Nucleotide-free MalK Drives the Transition of the Maltose Transporter to the Inward-facing Conformation*

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Background: ATP promotes closure of MalK2 and opening of MalFG toward the periplasm.

Results: Without MalK, MalFG exists in an intermediate conformation. Binding of apo-MalK reverses the conformation of MalFG toward the cytosol.

Conclusion: The NBDs exploit energy from ATP binding but also ATP hydrolysis to control the conformation of the transporter.

Significance: Tightly bound MalK blocks opening of MalFG to the periplasm.

The complex MalFGK2 hydrolyzes ATP and alternates between inward- and outward-facing conformations during maltose transport. It has been shown that ATP promotes closure of MalK2 and opening of MalFG toward the periplasm. Yet, why the transporter rests in a conformation facing the cytosol in the absence of nucleotide and how it returns to this state after hydrolysis of ATP is unknown. The membrane domain MalFG may be naturally stable in the inward-facing conformation, or the ABC domain may catalyze the transition. We address this question by analyzing the conformation of MalFG in nanodiscs and in proteoliposomes. We find that MalFG alone exists in an intermediate state until MalK binds and converts the membrane domain to the inward-facing state. We also find that MalK, if overly-bound to MalFG, blocks the transition of the transporter, whereas suppressor mutations that weaken this association restore transport. MalK therefore exploits hydrolysis of ATP to reverse the conformation of MalFG to the inward-facing conformation, a step essential for release of maltose in the cytosol.

ATP-binding cassette (ABC)2 transporters form a conserved family of membrane proteins involved in the transport of various substrates across diverse membranes (1–3). Their core structure is two transmembrane domains (TMDs) that create a translocation pathway, and two nucleotide binding domains (NBDs) that associate and dissociate upon binding and hydrolysis of ATP (2). Depending on the organism, the nature of the substrate and the direction of transport, NBDs and TMDs can be either fused or expressed as separate polypeptides. In all cases, the communication between NBDs and TMDs occurs through functionally conserved couplings helices located at the domain interface.

The maltose transporter, a prototypical type I ABC importer, has been crystallized in two distinct conformations: inward-facing (facing the cytosol) in the absence of nucleotides and outward-facing (facing the periplasm) with non-hydrolysable ATP analogs (4, 5). Biochemical analyses such as cross-linking, fluorescence, and EPR spectroscopy have confirmed that ATP binding induces dimerization (closure) of MalK and orientation of MalFG toward the periplasm (6–9). Yet, how the conformation of MalFG reverts to the inward-facing state after hydrolysis of ATP remains unknown. Similarly, why the transporter rests in an inward-facing conformation in the absence of nucleotide is unclear. At least two models are possible: the TMDs maintain the NBDs in a separated state, or the NBDs maintain the TMDs in an inward-facing state.

In the first model, MalFG may be naturally stable in the conformation facing the cytosol, due for example to the constraints imposed by phospholipids on the α-helical transmembrane segments. This resting conformation would prevent the closure of MalK, keeping the ATPase activity of the transporter to its basal level. In support of the first model, crystal structure and EPR data indicate that the maltose-binding protein MalE converts MalFG into an intermediate conformation, closer to the transition state and termed pretranslocation state (8, 10, 11). The intrinsic stability of MalFG would largely contribute to the return of the transporter toward the cytosol once ATP is hydrolyzed. In the second model, nucleotide-free MalK may be important for the stabilization of MalFG in the inward-facing conformation. Upon dimerization of MalK with ATP, MalFG would face the periplasm, a conformation that possesses high-affinity for open-state MalE (9). Upon hydrolysis of ATP, the MalK dimer would dissociate (open) and facilitate the reversion of MalFG toward the cytosol.

In this study, we address the two possible mechanisms by monitoring the effect of MalK on the conformation of isolated MalFG in nanodiscs and membrane bilayers. We also address how the binding of MalK2 to MalFG membrane domain controls the activity of the transporter. Together, the results reveal the inherent ability of MalFG to reach an outward-facing con-
formation and the critical role of MalK$_2$ for the reversal of MalFG toward the cytosol.

**EXPERIMENTAL PROCEDURES**

Reagents—Escherichia coli total lipids were purchased from Avanti Polar Lipids. N-Dodecyl-$\beta$-D-maltoside (DDM) and octyl-$\beta$-D-glucopyranoside were obtained from Anatrace. Biobeads were purchased from Bio-Rad. nitritotriacetic acid (Ni$_2^+$-NTA)-chelating Sepharose, Superdex 200, and Sephadex G-25 were obtained from GE Healthcare. [$^3$H]maltose (50 $\mu$Ci/μmol) was from American Radiolabeled Chemicals. All other chemicals, including bis-maleimidoethane (BMOE) were from Sigma.

**Protein Purification**—Purification of MalE and MalFGK$_2$ was performed as described previously (12). For purification of MalFG, genes of MalF, MalG, and MalK were inserted into the pTrc99A plasmid (Pharmacia). A His$_6$ tag was placed at the N terminus of MalF and a 23-amino acid histidine tag extension was placed at the N terminus of MalK (yielding pTrc$^{his}_{6}$FG$^{his}_6$K) (13). This plasmid was transformed into E. coli strain BL21. Transformants were grown at $37^\circ$C in 12 liters of LB medium, and protein expression was induced at $A_{600}$ $\approx$ 0.5 with 0.5 mM isopropyl 1-thio-$\beta$-D-galactopyranoside for 3 h. Cells were collected in TSG buffer (50 mM Tris-HCl, pH 8; 50 mM NaCl; 10% glycerol) containing 0.01% PMSF, and lysed through a French Press (8,000 psi, twice). After low speed centrifugation (100,000 g, 10 min, 4 °C), membrane fraction was isolated by ultracentrifugation (100,000 $\times$ g, 4 °C). After removal of Bio-Beads by sedimentation, nanodiscs were separated by size-exclusion chromatography on a Superdex 200 GL 10/300 equilibrated in TSG buffer. The quality of the reconstitution was tested by native gel electrophoresis. Proteoliposomes were prepared using a detergent dilution method (14). Briefly, MalFGK$_2$ (5 μM), with or without MalK (50 μM), were incubated on ice for 30 min in 500 μl of reconstitution buffer (50 mM Tris-HCl, pH 8.0, 1 mM MgCl$_2$) containing 1% octyl-$\beta$-D-glucopyranoside and E. coli total lipids (50 mM). Proteoliposomes were formed by rapid dilution into 20 ml of reconstitution buffer and collected by ultracentrifugation (100,000 $\times$ g, 4 °C). Proteoliposomes were resuspended in 20 mM Tris-HCl (pH 8) and frozen in liquid nitrogen. Proteoliposomes were extruded through a 400 nm polycarbonate filter before use.

**Cross-linking Experiments**—The cysteine mutants MalF$^{396C-}_{\text{His}}$, MalG$^{B185C}$, and MalF$^{446C-}_{\text{His}}$-MalG$^{234C}$ were generated as described previously (9). Cross-linking experiments were performed in 50 mM Tris-HCl, pH 8.0, 10 mM MgCl$_2$ containing 2 μM proteoliposomes. The samples were incubated with 50 μM BMOE (10 min at room temperature) and then treated with N-ethylmaleimide (5 mM) before analyses by SDS-PAGE.

**Maltose Transport**—For in vivo assays, strain ED169 (ΔmalB$_{I07}$), carrying pACYC-MalE, a pACYC184-derivative (New England Biolabs) expressing MalE, was transformed with pTrc-FGK, or pTrc$^{his}_{6}$FG$^{his}_6$K, or pTrc$^{his}_{6}$FGK. Transformants were plated on MacConkey agar supplemented with 0.4% maltose, 100 μg/ml ampicillin. Incubation was at 37 °C for 12 h (15). For in vitro assays, proteoliposomes (1 μM prepared from reconstitution buffer plus 5 mM ATP) were incubated at room temperature with MalE (10 μM) and [$^3$H]maltose (20 μM, 50 μCi/μmol). At the indicated time, samples (50 μl) were diluted into 1 ml of ice-cold reconstitution buffer plus maltose (10 mM) followed by filtration through a 0.22-μm nitrocellulose Millipore filter. The filter was washed by 3 ml of reconstitution buffer. Radioactivity was measured using a Perkin Elmer scintillation counter. Cpm were converted to nmol of maltose using a calibration curve made with known amounts of [$^3$H]maltose.

**Selection and Generation of MalK Variants**—The gene malK was subjected to error-prone PCR using Taq polymerase in a reaction mixture containing MgCl$_2$ (3.5 mM), MnCl$_2$ (0.5 mM), dATP (0.2 mM), dGTP (0.2 mM), dCTP (1 mM), and dTTP (1 mM). The amplified DNA fragment was replaced into pTrc$^{his}_{6}$FGK using restriction sites (XbaI and HindIII) and transformed into strain ED169 carrying pACYC-MalE. Transformants were plated on MacConkey agar supplemented with 0.4% maltose, 100 μM isopropyl 1-thio-$\beta$-D-galactopyranoside, 100 μg/ml ampicillin. Incubation was at 37 °C for 12 h.
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Urea Resistance of MalFGK₂ Bearing MalK Suppressor Mutations—Inner membrane vesicles (5 mg/ml, 200 μl) were treated with 6 M urea (room temperature, 30 min). Membranes were reisolated by ultra-centrifugation, resuspended in TSG buffer, and solubilized with 1% DDM (4 °C, 1 h). Insoluble debris were removed by ultracentrifugation, and the supernatant was subjected to pulldown assay, using Ni²⁺-NTA-Sepharose beads in TSG buffer containing 0.01% DDM and 5 mM MgCl₂. Proteins were eluted in 100 μl of TSG buffer containing 0.01% DDM and 500 mM imidazole. Samples were analyzed by blue-native gel electrophoresis.

Other Methods—The MalFGK₂ ATPase activity was determined by monitoring the release of inorganic phosphate using the malachite green method. Inner membrane vesicle preparations, protein iodination, and linear gradient blue-native and clear-native gel electrophoresis were performed as described (16). The binding of MalE to MalFG and MalFGK₂ was determined by fluorescence quenching assay (12). Fluorescence measurements were carried out at 25 °C using a Cary Eclipse spectrofluorometer.

RESULTS

Purification of the MalFG Membrane Domain—MalFGK₂ forms a tight complex in the membrane and, as is often the case for such membrane assemblies, it is difficult to separate the subunits or to express them individually. However, it was reported that MalK modified with a N-terminal 23-amino acid histidine tag extension (termed WT MalK) can dissociate from the complex with urea treatment (13). We therefore expressed WT MalK together with N-terminal His₆-tagged MalFG (hereafter termed WT MalFG). This transporter (WT MalFG-WT MalK) supports maltose transport and ATPase activities with kinetics similar to the wild type, both in vivo and in vitro (Fig. 1a and see Figs. 4a and 5b below). Thus, after treatment of the membrane with 4 M urea, MalFG was purified by Ni²⁺ affinity and gel filtration chromatography. The apparent molecular weight of MalFG is ~95 kDa in detergent solution, and this complex is devoid of MalK (Fig. 1b).

Without MalK, MalFG Orients Itself toward the Periplasm—We measured the conformation of MalFG in the membrane bilayer using engineered cysteine residues and the homobifunctional cross-linker BMOE (Fig. 2). The cysteine pairs MalF₃⁹₆C-MalG₁₈₅C and MalF₄₄₆C-MalG₂₃₄C served to monitor the conformation of the transporter near the cytosolic and the periplasmic sides of the membrane, respectively (Fig. 2a) (9). In agreement with the alternating access model, a decrease in cross-linking efficiency with one pair was mirrored by an increase with the other pair (9). This analysis confirmed that the MalFG-MalK complex is oriented toward the cytosol in the absence of ATP (lane 2, Fig. 2, b and c) and toward the periplasm upon addition of ATP (lane 3; Fig. 2, b and c). Strikingly, when MalK was removed from the membrane, a large fraction of MalFG (~40–70%) was already facing the periplasm (lane 5; Fig. 2, b and c). The cross-link efficiency was not modified with ATP as expected (lane 6). Thus, without MalK, MalFG is able to reach an outward-facing conformation. Apo-MalK therefore seems necessary to keep MalFG oriented toward the cytosol.

Apo-MalK Decreases the Affinity of MalE to the Transporter—We recently showed that inward-facing MalFGK₂ has a very low affinity for MalE (Kₐ > 50 μM). In contrast, MalFGK₂ oriented toward the periplasm has ~1,000 stronger affinity (Kₐ ~ 79 nM). These Kₐ values were obtained using a fluorescence quenching assay with MalFGK₂ reconstituted in nanodiscs (12). Here, using the same assay, the equilibrium affinity of MalE for MalFG devoid of MalK was found to be 1.3 ± 0.3 μM (Fig. 3, a and b). This result is consistent with the observation that MalE on its own rests in an intermediate conformation (Fig. 2). These results were confirmed by native gel electrophoresis (Fig. 3c). MalE did not form a stable complex with MalFGK₂, unless the transporter had been converted to the outward-facing conformation with AMPPNP (Fig. 3c, left panel). However, when MalK was absent, MalE was already able to form a stable complex with MalFG (Fig. 3c, right panel). We also tested whether binding depends on the conformation of MalE, as reported for MalFGK₂ (12). The MalE variant stabilized in the open state (MalE-254) formed a tight complex with MalFG (Fig. 3c). In contrast, the MalE variant stabilized in the closed state (MalE-DW) was unable to associate with MalFG (Fig. 3c). Thus, without MalK, MalFG acquires an outward-facing conformation with a high affinity for open-state MalE. Apo-MalK is therefore necessary for the conversion of MalFG to the inward-facing state, a state that has low affinity for MalE.

Apo-MalK Converts MalFG to the Inward-facing State—We tested whether apo-MalK can revert the conformation of MalFG to the inward-facing state. We showed first that the reassembled complex recovers close to wild-type maltose transport activity (Fig. 4a). We then monitored the conforma-
tion of MalFG in the membrane using the cysteine pairs MalF<sub>396C</sub>-MalG<sub>185C</sub> and MalF<sub>446C</sub>-MalG<sub>234C</sub> (Fig. 4b). Compared with MalFG alone, a significant fraction of the reassembled complex (>50%) reverted to the inward-facing conformation, as judged by the decreased cross-link efficiency for the pair MalF<sub>396C</sub>-MalG<sub>185C</sub> (compare lanes 2 with 4, upper panel), and the corresponding increase in yield for the pair MalF<sub>446C</sub>-MalG<sub>234C</sub> (compare lanes 2 with 4, lower panel). As expected, the reassembled complex regained sensitivity to nucleotides; it faced the periplasm in the presence of ATP (lane 5). These results were confirmed using the reassembled complex in nanodiscs (Fig. 4c). The presence of MalK was sufficient to abolish the binding of MalE to Nd-MalFG (Fig. 1a and b). Surprisingly, we observed that WT-MalK is perhaps overly bound to HT-MalFG and thereby inhibits the conformational freedom and the transition of the transporter to the outward-facing state. We assessed the interaction of WT-MalK with HT-MalFG with urea. In the detergent micelles, WT-MalFG-WT-MalK was resistant to dissociation compared with HT-MalFG-HT-MalK (Fig. 6a). Thus, the increased association of WT-MalK with HT-MalFG seems to hinder the transition of the transporter to the outward-facing state, which in turn, explains the reduced ability of the complex to hydrolyze ATP.

**Mutations That Destabilize MalK Binding to HT-MalFG Restore Transport**—To corroborate the hypothesis above, we searched for suppressor mutations. We reasoned that suppressors would weaken the interaction of WT-MalK with HT-MalFG and therefore restore the capacity of ATP to convert the transporter to the outward-facing conformation. The gene *malK* was
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![Diagram](https://example.com/diagram.png)

**FIGURE 3. MalK decreases the affinity of MalE to MalFG.**

(a) time courses of MalE binding to MalFG. The experiment was performed using ATTO-655-labeled MalE as described previously (12). b, binding affinity of MalE to MalFG. ATTO-655-labeled MalE (20 nM) was titrated with MalFG and MalFGK2 nanodiscs (Nd-FG and Nd-FGK2), with or without AMP-PNP (1 mM) and maltose (1 mM). The quenching data were fit to a single site binding equation, giving the dissociation constant of 1.3 \( \pm \) 0.3 \( \mu \)M for the MalE-MalFG complex. c, binding of MalE variants to MalFG. The quenching data were fit to a single site binding equation, giving the dissociation constant of 1.3 \( \pm \) 0.3 \( \mu \)M for the MalE-MalFG complex.

mutagenized, co-transformed with \( \mu t^T \) malF and, cells were plated on McConkey-maltose plates (Fig. 6b). Five mutants that formed bright red colonies were characterized. Mutants \( \mu t^T \) malK\(_{259} \), \( \mu t^T \) malK\(_{290} \), and \( \mu t^T \) malK\(_{295} \) produced complexes sufficiently stable for subsequent biochemical analysis. In agreement with the prediction, the protein MalK\(_{259} \) carries the L157P/A250S/M281V mutations and the protein MalK\(_{290} \) carries the P259R/L290I mutations. These mutations are located in the C-terminal region and Walker B motif of MalK, which is close to the EAA motif in MalF.

**DISCUSSION**

Why the maltose transporter is naturally stable in the inward-facing state and how it reverses from the outward-facing conformation after hydrolysis of ATP are important unresolved questions. These steps are critical to keep the ATPase activity to ground levels and to facilitate maltose release after hydrolysis of the nucleotide. The results presented in this study reveal that MalFG on its own exists in an intermediate conformation, neither fully inward nor outward. The addition of apo-MalK and ATP-bound MalK are sufficient to shift the MalFG equilibrium to the inward- or outward-facing conformation, respectively. This result is important as it demonstrates that the NBDs exploit the energy from ATP binding but also from ATP hydrolysis to alternatively stabilize the transporter in either conformation. In addition, because apo-MalK stabilizes MalFG in the inward-facing state, the results argue against a model in which MalFG is restraining MalK\(_{2} \) in an open dissociated state (8). Instead, we find that MalFG alone rests in a conformation closer to the transition state, supporting the notion that ATP binding to MalK provides enough energy to drive the conversion of MalFG toward the periplasmic-facing conformation (9). The observation that MalFG on its own rests near to the transition state may also explain why single mutations in MalF can increase thousand fold the basal ATPase activity of the transporter (17).

How the molecular motions at the NBDs are transmitted to the TMDs remains a key unresolved question. The crystal structures show that the transmission interface consists of a conserved coupling helix (EAA motif) from the TMDs docked onto a groove on the NBD helical subdomain, resembling a ball-and-socket joint (4, 5). Molecular dynamic simulations suggest that MalK contributes directly to the structural integrity of the coupling helices (18). It is thus reasonable to propose that any change in this interface will modify the rotation and therefore the movement of the TMDs. More specifically, we observe that the strength or avidity of the MalK-MalFG interface, as monitored by urea resistance and stability in detergent solution, bears direct consequence on the transporter activity. For instance, a mutant (\( \mu t^T \) MalFG\(_{\mu t T} \) MalK) in which MalK is tightly bound to MalFG is unable to hydrolyze ATP and unable to reach the outward-facing conformation. The tight association of MalK to MalFG apparently prevents the action of ATP. Accordingly, the rescue of the mutant is possible with secondary mutations in MalK that decrease its association to MalFG. Not surprisingly, these mutations are located near the helical subdomain of MalK, and earlier genetic studies have shown that mutations in the coupling helices on MalFG lead to dissociation of MalK from the membrane (15). Interestingly, these

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FIGURE 4. MalK reverses the conformation of MalFG to the inward facing state. a, maltose transport activities of MalFGK and reassembled MalFGK. Maltose transport was determined in the presence of MalE (10 μM) and [3H]maltose (50 μCi/μmol, 20 μM). Each data point is the mean of three independent experiments, with S.D. shown as error bars. b, effect of MalK on the conformation of MalFG was analyzed by cross-link experiments. Proteoliposomes (2 μM) containing MalFG and reassembled MalFGK, with the indicated cysteine pairs were treated with BMOE (50 μM; 10 min at room temperature). Free cysteines were modified with N-ethylmaleimide (5 mM) prior to protein analysis by SDS-PAGE (4–12%) and immunoblot against MalF. c, binding of MalK on MalFG nanodiscs was analyzed by native gel electrophoresis. Nd-MalFG (1 μM) was incubated with purified MalK (0–5 μM) for 5 min at room temperature. The reconstituted complex was visualized by native gel and immunoblot against MalF. d, effect of MalK on MalE binding to MalFG. Nd-MalFG (1 μM) was incubated with MalK (10 μM) before addition of 125I-labeled MalE (~5,000 cpm, 0.5 μM) in the presence of ATP or ATP and vanadate (1 mM). The complexes were visualized by native gel electrophoresis and autoradiography.

FIGURE 5. The complex hMalFG-WTMalK is blocked in the inward-facing state. a, maltose transporter activity of hMalFG-WTMalK was evaluated on MacConkey-maltose plates. Strain ED169 (ΔMalB) transformed with the indicated plasmids was plated on MacConkey-maltose for 12 h at 37 °C. b, ATPase activity of the transporter. Proteoliposomes containing the indicated complex were incubated at 37 °C in the presence of MalE (2 μM) and maltose (1 mM), as indicated. c, binding of MalE to the transporter. The nanodiscs containing the indicated complex (2 μM each) were incubated with [125I]MalE (~10,000 cpm, 2 μM) in the presence of AMPPNP or ATP and vanadate for 10 min at room temperature, as indicated. Binding of MalE to the nanodisc was detected by native gel electrophoresis followed by Coomassie Blue staining (top) and autoradiography (bottom). d, equilibrium binding affinity of MalE. Titrations with ATTO-655-labeled MalE were done in the presence of AMPPNP (1 mM). The fluorescence quenching data were fitted to a single site binding equation. The dissociation constant of MalE-hMalFG-WTMalK complex is 295 ± 34 nM. The dissociation constant of MalE-hMalFG-HTMalK complex is 85 ± 14 nM. e, stability of the transporter in urea. The purified MalFGK complex (10 μM) was incubated for 5 min at room temperature in TSG buffer containing 5 mM MgCl2, 0.01% DDM and urea (0–5 M). The transporter was analyzed by blue-native gel electrophoresis.
results bear some parallel with those reported by Ames and colleagues on the histidine transporter (19). It was observed that ATP binding decreases the affinity of HisP2 to HisQM. The authors proposed that a subtle cycle of engagement and disengagement of the NBDs with the TMDs may explain how ATP binding and hydrolysis is coupled to transport. In the case of the BtuCD transporter, an increased association between TMDs and NBDs was instead observed in the presence of ATP (20). This result is not contradictory because BtuCD undergoes reversed conformational changes compared with the maltose and histidine transporters.

Finally, we note that some of the suppressor mutations indentified in this study are localized at the C terminus of MalK, which is a binding site for the inhibitory protein EIIAGlc (21, 22). In methionine and molybdate transporters, the binding of the substrate to the C-terminal domain decreases ATPase and transport activities, a mechanism termed “trans-inhibition.” Thus, how the distant C-terminal domain on MalK exerts its regulatory effect on the core nucleotide binding domain remains an interesting question. It is also worth mentioning that the membrane domain MalFG cannot be expressed without MalK (23). The introduction of mutations at the interface of TMD-NBD severely disrupts the biogenesis the complex (15). Similarly, the folding and trafficking of other ABC transporters such as CFTR (cystic fibrosis transmembrane conductance regulator) and ABCC6 is particularly sensitive to mutations in this region (24, 25). The study of the MalFGK2 variants as those isolated in this work may help to understand why this interface

FIGURE 6. Mutations that weaken binding of MalK to MalFG restore transport. a, urea extraction of MalK from membrane-embedded MalFG. Inner membrane vesicles were treated with urea (0 – 6 M) in 50 mM Tris buffer, pH 8.0, for 10 min at room temperature. Membranes were re-isolated by ultracentrifugation and analyzed by SDS-PAGE and immunoblot anti-MalK. The percentage of membrane-bound MalK after urea treatment was determined by densitometry analysis, using membrane treated without urea as reference. b, selection and generation of MalK suppressors. The malK gene on plasmid pTrc-HisMalFGK was subjected to error-prone PCR and expressed into strain ED169 (H9004 ΔMalB). Cells were plated onto MacConkey-maltose and incubated for 12 h at 37 °C. c, urea resistance of MalFGK2 bearing MalK suppressor mutations. Inner membrane vesicles prepared from five distinct colonies (MalKmut1 to MalKmut5) were treated with 6 M urea and solubilized in TSG containing 1% DDM (4 °C, 1 h). Each complex was isolated on nickel-nitrilotriacetic acid-Sepharose beads in TSG buffer containing 0.01% DDM and 5 mM MgCl2. The eluted complex was analyzed by blue-native gel electrophoresis. The left two lanes show the stability of HTMalFG-WTMalK treated in the same condition. d, binding of MalE to the MalFGK2 mutants. Nanodiscs containing the indicated purified complex (2 μM each) were incubated with [125I]MalE (~5,000 cpm, 1 μM) in the presence of nucleotides, as indicated. Binding of MalE to nanodiscs was detected by native gel electrophoresis and autoradiography. Note that the complex MalFG-MalKmut3 easily dissociates during purification; therefore, a fraction of the reconstituted nanodiscs is devoid of MalK.

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is so critical for the biogenesis and stability of these proteins in the membrane environment.

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