X-RAY STRUCTURE OF THE COMPLETE ABC ENZYME ABCE1 FROM PYROCOCCUS ABYSSI
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Running head: ABCE1 crystal structure

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The ATP-binding cassette enzyme ABCE1 (also known as RNase-L inhibitor, Pixie and HP68), one of the evolutionary most sequence conserved enzymes, functions in translation initiation, ribosome biogenesis and HIV capsid assembly. However, its structural mechanism and biochemical role in these processes have not been revealed. We determined the crystal structure of Pyrococcus abyssi ABCE1 in complex with Mg$^{2+}$ and ADP to 2.8 Å resolution. ABCE1 consists of four structural domains. Two nucleotide binding domains are arranged in a head-to-tail orientation by a hinge domain suggesting that these domains undergo the characteristic tweezers-like powerstroke of ABC enzymes. In contrast to all other known ABC enzymes ABCE1 has a N-terminal iron-sulfur-cluster (FeS) domain. The FeS domain contains two [4Fe-4S] clusters and is structurally highly related to bacterial-type ferredoxins. However, one cluster is coordinated by an unusual Cys-X$_4$-Cys-X$_{34}$-Cys triad. Surprisingly, intimate interactions of the FeS domain with the adenine and ribose binding Y-loop on nucleotide binding domain 1 suggest a linkage between FeS domain function and ATP induced conformational control of the ABC tandem cassette. The structure substantially expands the functional architecture of ABC enzymes and raises the possibility that ABCE1 is a chemomechanical engine linked to a redox process.
translation initiation components (9,14-19). In this process, yeast ABCE1 interacts with eukaryotic translation initiation factors as well as ribosomal subunits and is required for rRNA maturation and nuclear export of 40S and 60S subunits (14-16,18,19). The mechanistic basis for the role of ABCE1 in all of these processes is not known but it has been recently observed that drosophila ABCE1 (pixie) binds to 40S ribosomes in an ATP dependent manner (18).

ABCE1 is a 68 kDa ABC enzyme, consisting of a cysteine rich N-terminal region, followed by two ABC type nucleotide binding domains (NBDs). The crystal structure of this tandem cassette NBD region of ABCE1 has been determined (20). Both NBDs possess the typical bi-lobed fold of ABC type ATPase domains (21) and contain ATP binding sites with Walker A, Walker B, Q-loop and signature motifs. The two NBDs are arranged in the typical ABC enzyme head-to-tail orientation (22-27) by a “hinge” domain. This arrangement creates two composite nucleotide binding sites formed by Walker A/B and Q-loops from one NBD and by the signature motifs from the respective opposing NBD. In the ATP binding subunit of the bacterial maltose transporter MalK, ATP binding by the two composite active sites induces a tweezers like powerstroke between the two NBDs (28) that is suggested to trigger conformational changes in associated function specific domains(29-31).

The highly conserved cysteine-rich N-terminal region of ABCE1 is unique among ABC enzymes. Sequence analysis as well as genetic interactions with iron-sulfur ([Fe-S]) cluster biogenesis enzymes indicated that this motifs of yeast ABCE1 are [Fe-S] binding sites, most likely comprising a ferredoxin like fold and coordination (15,32). [Fe-S] clusters, composed of non-heme iron and acid-labile inorganic sulfide, constitute one of the most ancient, widely distributed and structurally and functionally diverse class of prosthetic groups. [Fe-S] clusters can occur in a variety of stoichiometries, including [2Fe-2S], [3Fe-4S] and [4Fe-4S] clusters. Many [Fe-S] clusters mediate electron transfer in redox reactions. However, other functions can include fold stabilization, substrate binding and substrate activation (33-35). Mutations in the predicted [Fe-S] cysteine ligands of the ABCE1 in general lead to cell inviability in yeast, demonstrating that not only the NBDs but also the cysteine rich domain is essential for ABCE1 function (15,32). Thus a structural framework for this domain and its physical and functional interaction with the NBDs is the key for understanding the biochemical function of ABCE1.

To derive the complete multidomain functional architecture of ABCE1, we determined the crystal structure of the complete ABCE1 protein from Pyrococcus abyssi in complex with Mg$^{2+}$ and ADP to 2.8 Å resolution. The fold and double [4Fe-4S] binding environment of the N-terminal FeS domain is structurally similar to bacterial-type ferredoxin domains, which are found in a variety of oxidoreductases. However, the FeS domain of ABCE1 also possesses some uncharacteristic features, such as unique loop insertions that could indicate functional sites. The short 12 Å distance of the two [4Fe-4S] clusters, typically associated with efficient electron transfer reactions, raises the possibility that ABCE1 is involved in an electron transfer process reaction.

**Experimental Procedures**

**Protein Expression and Purification.** The coding sequence of P. abyssi ABCE1 (pabABCE1) was amplified from genomic DNA by the polymerase chain reaction using oligonucleotides

AAAAAAACATATGGTGAGGAAAATGGGATCGCG  and  TTTTTTGCGGCCGCGCGTAGTAGTATT
CTCCCCTTGC. The purified PCR product was cloned into a modified pET28 vector (Novagen). The vector pET28-N-strep containing the pabABCE1 gene was constructed in several steps. Two annealed oligonucleotides (5'-CATGGCTAGCTGGAGCCACCCGCAGTT CGAAAAAGGCGCTCA-3' and 5'-TATGAGCGCCTTTTTCGAACTGCGGGT GGCTCCAGCTAGC-3') that contain the DNA sequence for the short Strep-tag II peptide (MASWSHPQFEKGAH) were ligated into a pET28 vector (Novagen) using NcoI and NdeI restriction sites. The coding sequence for pabABCE1 was inserted into the plasmid using NdeI and NotI restriction sites. Escherichia coli Rosetta (DE3) (Novagen, T7 promoter) cells were transformed with the resulting plasmid. Cells were grown at 37°C in the presence of the appropriate antibiotics to an OD600 = 0.6-0.8. Gene expression was induced by adding 0.4 mM IPTG. After incubation at 37°C for four hours, cells were harvested by centrifugation, resuspended in 20mM Tris (pH 8.0), 200mM NaCl, 4 mM DTT and disrupted by sonication. After sedimenting cell debris and other insoluble material, pabABCE1 was purified aerobically by heat denaturation of the lysate (70°C for 10 minutes), Resource Q ion exchange chromatography (Amersham) and Strep-Tactin (IBA) affinity chromatography using standard/manufacturer suggested protocols.

Reconstitution of the [Fe-S] cluster. All experimental steps of the reconstitution procedure were made anaerobically inside a glove box (Coy Laboratories), in an atmosphere containing 95% nitrogen and 5% hydrogen. The atmosphere contained less than 2 ppm 02 and all buffer solutions were degassed and preincubated in the glove box for at least 48 hours. Purified pabABCE1 was transferred into the anaerobic chamber and the buffer containing purified ABCE1 was exchanged to 50 mM Tris (pH 8.0), 200 mM NaCl, 5mM DTT, using a disposable PD10 gel filtration column (Bio-Rad). For reconstitution of the [Fe-S] cluster, pabABCE1 was concentrated to 5 mg/ml (1.5 ml) and incubated overnight at 6°C in the presence of 0.2 mM pyridoxal phosphate, 2 mM L-cysteine, 2 mM FeCl2, and 2.5-5 µM E. coli IscS (separately expressed and purified as described (36)). Reconstituted pabABCE1 was purified in the anaerobic chamber by Strep-Tactin (IBA) affinity chromatography. After buffer exchange (PD10, BioRad) to 50mM Tris (pH 8.0), 150 mM NaCl, 5mM DTT, the protein was concentrated with Centricron-10 ultrafiltration units (Amicon) to a final concentration of 15 mg/ml and used in crystallization trials.

Crystallization, crystallographic data collection, model building and refinement. pabABCE1 crystallized in the space group p4321 with cell dimensions a = b = 63.2, c = 319.4 Å. Crystals were obtained by mixing 1 µl of protein solution (50mM Tris (pH 8.0), 150 mM NaCl, 5mM DTT) with 1 µl of reservoir solution (0.2 M Ca-acetate (pH 7.3) and 20% PEG 3350) and grew after incubation for several days at 20°C under anaerobic conditions inside a glove box (Coy Laboratories). Crystals were transferred to a stabilizing buffer (0.2 M Ca-acetate (pH 7.3) and 20% PEG 3350, 20% glycerol) and flash frozen in liquid nitrogen. A data set to 2.8 Å resolution, collected at beamline PX1 at the Swiss Light Source (SLS, Villingen, Switzerland), was processed with XDS (37). Phases were determined by molecular replacement with the program MolRep (38), using the coordinates of the previously determined P. furiosus ΔFeS-ABCE1 structure as the search model (20). Electron density for the protein residues as well as for the two [Fe-S] clusters was clearly apparent in 2Fo-Fc and Fo-Fc density maps and was used to trace the missing N-terminal 75 residues with MAIN (39). The model was completed by rounds of manual model building with MAIN and automated refinement with CNS (40).
Refinement included overall anisotropic B factor and bulk solvent corrections, simulated annealing, positional refinement and restrained individual B factor refinement. The solvent was generated with CNS and verified by manual inspection. We omitted 5% of the reflection data from the beginning of refinement on to calculate R_free for cross validation. Crystallographic data and model statistic are summarized in Table 1.

Three-dimensional alignment of coordinates. Three-dimensional alignment of coordinates and calculation of root mean square deviations were performed with LSQMAN (Uppsala Software Factory). After initial “brute force” alignment, the dynamic programming based operator was used with standard settings. The quality and correctness of the alignment was verified by graphical inspection.

Figures and coordinates. Structural figures were prepared with PYMOL (DeLano Scientific). Coordinates and structure factors (accession code 3BK7) have been deposited to the Protein Data Bank (PDB).

Results

Purification and reconstitution of the [Fe-S] cluster containing ABCE1. Initial attempts to crystallize full length P. abyssi (pab)ABCE1 out of a standard E. coli overexpression system were unsuccessful, presumably because of incomplete [Fe-S] cluster formation and additional oxidation of the [Fe-S] clusters during protein purification. To facilitate [Fe-S] cluster formation during overexpression, we co-overexpressed the E. coli ISC (iron-sulfur-cluster) operon on a separate plasmid (41). Furthermore, we subsequently reconstituted/repaired the [Fe-S] clusters of the purified protein in vitro in an anaerobic chamber, containing a 95% nitrogen plus 5% hydrogen atmosphere, by adding the E. coli IscS protein (separately expressed and purified (36)), along with Fe^{2+}, cysteine and pyridoxal phosphate. IscS catalyzes the formation of S^{2-} from cysteine. S^{2-} and Fe^{2+} assemble into the [Fe-S] clusters. The reconstituted ABCE1 showed a strong 418 nm absorption peak characteristic for proteins containing [4Fe-4S] clusters and was further used for crystallization.

Crystallization and structure determination. pabABCE1 crystallized under anaerobic conditions in the space group P4_3_2_1, with one molecule per asymmetric unit (Table 1). The crystals diffracted X-rays to a resolution of 2.8 Å. The structure was solved by the molecular replacement method, using the previously determined pfuΔFeS-ABCE1 structure as the search model. The residues for the iron-sulfur cluster domain (residues 1-75), along with two [4Fe-4S] clusters were clearly visible in 2F_o-F_c and F_o-F_c electron density maps and could be readily interpreted (Fig. 1). The final model, refined at 2.8 Å resolution, comprised all 593 protein residues along with two Mg^{2+}-ADP moieties, two [4Fe-4S] clusters and 124 solvent molecules (Fig. 2).

Structural overview. ABCE1 is a bowl shaped molecule with overall dimensions of app. 80 Å x 65 Å x 50 Å. It consists of four structural domains: an N-terminal [Fe-S] cluster containing domain (FeS domain), two ABC ATPase type nucleotide binding domains (NBD1 and NBD2), and a “hinge” domain (Fig. 2). The structures of the NBDs and the hinge region are similar to the equivalent regions in the previously reported structure of pfuΔFeS-ABCE1 and are described in detail in ref. (20). NBD1 and NBD2 face each other in the typical head-to-tail orientation of ABC enzymes, creating a roughly 10-14 Å wide interface cleft (backbone positions). This interface cleft harbors two composite ATP binding sites, which are formed by the conserved Walker A, Walker B/D-Loop and Q-loop motifs of one
NBD and the signature motif of the opposing NBD.

In the obtained crystal form, both active sites contain an Mg\(^{2+}\) -ADP moiety (or Ca\(^{2+}\) -ADP because of the crystallization solution), retained from the heterologous expression (Fig. 1b, 2). Stably bound ADP molecules have also been found in other ABC type ATPases (23,42), and have been observed with other crystal forms of ABCE1 from different organisms (ref (20) and AK, unpublished data). It is possible that nucleotide exchange in ABCE1 necessitates allosteric control by binding to a yet to be identified substrate or interacting partner, perhaps similar to the mismatch DNA provoked ADP->ATP exchange of the mismatch sensor MutS (43).

The orientation of NBD1 and NBD2 is mediated by the “hinge” domain, formed by highly conserved sequence regions between the two NBDs and at the C-terminus of ABCE1 (Fig. 3 and suppl. Fig. 1). The hinge domain is tightly bound along the NBD1:NBD2 interface and may form a pivot point for the putative ATP-driven conformational changes between NBD1 and NBD2. The signature motifs, which bind to the opposing ATP γ-phosphates in the ATP-bound conformation of ABC enzymes, are app. 11 Å away from their expected position in the presence of ATP. Since mutations in both invariant serine residues of the signature motifs of S. cerevisiae RLI1p are lethal in S. cerevisiae (20) it is likely that the NBDs of ABCE1 undergo a tweezers like motion similar to that of MalK (28).

The most intriguing and unique feature of ABCE1 is the FeS domain (residues 1-75, Fig. 2). The FeS domain is a small ellipsoid domain of 20 Å x 20 Å x 30 Å dimensions. It is positioned at the lateral opening of the cleft between NBD1 and NBD2 and directly binds to the outside of Lobe I of NBD1 (β5, β6, β11, β12). We observe no direct contacts of the FeS domain to the clamp and NBD2. However, lobe II of NBD2 and the FeS domain are only separated by 5 Å and it is possible that the FeS domain also interacts with NBD2 during the ATP dependent conformational cycle of ABCE1.

The FeS domain has a ferredoxin fold and possesses two [4Fe-4S] clusters. The structure of ABCE1 reveals that the FeS domain has the (βαβ)\(_2\) fold of the “bacterial type” ferredoxins (Fig. 4a). Ferredoxins function as electron-transfer mediators in diverse biological redox systems and are grouped into “plant” and “bacterial” types (44). The two-stranded antiparallel β-sheets β1+β4 and β2+β3 along with two α-helices α1 and α3 form the core ferredoxin fold (Fig. 4a). However, ABCE1 has two notable differences from the canonical fold. One is an additional helical turn (α2) that is inserted after Cys29, the first coordinating cysteine of cluster II (Fig 4a). The second difference is the geometry of the cysteine-triad that coordinates cluster I (Fig. 4b). Both insertions are located at positions that have been suggested to mediate electron entry and exit in electron relay active ferredoxin like domains (45,46) and may be a specific functional adaptations with ABCE1.

The two [4Fe-4S] clusters are situated in the core of the FeS domain. The electron density shows an eight atom distorted cube for each of the two clusters (Fig. 1a). Distances of 2.2-2.3 Å between the iron and sulfur atoms and 2.7-2.8 Å between the iron atoms correspond well with values derived from X-ray absorption spectroscopy (32). Both [4Fe-4S] clusters are surrounded by a hydrophobic cocoon (Ile10, Pro17, Phe23, Leu24, Pro48, Pro66, Phe67, Ala69, Ile70 for cluster I and Pro30, Val31, Ala38, Ile39, Ile50, Ile60, Ile72 for cluster II) and do not directly interact with solvent (Fig. 4c). The lethal pixie mutants L17 (Ala77\(^{dme}\)->Thr, Ala69 in P. abyssi) and L24 (Pro38->Leu, Pro30 in P. abyssi) map to the hydrophobic cocoon of cluster II and I, respectively (8). These mutations likely
perturb the cluster environment, if not prohibit cluster assembly. In any case, these mutations demonstrate the requirement of both clusters and the surrounding cocoon for ABCE1 function in flies.

The sequence invariance of the eight coordinating cysteine ligands among known ABCE1 sequences suggests that the two [4Fe-4S] clusters are conserved in ABCE1 across archaea and eukaryotes (Fig. 3). The cysteine ligands are grouped in two motifs of four cysteines. In di-cluster bacterial type ferredoxin domains, these motifs usually have a Cys-X3-Cys-X2-Cys-Xn-Cys consensus. Hereby, the first three cysteines bind one cluster, whereas the fourth binds to the other cluster. In ABCE1, [4Fe-4S] cluster I is ligated by Cys15, Cys20, Cys25 of motif I and by Cys65 of motif II. [4Fe-4S] cluster II is ligated by Cys55, Cys58 and Cys61 of motif II, and by Cys29 of motif I. Thus, the second cysteine motif of ABCE1 has the consensus spacing of ferredoxins, while the first set diverged into the highly unusual Cys-X4-Cys-X3/4-Cys-Xn-Cys spacing (Fig. 4b), a feature that also has been observed by bioinformatics analysis (32).

Careful mutational analysis in yeast indicated that six out of eight coordinating cysteines are essential for ABCE1 function (32). Notable exceptions were Cys58exc and Cys29exc. The former coordinates cluster I while latter coordinates cluster II. Mutational analysis (32), supported by our structural analysis, suggests that the coordinating function of Cys29 can be taken over by Cys38 in yeast. This cysteine residue (which is an alanine in P. abyssi) is well positioned to bind to the iron atom and could easily restore coordination of cluster II in the absence of Cys29. Cys58, on the other hand, is located between two conserved glycine residues and this loop appears to be stabilized predominantly by the Fe-Cys interaction. Thus, a lack of Cys58 might not interfere with FeS domain fold and could - for instance - still allow formation of a partially active [3Fe-4S] cluster.

Similar to some bacterial ferredoxins, the [4Fe-4S] clusters of ABCE1 are stable down to redox potentials below -500 - -600 mV (32,47). Reasons for such low potentials are solvent shielding of the clusters, side chain features, cavity sizes and N-H…S hydrogen bonds (48). For instance, a bulky amino acid (e.g. Leu, Met) at X4 of the C-X1-X2-C-X3-X4-C ligation triad motif has been associated with a low redox potential. Cluster II of ABCE1 carries a conserved isoleucine (Ile60) at this position, the bulky nature of which could contribute to the negative potentials.

The FeS domain and the ATPase active site of NBD1 are tightly linked. The FeS domain binds to NBD1 via a 1360 Å² substantial interface (Fig. 2). Strands β1 and β4 form a flat antiparallel sheet that covers the outside sheet (strands β5, β6, β11, β12) of NBD1. In addition, a prominent loop (residues 87-92; denoted Y-loop; Fig. 3) between β5 and β6 bridges FeS and NBD1 with a net of polar interactions (Fig. 5a). Notably, Asn90 at the tip of the Y-loop interacts with the main chain of the FeS domain, while the base of this loop is attached to the FeS domain via interaction between Arg86 and Glu52. The Y-loop was previously identified as a central element of the ATP-binding site of ABCE1 (20). Tyr87 forms the major interaction site for ADP via an aromatic stack with the adenine moiety, while Phe92 provides a hydrophobic face for the ADP ribose. Thus, the Y-loop appears to be a structural bridge between the ATP/ADP binding moiety on NBD1 and the FeS domain. Such a tight direct link between the ATP binding site and an associated domain has - to our knowledge - not been observed in ABC enzymes and could have important functional consequences for the often seen allosteric control of the ATPase activity of ABC enzymes by substrate interactions.
Model for ATP induced conformational changes. Previous results from others and us have shown that ATP binding and hydrolysis motifs in both NBDs are essential for the function of ABCE1 in yeast and drosophila in vivo. The precise role of ATP binding and hydrolysis for the functional cycle of ABCE1 remains to be established. However, since drosophila ABCE1 binds to the 43S ribosomal subunit in an ATP dependent manner (18), a likely explanation is that ATP could control the interaction with molecular partners of ABCE1 by inducing different conformational states in the enzyme. We have not been able to crystallize ABCE1 in the presence of ATP or ATP analogs. However, the ATP bound conformations of NBDs from several ABC enzymes are structurally characterized and can be used to obtain a preliminary model ABCE1 in the presence of ATP (Fig. 5b). To obtain such a model, we used the structure of the homodimeric ABC domains of the bacterial ABC transporter MJ0796 (25) and the procedure as described for ΔFeS-ABCE1 (20). We rigid-body superimposed MJ0798 and ABCE1 via NBD1 (the resulting root mean square deviation is 1.3 Å over 150 Cα atoms). We then superimposed NBD2 of ABCE1 with the second NBD of MJ0796 (root mean square deviation of 1.3 Å over 159 Cα atoms), leaving the remainder of ABCE1 fixed. The low root mean square deviations demonstrate the overall good fit.

In our modeled orientation, NBD2 swings app. 40° with respect to the FeS-NBD1-hinge. This movement is very similar to the observed tweezers-like motion of the NBDs of the ABC transporter MalK (28). Only small side chain rearrangements are necessary in the D-loop between the two NBDs to remove close contacts. The superposition appropriately places the signature motifs of NBD1 and NBD2 to bind the opposing ATP γ-phosphates during ATP-hydrolysis consistent with the in vivo mutational analyses (20).

Interestingly, in the modeled orientation, lobe II of NBD2 substantially clashes with the FeS domain (Fig. 5b). We cannot formally rule out that ATP binding and hydrolysis by ABCE1 involves conformational changes between NBD1 and NBD2 not seen in other ABC enzymes. However, such a different mechanism would not explain the functional significance of the signature motif mutants. A plausible and perhaps more likely explanation is that ATP induced conformational changes between the NBDs could be linked to a repositioning of the FeS domain. Such structural changes could also explain the effect of Lys453csc->Ala point mutation on the surface of lobe II of NBD2. The lethal effect of this Lys453csc->Ala mutation in S. cerevisiae has remained unexplained, because this surface residue has no evident architectural function and is not close to any known functional motif. Since Lys443pab (corresponding to Lys453csc) is located near the cleft between NBD2 and the FeS domain the lethal effect of Lys453csc->Ala could stem from deunct ATP conformational changes or a disruption of macromolecular interactions in this region.

Surface features of pABCE1. To learn more about the functional architecture of ABCE1 and potential macromolecular interfaces we analyzed the molecular surface of pABCE1. Mapping of sequence conservation across archaeal and eukaryotic species revealed at least three notable conserved patches (Fig. 6a). Two of them, the nucleotide binding cleft (patch 1) between the two NBDs and the conserved patch 2 on the hinge domain have been previously observed and attributed to functional sites associated with nucleotide dependent conformational changes (patch 1 and 2) and potential macromolecular interactions (patch 2) (20). We noticed a third patch on the surface of the FeS domain at the side facing NBD2.
patch could be an important binding site for macromolecular partners. The opposing side of NBD2 harbors Lys443, the alanine mutation of which is lethal in yeast (Fig 6a). Interestingly, this conserved patch co-localizes with strong positive electrostatic surface potential (Fig. 6b), a feature that could be important in the interaction with negatively charged components of 43S ribosomal particles, RNA molecules or other translation initiation intermediates.

Discussion

ABCE1 is one of the most conserved enzymes in evolution and present in all archaea and eukaryotes. Current data suggest that ABCE1 has key cellular roles in translation or ribosome biogenesis and interacts with several translation initiation factors (eIF3/Hcr1, eIF5, eIF2B) and ribosomal subunits (14-16,18,19). Additional functions of ABCE1 demonstrated are the assembly of HIV1 capsids (13) and inhibition of RNase-L (10). A second paralog of ABCE1 in plants is an endogenous suppressor of RNA interference (49). However, despite efforts in several laboratories, understanding the essential biological role of ABCE1 is currently hampered by several shortcomings, including the unknown target for the mechanistic action of ABCE1 and the unknown biochemical function of ABCE1.

To guide future research, we provide here a structural framework for the complete multidomain ABCE1 molecule from P. abyssi. The overall high conservation of the entire polypeptide chain argues that our structure is also a valid model for eukaryotic ABCE1. The unique N-terminus of ABCE1, distinct from all other known ABC enzymes, has striking structural similarity to bacterial ferredoxin domains, which are typically involved in electron transfer processes. As judged from topological similarities to ABC transporters the three C-terminal domains NBD1, NBD2 and “hinge” are well positioned to undergo the nucleotide dependent structural switch and probably form a “chemomechanical engine” module. The mutual interaction of the putative “engine” module with the FeS domain distinguishes ABCE1 from other ABC enzyme structures. In particular, the intriguing position of the FeS domain at the lateral opening of the ATP binding cleft and the tight, unexpected structural connection between the nucleotide binding Y-loop of NBD1 and the FeS domain suggest a mechanistic link between the putative ATP dependent structural changes in the NBD1-NBD2-hinge “engine” and the FeS domain.

The role and function of the FeS domain in the cellular biology of ABCE1 is unclear. Many mutations in structurally important residues in the FeS domain are lethal in flies and yeast, indicating the essential role of this domain in the function of ABCE1. Functions of [Fe-S] clusters include electron transfer, substrate binding and a direct role in catalysis. In addition, some [Fe-S] clusters appear to have a more structural function. Since both [4Fe-4S] clusters of ABCE1 are quite well shielded from solvent and are coordinated by four cysteines each it is unlikely that the clusters of the FeS domain have a direct role in some sort of catalysis or in substrate binding. This does not rule out that the FeS domain itself interacts with substrates or macromolecular partners. Therefore, an obvious question with regard to the [Fe-S] clusters of ABCE1 is whether they participate in an electron transfer process or whether they have an architectural function. Previous biochemical studies showed that the [4Fe-4S] clusters of ABCE1 from the hyperthermophilic archaeon Sulfolobus solfataricus are resistant to reduction with dithionite and are electronically stable down to redox potentials of -560 mV. This considerable stability could indicate that the [4Fe-4S] clusters of ABCE1 have structural
rather than electron transfer functions (32). Here, the FeS domain and its positive surface patch could form an important site for macromolecular interactions and ABCE1 may act as a chemomechanical engine, for instance to help assemble ribosomal particles, immature HIV capsids or 48S translation initiation intermediates.

The stability against reducing agents of the ABCE1 clusters is similar to that of clusters in the DNA repair enzymes Endo III and MutY (50,51). Initially, the clusters of the DNA repair enzymes have been suggested to fulfill a mainly structural function. However, recent data show they become redox active, once Endo III and MutY bind to DNA substrates (52). It is therefore possible that the clusters of ABCE1 also become redox active only after association with biological partners.

To our knowledge, most if not all bacterial ferredoxin type two [4Fe-4S] clusters with known biological functions are associated with electron transfer reactions. In support of an electron transfer role, the two clusters of ABCE1 are separated by the ideal 12 Å (center-to-center) distance that allows magnetic interaction of the clusters and is characteristic of biological relevant electron transfer reactions. Thus, an alternative or additional function of ABCE1 could be the catalysis of electron transfer in a translation/ribosome associated process. Such a possible a link between ATP dependent conformational changes and [4Fe-4S] cluster-mediated electron transfer is not unprecedented and well characterized for the nitrogenase enzyme. Here electron transfer is coupled to nucleotide dependent conformational changes at two ATP/ADP binding domains (53). In this regard, ABCE1 may act as a chemomechanical enzyme with structurally-linked NBD- and FeS-associated functions, for instance by linking nucleotide driven association to translation components with a possible role in an electron transfer process. Thus future experimental approaches should not only address a possible function in the assembly of protein-nucleic acid complexes but should also address the possibility of an ABCE1 mediated electron transfer reaction.

In sum, our crystallographic analysis uncovers new structural features of ABC enzymes, including a surprisingly direct link between the ATP-binding site of an NBD to an associated function specific domain and the location of a FeS domain at the lateral opening of the active site cleft. These results could be important in the light of the poorly understood allosteric control of the ATPase of ABC enzymes by substrate interactions. In addition, our results considerably expand the known functional architecture of ABC enzymes and indicate a new mode for how the engine module of ABC enzymes may communicate with function specific domains.

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Figure Legends

Figure 1. Electron densities. a) Stereo view of a portion of the electron density at the two [4Fe-4S] clusters with superimposed final model. The model is shown as stick representation with green (FeS) or orange (NBD1) carbons, blue nitrogens, red oxygens, yellow sulfurs and ruby iron atoms. The final 2Fo-Fc density, contoured at 1σ, is shown in light brown. A Fo-Fc density, contoured at 3σ and calculated after simulated annealing with omitted [4Fe-4S] clusters atoms and coordinating cysteine sulfurs is shown in dark magenta. Both [4Fe-4S] clusters as well as their coordinating residues are well defined. b) Stereoview of a portion of the electron density (2Fo-Fc, contoured at 1σ) around the ATP binding site (Walker A motif). The final model is superimposed and shown as color coded sticks. Me2+ represents the active stite metal, either Mg2+ or Ca2+ from the crystallization conditions.

Figure 2. Structural overview. a) Stereo plot of a ribbon representation of pabABCE1 with highlighted secondary structure (“top view”). The FeS domain is shown in green, along with CPK models of the two [4Fe-4S] clusters (red iron, yellow sulfur). The two nucleotide binding domains NBD1 and NBD2 are displayed in yellow and orange, respectively. NBD1 contains a helix-loop-helix insertion (HIH, blue). The two NBDs are oriented into the head-to-tail orientation by the ABCE1 specific hinge domain (pale blue). The FeS domain binds to NBD1 and is situated at the lateral opening of the nucleotide-binding cleft. The two experimentally bound ADP molecules (color coded stick model with cyan carbon, red oxygen, blue nitrogen and white phosphor atoms) and magnesium ions (magenta spheres) highlight the position of the two composite nucleotide-binding sites (P: P-loop/Walker A; S: signature motif). b) Same as a) but viewed along the nucleotide binding cleft (“front view”).

Figure 3. Sequence alignment of ABCE1 from Pyrococcus abyssi (Pab), Sulfolobus solfataricus (Sso), Saccharomyces cerevisiae (Sce), Homo sapiens (Hsa) and Drosophila melanogaster (Dme). Domains are highlighted by color coded shading (using the color code of Fig. 2). Annotated secondary structure is shown above the alignment, functional motifs below (most functional motifs are present in both NBDs). The coordinating cysteines for the [4Fe-4S] clusters I and II are annotated.
Figure 4. The FeS domain. a) Comparison of the FeS domain of *P. abyssi* ABCE1 (green with color coded cysteine side chains and [4Fe-4S] clusters) with the bacterial type ferredoxin domain from adenyllylsulfate reductase (purple, PDB code 1JNR, (46)), which is the closest structural match according to a DALI search of the protein data base. Two insertions in the ABCE1 FeS domain compared to the canonical ferredoxin fold are shown in magenta. One notable cysteine coordination difference is indicated (arrow). These loop insertions are near the proposed locations of electron entry and exit in bacterial-type ferredoxin domains with electron relay functions. b) Detailed view of the C-X4-C-X4-C cysteine triad at cluster I of ABCE1 (green, top) with the canonical C-X2-C-X2-C motif (wheat, bottom, taken from PDB entry 1IQZ, (54)). Only backbone atoms along with cysteine side chains and [4Fe-4S] cluster atoms are shown as stick model. c) Stereoplot of the FeS domain (tube model) with highlighted and annotated cluster coordination cysteines and hydrophobic cocoon residues (magenta sticks).

Figure 5. FeS–NBD1 interaction and model for ATP bound conformation. a) Stereoplot of the interface between FeS and NBD1, shown as stick model with the color code of Fig. 2a. NBD1 (orange) interacts with the FeS domain (green) via a small hydrophobic interface (e.g. V9 and I7) and an intimate hydrogen bonding network as well as salt bridges of the Y-loop (R86-F92) to side chain and backbone atoms of the FeS domain. The Y-loop binds ADP via aromatic stacking (Y87) and hydrophobic interactions (F92) to adenosine and ribose, respectively, and structurally couples the nucleotide-binding site to the FeS domain. b) Model for the ATP bound engaged conformation of the two NBDs of ABCE1 (orange and yellow ribbons) obtained by superimposing them onto the structure of the ATP-bound NBDs of the ABC transporter MJ0973 (gray ribbon). The putative orientation of NBD2 in the presence of ATP reveals a structural clash with the FeS domain (dashed circle), raising the possibility that nucleotide dependent conformational changes of the NBDs and functions of the FeS domain are mechanistically linked.

Figure 6. Molecular surface analysis. a) Molecular surface of pabABCE1 colored according to sequence conservation among different ABCE1 species. Conservation was derived from the AMAS server and varies from dark red (invariant) to white (unconserved). Two views of pabABCE1 are show. The left view shows the “top” side of the particle using the orientation of Fig. 2a. The right view shows the “bottom” side of the particle, obtained by rotating the model around the vertical axis. We observed three conserved regions. Patch 1 is located in the active site cleft, while patch 2 is located at the hinge domain. Patch 3 is on the FeS domain, near the interface cleft to NBD2 b) Molecular surface of pABCE1 colored according to electrostatic potential at the solvent accessible region (red -5 kT/e, blue +5 kT/e). The electrostatic potential was calculated with APBS, using the experimentally determined charge of +2 for each of the clusters (32). Strong positive patches co-localize with the conserved surface patches, indicating they could have important functions in e.g. nucleic acid interactions.
Table 1: Crystallographic data collection and model refinement statistics

**Crystallographic data collection and analysis**

Space group: P4₁2₁2; unit cell: a=b=63.2 Å c=319.4 Å

| Description                      | Value                  |
|----------------------------------|------------------------|
| X-ray source                     | PXI (SLS)              |
| Wavelength (Å)                   | 0.9793                 |
| Data range (Å)                   | 50.0-2.8               |
| Observations (unique)            | 128560 (16784)         |
| \( I / \sigma \) (last shell)    | 15.2 (4.7)             |
| Completeness (%) (last shell)    | 97.5 (95.8)            |
| \( R_{\text{sym}} \) (last shell)| 0.148 (0.504)          |

**Refinement and model statistics**

Data range (Å) 20.0-2.8
Reflections F>0 (cross validation) 16308 (796)
Protein residues 1-593
Non-hydrogen protein atoms 4838
Water molecules 124
Adenosine diphosphates 2
Magnesium ions 2
Iron atoms 8
Non-protein sulfurs 8
\( R_{\text{work}} \) (\( R_{\text{free}} \)) 0.208 (0.275)
Rms bond length (Å)/bond angles 0.009 (1.3)
% core, allowed, partially allowed and disallowed in Ramachandran plot 83.0, 15.5, 1.5, 0.0

\( R_{\text{sym}} \) is the unweighted R value on I between symmetry mates
\( R_{\text{work}} = \sum_{hkl} \frac{|F_{\text{obs}}(hkl)|-|F_{\text{calc}}(hkl)|}{\sum_{hkl} |F_{\text{obs}}(hkl)|} \)
\( R_{\text{free}} \) = the cross validation R factor for 5% of reflections against which the model was not refined
Figure 1, Karcher et al (2007)
Figure 2, Karcher et al (2007)
Figure 3, Karcher et al (2007)
Figure 4, Karcher et al (2007)
Figure 6, Karcher et al (2007)
