For each construct, 100 cells were scored in biological triplicate; representative images are shown. Error bars indicate s.e.m. Scale bars, 10 μm. Note that the clumping mitochondria for MFN1(D189A) and anticipated normal mitochondria for MFN1(D193A). Arg455, Arg460, Gln473 and Arg594 are conserved residues in the predicted HD2 which were screened for contacting Asp189 based on sequence alignment of mitofusins and BDLP. Corresponding mutants increased mitochondrial fragmentation or aggregation. It seems that either they are not the right residues interacting with D189, or a single point mutation was not sufficient to break the plausible interaction.
Extended Data Figure 9 | Proposed model for MFN1-mediated OMM fusion. a, Model for nucleotide-regulated OMM fusion mediated by MFN1. The G domain, HD1, predicted HD2 and transmembrane domain are indicated in the top left MFN1 molecule, and coloured orange, green, grey and blue, respectively. During GTP hydrolysis, HD2s of tethered MFN1 molecules may fold back via intrinsic mechanistic potential analogous to the BSE-stalk of MxA protein (Y.C. et al., unpublished observations) to bring opposing membrane in close proximity. Repeating tethering reactions by appropriate numbers of MFN1 would promote docking of opposing OMMs, presumably as described in a recent in vitro electron cryo-tomography study where discrete electron densities representing yeast FZO1 displayed a ring-like arrangement surrounding docked OMMs. If this ‘docking ring’ exists in mammals, MFN1 may contribute to its formation through hydrolysis-dependent in trans oligomerization (shown in c). Subsequent membrane merger may rely on local membrane curvature, as reported in many cellular events such as synaptic vesicle fusion and cell-to-cell fusion. As the space between docked OMMs (approximately 2 nm) is too small to accommodate MFN1 molecules, these molecules may gather at the rim of the docking site, resulting in a crowding effect that possibly generates bending on local OMMs to facilitate fusion. b, Schematic drawing shows the GTP-loading-induced conformational rearrangement of the MFN1 HD1–HD2 region via the Asp189 trigger. c, Possible organization of the plausible in trans cross oligomer of MFN1 around the docking site. This process is dependent on GTP hydrolysis.
Extended Data Table 1 | Crystallographic data collection and refinement statistics

|                  | MN1αB | MN1αB | MN1αC<sup>THPA</sup> | MN1αC | MN1αC<sup>THPA</sup> | MN1αA | MN1αA |
|------------------|-------|-------|-----------------------|-------|-----------------------|-------|-------|
| Initial ligand   | GMPCPP | GMPCPP | GTP                   | GDP-AF<sub>1</sub> | GDP-AF<sub>1</sub> | GTP   | GDP   |
| State            | apo    | apo    | GTP-bound             | Transition-like | GDP-bound             | GTPyG | GDPyG-bound |
|                  | P2<sub>1</sub>,21 | P2<sub>1</sub>,21 | C2                   | P2<sub>1</sub>,21 | C222<sub>2</sub>   | P2<sub>1</sub>,21 | P2<sub>1</sub>,21 |
| **Data collection** |       |       |                       |                   |                       |       |       |
| Data Set         | Native | SeMet derivative | Native             | Native | Native                | Native | Native |
| Source group     | P2<sub>1</sub>,21 | P2<sub>1</sub>,21 | C2                   | P2<sub>1</sub>,21 | C222<sub>2</sub>   | P2<sub>1</sub>,21 | P2<sub>1</sub>,21 |
| Cell dimensions  |       |       |                       |                   |                       |       |       |
| a, b, c (Å)      | 51.8, 110.9, 112.4 | 51.6, 110.2, 111.3 | 70.6, 72.4, 95.3 | 104.1, 45.0, 146.2 | 70.4, 72.9, 95.0 | 127.6, 143.1, 159.0 | 93.6, 93.6, 114.1 |
| α, β, γ (°)      | 90     | 90     | 90                   | 92.2   | 90                    | 90    | 120   |
| Wavelength (Å)   | 0.91800 | 0.97915 | 0.97515              | 0.91800 | 0.97915              | 0.91800 | 0.91800 |
| Resolution (Å)   | 47.0-2.2 (6.55-2.2) | 49.7-2.3 (6.94-2.3) | 44.7-1.6 (4.78-1.6) | 48.7-2.8 (8.39-2.8) | 44.7-1.8 (5.37-1.8) | 47.7-6.1 (16.77-6.1) | 46.8-4.3 (12.48-4.3) |
| R<sub>free</sub>* | 0.068 (0.438) | 0.090 (0.494) | 0.043 (0.583) | 0.095 (0.629) | 0.048 (0.517) | 0.0470 (0.522) | 0.0564 (0.537) |
| I / σ(I)         | 18.08 (4.27) | 21.22 (4.14) | 25.54 (3.33) | 20.55 (3.02) | 25.08 (4.12) | 19.28 (3.32) | 17.81 (4.62) |
| Completeness (%) | 99.4 (98.1) | 99.5 (97.7) | 89.4 (99.7) | 99.1 (97.6) | 98.9 (98.8) | 98.3 (98.8) | 97.2 (96.0) |
| Redundancy       | 7.9 (8.0) | 7.7 (7.5) | 7.1 (7.2) | 7.4 (7.4) | 7.9 (8.1) | 5.4 (5.4) | 10.4 (10.8) |

**Refinement**

|                  | MN1αB | MN1αB | MN1αC<sup>THPA</sup> | MN1αC | MN1αC<sup>THPA</sup> | MN1αA | MN1αA |
|------------------|-------|-------|-----------------------|-------|-----------------------|-------|-------|
| Resolution (Å)   | 39.5-2.2 | 29.1-1.6 | 48.7-2.8 | 29.0-1.8 |
| No. reflections  | 33374 | 57847 | 24264 | 45448 |
| R<sub>merge</sub> / R<sub>free</sub> | 0.176/0.214 | 0.179/0.215 | 0.201/0.258 | 0.177/0.204 |
| No. atoms        | Protein | 3291 | 3224 | 6484 | 3137 |
|                  | Ligand/ligand | 34 | 64 | 28 |
|                  | Water   | 184 | 427 | 4 | 273 |
| B-factors        | Protein | 57.5 | 33.6 | 106.9 | 43.4 |
|                  | Ligand/ligand | 30.8 | 84.0 | 36.3 |
|                  | Water   | 55.3 | 43.2 | 68.9 | 48.3 |
| R.m.s. deviations | Bond lengths (Å) | 0.007 | 0.005 | 0.012 | 0.006 |
|                  | Bond angles (°) | 0.753 | 0.812 | 1.471 | 0.763 |

*Numbers in parentheses represent values from the highest resolution shell.