Architectures and mechanisms of ABC proteins

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Abstract

ATP binding cassette (ABC) ATPases form chemo-mechanical engines and switches that function in a broad range of biological processes. Most prominently, a very large family of integral membrane NTPases – ABC transporters – catalyzes the import or of a diverse molecules across membranes. ABC proteins are also critical components of the chromosome segregation, recombination and DNA repair machineries and regulate or catalyze critical steps of ribosomal protein synthesis. Recent structural and mechanistic studies draw interesting architectural and mechanistic parallels between diverse ABC proteins. Here I review the current state of our understanding how NTP-dependent conformational changes of ABC proteins drive diverse biological processes.

Introduction

ATP binding cassette (ABC) proteins form a very large and diverse family with P-loop NTPases and are found in all kingdoms of life. They are among the most abundant class of proteins and utilize the energy of nucleoside triphosphate (NTP) hydrolysis to catalyze a broad range of biochemical reactions. In particular, they are implicated in three fundamental processes of the cell, the transport of substances across membranes, the maintenance and distribution of the genetic information and the translation of the genetic information. For instance, ribosome-associated ABC proteins are found to catalyze critical steps translation initiation, elongation and ribosome recycling; DNA-associated ABC proteins structurally and logistically organize the distribution of chromosomes or are involved in different DNA repair and recombination pathways; perhaps most well know, ABC transporters export or import a large range of molecules vectorially against concentration gradients across membranes, but can also form regulated ion channels or flip lipids between the leaflets of membrane bilayers. For a comprehensive overview of the different ABC proteins and their biological functions, the reader is referred to excellent books on the subject[1 2].

In the last two decades, biochemical and structural studies determined the functional architecture and mechanism of many ABC proteins, revealing a fascinating wealth of different architectures (Figure 1). Despite their obvious architectural and functional diversity, ABC proteins share some common features and mechanistic principles. Most importantly, ABC proteins possess homodimeric or heterodimeric pairs of ABC type nucleotide binding domains (NBDs) along with different function-associated domains, a feature that has been noted early on[3-5]. Modelling[6] and structural analysis[7] revealed that in ABC proteins the two NBDs cooperate to form composite active sites. NTPs are sandwiched in the NBD dimer

interfaces and binding and hydrolysis of NTPs leads to conformational changes between the two NBDs, which is referred to as the “powerstroke” of ABC proteins. The free energy released from the hydrolysis of NTPs to NDPs and P_i (ΔG -40 – -50 kJ/mol per NTP) and the conformational changes of the NBDs are coupled to conformational changes in associated domains or interacting ligands, thereby chemo-mechanically catalyzing the respective biomolecular reaction. Ligand binding in turn often allosterically activates ABC proteins. However, while many ABC transporters transport solutes against a concentration gradient, requiring free energy from NTP hydrolysis, the need for energy conversion in the functions of other ABC proteins such as DNA damage and mismatch recognition, DNA cleavage, sister-chromatid cohesion is less clear. In some of the latter cases, NTP hydrolysis appears to increase for instance the specificity of the reaction by e.g. kinetic proofreading. In summary, ABC proteins show – to a varying degree – features of NTP-controlled “molecular machines” and “molecular switches”.

Biomedical relevance of ABC proteins

The structural and functional investigation of ABC proteins is of considerable biomedical importance and a variety of ABC proteins are associated with human disease.

Mutations in the ABC protein cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel that is involved in the production of mucus and sweat, leads to cystic fibrosis, the most widespread, genetically inherited and life shortening disease in North America and Europe.

The transporter associated with antigen presentation (TAP) is a central protein of the adaptive immune system[8]. It transports peptides across the ER membrane, where they are loaded onto MHC class I receptors. Besides being implicated in immune deficiency syndromes, TAP is a target of several viral proteins or peptides that inhibit TAP to limit the antiviral immune response[9]. A recent cryo-electron microscopy structure visualized TAP bound to a viral peptide at sub-nanometer resolution, revealing unprecedented insight into immune evasion by targeting TAP[10].

Multidrug resistance (MDR) proteins constitute a family of ABC efflux pumps in both eu- and prokaryotes[11]. These proteins are related to TAP and export a broad range of small molecules to detoxify cells as defense against xenobiotic agents. Upregulation of the expression of e.g. P-Glycoprotein (the product of the multidrug resistance-associated gene 1 MDR-1), the multidrug resistance protein (MPR) or the breast cancer resistance protein (BCRP) is a mechanism of cancer cells to gain resistance against chemotherapeutic agents[12]. Multidrug resistance-associated ABC efflux pumps are also found in bacteria[13] and were model systems for pioneering structural studies of efflux pumps[14]. For instance, Lactococcus lactis LmrA, a homolog of the human MDR1 protein, extrudes a variety of

cationic amphiphilic compounds[15]. A variety of other human diseases are associated with misregulation or genetic defects in ABC transporter genes, including Tangier and Alzheimers diseases[16].

In addition to the many disease-associated ABC transporters, other ABC proteins are also relevant from a biomedical point of view. The DNA double-strand break repair protein Rad50 forms a complex with the nuclease Mre11 and the DNA damage response checkpoint factor NBS1. Mutations in these three proteins lead to disease syndromes such as Nijmegen breakage syndrome (NBS), NBS like syndrome and ataxia telangiectasia like disease[17-19]. These syndromes exhibit – to a varying degree –neurological disorders, immunodeficiency and cancer predisposition as a result of defective cellular responses to DNA double-strand breaks. Furthermore, mutations in the ABC protein MSH2, which together with the related ABC proteins MSH6 or MSH3 form the heterodimeric Mutsα and Mutsβ DNA mismatch sensors, respectively, are associated with hereditary non-polyposis colon cancer[20 21].

Finally, mutations in subunits of the cohesin complex, an ABC protein complex that sustains a cohesion of the two sister-chromatids between DNA replication and mitosis, or mutations in other proteins in the cohesion establishment pathway are associated with Cornelia de Lange syndrome[22], Roberts syndrome[23 24] as well as cancer[25]. The term "cohesinopathy" is used to describe these defects in chromosome organization and chromatin structures[26].

Interestingly, many of the underlying mutations, e.g. those in MSH2 associated with HNPCC, do not map to identifiable functional ATP and DNA binding motifs, suggesting defects in global conformational changes and in interaction with other proteins[27]. Thus, comprehensive mechanistic and structural analysis of the complete ABC protein systems is often necessary to understand the underlying pathomechanisms of the disease-associated mutations.

Architecture of ABC proteins

ABC transporters

The best know and most intensely studied ABC proteins are ABC transporters (also known as "traffic ATPases"). ABC transporters contain two transmembrane domains (TMDs) that form a central pathway for solute transport, along with two NBDs (Figure 1). TMDs and NBDs can be genetically fused together or can be encoded by separate open reading frames. ABC transporters are classified into four different families (type I, II and III importers, and exporters) on the basis of their biochemical activity and architecture. All four family members are found in prokaryotes, while eukaryotes only contain exporter type transporters (not all are involved in active transport). The NBDs are situated in the cytosol or organelle matrix where they can access NTPs. The side of the membrane where the NBDs are located is denoted

the “cis” side. Exporters transport cis->trans, importers trans->cis. Exporters transport hydrophobic or amphiphilic compounds out of the cell or membrane bilayer. They can also flip lipids, transport peptides or export e.g. bacterial toxins and other proteins.

The general mechanism of ABC transporters is reminiscent of the “alternating access” model, formulated 50 years ago[28], but there are also exceptions[29]. In the alternating access model, the TMDs have at least two principal states, “inward-facing” and “outward-facing”. In the inward-facing conformation, the substrate can access the binding site in the TMD channel from the “cis” side, in the outward-facing conformation from the “trans” side. NTP binding and hydrolysis by the NBDs switches the TMDs between these states, transporting the substrate across the membrane.

Pioneering structural work provided a first architectural framework for an ABC transporter – the bacterial type II importer BtuCD in an outward-facing conformation[30]. This revealed a 10+10 transmembrane helix topology in the BtuC dimer. In subsequent structural analyses, several groups provided structures of exporters and flippases[10 29 31-36], type II importers[37-41], and type III importers[42 43] in different functional states and complexed substrate delivering or regulatory proteins (for an excellent overview of available structures, see e.g. ref [44]). The first structure of an exporter, the bacterial Sav1866 protein in outward-facing conformation, revealed a very different topology of the TMDs (6+6) compared to BtuCD[31]. In addition, the TM helices were considerably longer, placing the NBDs at approx. 20-30 Å away from the membrane. It became apparent that ABC transporters exhibit strikingly different topologies in the TMDs, while the specific arrangement of the NBDs remains conserved (Figure 1).

ABC transporters are often subject to allosteric control. For instance, importers of the type I and II generally rely on substrate-binding proteins to deliver the ligand. The binding proteins form a tight complex with the TMDs on the trans side, positioning the substrate at the entrance of the outward-facing channel[39 41 45]. Interaction of the binding protein can allosterically promote NTP binding and hydrolysis of the NBDs in a long-range fashion by inducing a pre-translocation state in the NBDs[38 45]. In addition to substrate-binding protein mediated activation, ABC transporters can also be regulated by additional domains, e.g. regulatory domains that control the interaction of the NBDs in cis[46 47]. Exporters can also be allosterically regulated by the respective ligands[48-50]. In the inward-facing conformation, the NBDs can be quite separated, suggesting that ligand binding at the inward-facing cavity in the TMDs helps trigger a conformational change that brings the NBDs in closer proximity.

Not all ABC transporter-like proteins are involved in the vectorial transport of molecules. The CFTR protein is a regulated ion channel, allowing the passive flow of chloride ions along the concentration gradient. The ABC ATPase FtsE and the transmembrane protein FtsX constitute the FtsEX ABC system, which has homology to ABC transporters but

functions as a regulator of bacterial cell division[51] and cell morphology changes[52 53]. FtsEX interacts with FtsZ at the cytosolic Z ring at the site of cell division and with the peptidoglycan peptidases at the periplasm to trigger hydrolysis of the peptidoglycan layer [54]. Current models suggest that FtsEX, depending on its nucleotide state and interaction with FtsZ, recruits and activates the peptidases[55] for a local burst of peptidoglycan hydrolysis. Thus, FtsEX appears not to transport molecules but "information". It will be interesting to structurally characterize this system and compare its architecture and mechanism to bona-fide transporters.

Translation-associated ABC proteins

Soluble ABC proteins are found at various ribosome and translation-associated processes. These ABC proteins contain tandem NBDs in a single polypeptide chain, separated by linker regions and associated domains. In general, the ATP cycle regulates the interaction with the ribosome and – depending on the nucleotide state – the ABC proteins can either arrest ribosomal states or break them. For three of these ribosome or translation-associated ABC proteins, ABCE1, EttA and eEF3, detailed structural information of the proteins and their complexes with the ribosome has been obtained, revealing their mode of action at the ribosome. Unexpectedly, all three ABC proteins were found to bind to different sites at the ribosome. eEF3 and EttA are a member of the ABCF family that includes also ABC50. This family of proteins is involved in translation initiation as well as elongation[56 57]. eEF3 is a yeast specific translation elongation factor that binds near the E (tRNA exit) site of ribosomes and helps to remove tRNA[58]. Recently, EttA was crystallized and found to bind to the ribosome at the E site, showing along with biochemical studies how bacteria can regulate translation as a function of cellular energy[59 60]. In the presence of ADP (low energy cell state), EttA binds to the E site and restricts the movements of tRNA and ribosome, inhibiting translation. Exchange of ADP with ATP (increasing energy state) induces a structural switch that allows dipeptide formation. ATP hydrolysis finally removes EttA from the ribosome.

While eEF3 and EttA appear to be more phylogenetically restricted, another ribosome ABC protein – ABCE1 (also denoted RNase-L inhibitor, Rli1) is highly conserved in all archaea and eukaryotes a plays a fundamental role in the eukaryotic-archaeal translation cycle by recycling terminated ribosomes[61 62]. ABCE1 possesses a twin 4Fe-4S cluster domain precedes the tandem NBDs[63] (Figure 1b). ABCE1 binds like canonical translation factors and the NBDs and 4Fe-4S cluster domains are observed in a position where they can help place termination factors for peptide-release activity and split ribosomes as a result of ATP-dependent conformational changes in ABCE1[64]. The 4Fe-4S clusters are essential for the ABCE1s function but not redox-active[65]. It is unclear why such costly cofactors are

universally conserved in archaeal and eukaryotic ABCE1. Possibly, they are necessary from a structural perspective e.g. to ensure a rigid fold but could also have global regulatory roles.

Chromosome segregation and DNA repair ABC proteins

All kingdoms of life contain a number of chromosome or DNA-associated ABC proteins that function in the structural organization of chromosomes and the repair of DNA lesions. The UvrA and MutS DNA damage sensors detect helix distorting or bulky lesions to elicit nucleotide excision repair (UvrA) or DNA mismatches as well as small bulges to correct replication errors (MutS) (Figure 1c). UvrA is a DNA damage recognition factor in prokaryotes and together with the helicase UvrB and the endonuclease UvrC constitutes the UvrABC nucleotide excision repair system[66]. The UvrA protein has two NBDs and additionally forms a dimer, generating double NBD dimer along with zinc-finger domains[67]. The UvrA dimer binds DNA at both sides of the lesion, spanning approx. 10nm of DNA[68]. ATP-dependent conformational changes within the $UvrA_2UvrB_2$ heterotetramer, which scans the DNA, are proposed to modulate the DNA binding groove and overall shape of the damage recognition complex[66 68]. ATP-dependent structural modulation of complex may distinguish undamaged from damaged DNA in a mechanism that is consistent with kinetic proofreading[66 68]. ATP hydrolysis ejects of $UvrA_2$ from the recognition complex for further repair steps[69].

The MutS homodimer and its eukaryotic homologous heterodimers MutSα and MutSβ together with the MutL family of ATPase/endonuclease[70] detect replication errors in DNA[71 72]. ATP/ADP induced conformational changes in the two ABC domains of the MutS dimers control distal DNA and mismatch binding domains and the overall structure of the damage sensor[73 74]. ATP switches the sensor into a sliding clamp[75 76] that can diffuse along DNA on its own but remains attached to a mismatch in the presence of MutL[77]. MutS:DNA complexes were the first atomic resolution structures of “full” ABC protein complexes and also hinted an asymmetry between the two chemically equivalent ATP binding sites, because ADP was firmly bound at one NBD, but not the other in the homodimer[73 74].

Despite their overall structural differences, there are some interesting parallels between UvrA and MutS ABC systems. In both cases, DNA bending or melting – facilitated by the presence of the particular lesions – rather than the chemical nature of the lesion is an important part of the sensing reaction. Such a mechanism requires global conformational switches, which appear to be a hallmark of DNA repair ABC proteins.

An evolutionary highly conserved family of ABC proteins is broadly involved in the segregation and recombination of the genetic material. In the SMC/Rad50/RecN/RecF family, structural maintenance of chromosome (SMC) proteins and the DNA double-strand break (DSB) repair protein Rad50 are conserved in both eu- and prokaryotes while the DNA

repair/recombination factors RecN and RecF are only found in bacteria. In this family, the NBDs can directly bind to DNA, but also interact with other subunits and domains, including a long coiled-coil domain that is a characteristic of most but not all members of this family. SMC proteins function in the cohesion and/or structural organization of chromosomes, but also have roles in DNA repair. They consist of an ABC type NBD, a long coiled-coil domain and a dimerization domain (forming a "hinge"). NBD and dimerization domains are situated at both ends of the coiled-coil domain. Two SMC proteins form constitutive homo- (prokaryotes) or heterodimers (eukaryotes) via their dimerization domains[78]. The NBDs are additionally bridged by "kleisin" subunits[79 80], generating large proteinaceous SMC- kleisin-SMC rings that encircle DNA[81-83]. The encircling of DNA is a critical step in the cohesion of sister-chromatids by cohesin. A central question is how DNA enters the proteinaceous ring? The loading and unloading reactions were recently reconstituted *in vitro*[84,85]. These and other data[86 87] show that DNA-stimulated ATP binding and hydrolysis of the two opposing NBDs result in a transient dissociation of the kleisin subunit, resulting in the "transport" of DNA into and out of the rings. An alternative point of entry is proposed to be the dissociation of the hinge[86].

Rad50 is a DNA binding ABC protein that forms an evolutionary conserved complex with the DNA endo/exonuclease Mre11. Like SMC proteins, Rad50 possesses a long coiled-domain with an apical dimerization domain, forming ring like or extended tethered structures[88 89] (Figure 1c). The NBDs directly bind to dsDNA[7 90 91] and also interact with the nuclease dimer Mre11[92-94]. In the presence of hydrolysable ATP, Rad50's NBDs activate Mre11's exo- and endonuclease activities to cleave 5' strands near DNA double-strand breaks[95]. The precise mechanism is still rather unclear and may include unwinding of DNA[90] or structural modulation of the Mre11 dimer[93].

Of note, in the context of chromosome-associated ABC proteins of the SMC/Rad50/RecN/RecF family, the NDBs emerge as direct DNA binding modules, which is a remarkably different function compared to the protein-protein interactions of NBDs in most other ABC proteins. Several members of the SMC/Rad50/RecN/RecF family are highly conserved in all phyla, raising the important question whether nucleic acid binding or protein-protein interacting NBDs arose first in evolution.

The ABC type nucleotide-binding domain

The first atomic structure for an ATP binding cassette NBD was obtained for the bacterial histidine permease HisP[96]. The crystal structure in complex with ATP revealed an L-shaped, two-lobed structure for ABC type P-loop NTPases (Figure 2). At the core of the NBD is a bent, mostly parallel six-stranded β-sheet with four flanking α-helixes. Three helices ("B","C","D" in Figure 2) are situated on the convex side of the sheet, while helix "A" – immediately following the phosphate-binding loop (P-loop) - is situated on the concave side.

The P-loop connects strand "1" and helix "A" and binds the nucleotide phosphate groups. This NTP binding core is conserved to other P-loop NTPases, e.g. the "RecA" recombinase[97] (Figure 2). ABC type NBDs possess two large insertions/additions that typically mediate macromolecular interactions. The first insertion/addition forms an antiparallel β-sheet on the other side of helix "A". In ABC exporters, the TMDs are fused to this N-terminus via a linker loop. Most significantly, the large loop connecting the two β-sheets forms the tip of Lobe I and is a central element in the specific interaction and chemo-mechanical coupling of the NBD with other protein domains or macromolecular ligands (see below).

A second, large insertion is situated between strand "2" and helix "B". This helical insertion forms e.g. the large coiled-coil domains in chromosome-associated ABC proteins, or a helical subdomain in ABC transporters and also mediates interactions with associated domains and macromolecular ligands. However, it also contains the signature motif at the N-terminus of helix "B" that is a critical transactivation element for ATP-dependent conformational changes and ATP hydrolysis.

NBD dimer structure and mechanism of NTP hydrolysis

The structure of the NDB of Rad50 in complex with a non-hydrolyzable ATP analog[7], structures of the MutS mismatch proteins in complex with ADP[73 74], and subsequent structures of the isolated NBDs of an ABC transporter in complex with ATP[98] as well as the structure of the full BtuCD ABC transporter[30] provided a first architecture of NBD dimers in apo/ADP and ATP states.

In the ATP state, two NBDs oppose each other face-to-face and sandwich two ATP molecules in their dimer interface (Figure 3a,b). In the "engaged" conformation, ATP bound to the Walker A motif of one NBD is bound in "trans" by the highly conserved "signature motif" (also denoted C motif or LSGGQ motif) of the other NBD (Figure 2b). More precisely, the signature motif binds the ATP γ-phosphate via the conserved serine and 2nd glycine, providing a mechanism how hydrolysis of ATP to ADP leads to separation of the tight NBD dimer. Mutations in the signature motif that prevent NBD dimer engagement typically inactivate ABC proteins[99-102].

Besides the Walker A and signature motifs, several other motifs are highly conserved in the NBDs and are important for conformational control and NTP hydrolysis (Figure 3c). In particular, the catalytic water is positioned by the "dimerization" D-loop and the Walker B glutamate from the two opposing protomers. Furthermore, a highly conserved histidine (H-switch) can stabilize the negative charge of the glutamate and catalytic water, and that of the γ-phosphate in the transition state, as observed in the structure of maltose transporter in complex with the transition state analog ADP-VO_4^{3-} [103]. However, while this histidine is

conserved in most ABC proteins, SMC proteins lack it. In general, ABC proteins hydrolyze ATP but some can utilize - promiscuously or even preferentially - other NTPs as well[62 104]. The base moiety is specifically stacked to the A-loop element. The A-loop region can also forms specific hydrogen bonds to the base and ensure adenine base specificity[7 73 74 96 105]. Finally, a glutamine residue on lobe II forms an active site magnesium ligand and repositions lobes I and II as a function of NTP[7] and possibly also NDP[106] binding. Indeed, Rad50 NBDs can adopt two conformations that are stabilized, as a function of bound nucleotides, by two alternative ion pair and hydrogen bonding networks[107]. In one conformation, promoted by NTP/NDP binding and formation of the Q-loop:magnesium interaction, lobes I and II are oriented in such a way that the signature motif helix and the P-loop properly align in the closed dimer. Thus, NTP binding and hydrolysis can lead to both intra- and inter-NBD conformational changes as molecular basis for the powerstrokes.

Apart from ATP hydrolysis activity, the CFTR chloride channel exhibits adenylate kinase activity[108] (ATP + AMP ⇔ 2 ADP). The adenylate kinase reaction releases little free energy under physiological conditions and is in principle suitable for CFTR, which regulates the passive flow of ions. It is interesting to note that pioneering structural modeling of the ATP binding cassette was based on a structure of adenylate kinase[109]. Subsequently, adenylate kinase activity in addition to ATP hydrolysis activity has been reported for the DNA repair enzyme Rad50[100] as well as for the archaeal SMC protein[110]. Since the ATP hydrolysis reactions nevertheless dominate in these proteins, a physiological role for adenylate kinase reactions is not established. Even for CFTR, the relevance of adenylate kinase versus ATP hydrolysis remains controversial and there are arguments for both types of activities[111 112]. Definite mechanistic insights would tremendously benefit from a structural framework for CFTR, which is still lacking with the notable exception of a lows resolution EM structure[113] and a high-resolution structure of NBD1[114].

A key point of ABC enzyme mechanism is the question how many NTPs need to be hydrolyzed per cycle and whether the two active sites act in a concerted or a sequential manner. Many ABC proteins are assembled as homodimers and strong cooperativity of NTP hydrolysis argues for a concerted mechanism of NTP hydrolysis in these cases[115-119]. In addition, numerous structures revealed symmetric NTP-bound states, arguing for concerted NTP hydrolysis as well. However, it is also well established that some ABC proteins such as the mismatch repair proteins use a sequential, asymmetric mechanism of NTP binding and hydrolysis through its functional cycle[72]. In accordance, structures of some ABC proteins revealed a clear asymmetry with respect to conformation or nucleotide state[73 74 120 121]. Thus, at least for several heterodimeric NBDs, sequential NTP binding and hydrolysis might be a key aspect of the functional cycle.

Some ABC exporters such as for instance TAP only contain one functional ATPase site. In these cases, presumably only one ATP is hydrolyzed per functional cycle. Mutational analysis furthermore suggested that in the homodimeric ABC protein histindine permease, inactivation of one of the two NTP binding sites still results in a functional protein[122]. This suggests that the two active sites can be redundant and the hydrolysis of two NTPs per cycle is not always necessary. But this is not a general rule because in the maltose importer, inactivation of one NTP binding site results in an inactive protein[123]. Thus, it seems that the requirement for one or two NTPs per conformational cycle, the cooperation of the two NTPase active sites, and a concerted or sequential NTP binding and hydrolysis is realized in different ways, showing a remarkable adaptability of the chemo-mechanical engine.

In any case, the motifs required for NTP hydrolysis originate from both NBDs and NTP hydrolysis is mechanistically strictly coupled to NBD dimer formation. The potential charge by the signature helix dipole and the ordering of D-loops by protein-protein contacts in the NBD dimer are reminiscent of the transactivation of the hydrolysis of sandwiched NTPs between protomers in RecA filaments[124] and in the complex of small GTPases with activator proteins[125] (Figure 4).

Chemo-mechanical coupling and allosteric control

The intra and inter-NBD conformational changes are coupled to the conformation of function-associated domains in ABC transporters via an element that is denoted “coupling helix”, which is formed at a loop at the cis side of each TMD (Figure 5a). This helix binds to lobe I near the interface to lobe II and directly couples the position and orientation of the NBDs to the conformation of the TMD dimer[30 31]. Closing of the NBD dimer will induce a more closed state of the TMDs at the cis side and therefore promote the outward-facing conformation. The coupling helix can also transmit allosteric changes induced by ligand binding to the TMDs to the NBDs[38], it works both ways by thermodynamic reasoning. Mutations in the coupling helix result in defective ligand transport in a patho-physiological context[126].

Chromosome-associated ABC proteins of the SMC/Rad50/RecN/RecF family do not possess a bona-fide equivalent of the coupling helix. A “signature coupling” helix, which is situated between the Q-loop and the adjoining coiled-coil domains plays together with the coiled-coil domain important functional roles in the allosteric coupling of the ATPase with the DNA binding and nuclease activities[94 107 127]. Recent results showed that also DNA binds via its backbone strands along the NBD dimer groove on lobes I and II (Figure 5b). Intriguingly the coupling helices of ABC transporters and the DNA strands at Rad50 occupy similar positions and reveal an unexpected convergent functional architecture with respect to allosteric control and chemo-mechanical coupling (Figure 5c). One DNA strand directly bridges the NBD dimer, providing a rational for the coupling of ATP induced Rad50 dimer

formation and DNA binding. Whether NTP hydrolysis induced conformational changes in turn modulate the structure of DNA requires further investigation but ATP-dependent DNA melting is indeed observed for Rad50-Mre11[90 128 129].

Comparative mechanisms of transport and chromosome-associated ABC proteins. Given the recent progress on the ATP cycle of chromosome-associated ABC proteins of the SMC/Rad50 family and ABC transporters, it is worthwhile to compare the two systems and derive conserved principles. It should be noted that different ABC transporters exhibit significant mechanistic variations with respect to dominant structural states and thermodynamics[44 130]. Here I will compare the mechanism of the maltose importer, which is understood quite in detail[131], with the Rad50/SMC protein family of chromosome ABC proteins (Figure 6). Both systems show ligand dependent allosteric activation, enabling closure of the NBDs and subsequent ATP hydrolysis.

The maltose transporter cycle can be formulated with four states. We start with the inward-facing conformation with separated NBDs (I)[132]. Binding of the maltose binding protein (MBP) in complex with maltose to the trans side of the TMDs induces a pre-translocation conformation where the NBDs are brought into close proximity (II)[38]. ATP binding to the NBDs can now switch the TMDs to the outward-facing conformation (III)[41]. This change also impacts the structure of MBP. As a consequence maltose is released and binds to the maltose-binding site in the TMDs. The transition from I to II to III would correspond to the allosteric activation of the ATPase. ATP hydrolysis induces a high-energy state that switches the enzyme to post-hydrolysis state (VI)[103] and, after ADP+Pi and MBP release, to the inward-facing conformation.

A related multi-step mechanism can be drawn for DNA repair ABC protein Rad50 in complex with the nuclease Mre11. In the absence of ATP, Rad50 does not bind DNA with high affinity and the NBDs are in an open state (I-II)[93]. ATP binding leads to the closed, DNA binding state of the NBDs[7 90 91] and DNA binding allosterically activates Rad50's ATPase[133]. These steps would be equivalent to steps I-III of the maltose transporter. It is unclear what happens next, though and the closed state leads to a paradox: while in this state the Rad50 NBD dimer binds DNA, at the same time it blocks Mre11's nuclease active sites although DNA cleavage requires ATP. Thus, another conformation, possibly induced by ATP hydrolysis, is needed where the nuclease has access to the DNA. ATP hydrolysis and conformational changes are suggested to melt DNA[90] or induce a conformational change in the nuclease dimer[93 134]. Both mechanisms would be somewhat analogous to the mechanism of ABC transporters, because the NBDs modulate the conformations of interacting macromolecules in an ATP-dependent fashion. A equivalent role of ATP induced NBD conformations in modulating the structure and interaction of associated macromolecules is

observed for the related cohesion complex. Here, DNA-stimulated ATP binding to or ATP hydrolysis by the SMC protein NBDs are required to load and unload DNA into and out of the cohesion ring[84] through modulation (i.e. opening by dissociation) of the interaction of SMC and kleisin subunits[86]. In summary, the NBD dimer engine emerges as a universal module to control the association and conformation of interacting macromolecules.

Outlook and future questions

Tremendous work over the past ten years outlined the architecture and basic transport mechanism of many ABC proteins, in particular ABC transporters. The majority of those were determined by crystallography of detergent solubilized proteins prokaryotic proteins. With the advancements of cryo-electron microscopy, as demonstrated e.g. with the structure of TAP, it will be very informative to visualize at high resolution ABC transporters in more physiological environments, perhaps utilizing nanodiscs. Of particular interest are of course P-glycoprotein and CFTR for their medical relevance. Less complete is the analysis of chromosome-associated ABC proteins and there are several important mechanistic aspects to be revealed, including loading complexes of SMC proteins and the nuclease active complex of Mre11-Rad50. In addition, the mechanistic cycle of ribosome-associated ABC proteins, such as for instance the ATP state of ABCE1 in the splitting reaction will need further studies. ABCE1 was observed in an unexpected asymmetric nucleotide state bound at the ribosome, raising the possibility that asymmetric nucleotide states could have a more widespread role in other ABC systems as well. I am confident that with the further development of cryo-electron microcopy and the reconstitution and structural analysis of more complex ABC protein assemblies and native states, we will see many exciting and unexpected results in the coming years.

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Figure Legends:

Figure 1. ABC protein architectures

Selected architectures of ABC proteins: a) ABC transporters, b) translation ABC proteins, c) DNA repair ABC proteins, shown as cartoon models. Nucleotides in some of the structures and the iron-sulfur clusters of ABCE1 are shown as colored spheres. DNA is depicted in light brown. The two nucleotide binding domains (NBDs) are shown in yellow and orange, highlighting a shared architecture of ABC proteins. Other domains of various ABC proteins are shown in blue. A large part of the coiled-coil of Rad50 is modeled for illustrative purposes and shown in grey. Protein data bank accession codes: *Staphylococcus aureus* Sav1866 Exporter: 2HYD; *Escherichia coli* MalKFGK$_2$ maltose importer bound to maltose binding protein MalE (green): 2R6G; *Pyrococcus abysii* ABCE1: 3BK7; *Escherichia coli* MutS: 1E3M; *Thermotoga martimia* UvrA: 3PIH; *Methanocaldococcus jannashii* Rad50-Mre11: 3AV0; *Pyrococcus furiosus* Rad50 zinc hook: 1L8D.

Figure 2. The ABC type nucleotide-binding domain

Comparison of the ABC type NBD (PDB accession number 1B0U) with bacterial RecA (PDB accession number 2REB). The ABC type NBD shares a conserved core (red/yellow secondary structures) with other P-loop NTPases but also possess a variety of specific insertions (blue). Two large insertions generate an L-shaped structure with lobes I and II. ATP bound at the P-loop is shown as color coded stick model.

Figure 3. NBD dimer structure and mechanism of NTP hydrolysis

a) The two NBDs in an ABC transporter are positioned in a face-to-face orientation. ATP is sandwiched between opposing phosphate-binding Walker A and signature motif helices (red and purple). Shown is the structure of the BtuCD importer in complex with AMPPNP and substrate binding protein (SBP, green) (PDB accession code: 4FI3[135].)

b) Same as a) but rotated with omitted TMDs, showing the cooperative binding of ATP to two equivalent sites.

c) Detailed view of a composite ATP binding site. The structure is taken from the NBDs of an archaeal ABC transporter (PDB accession code: 1L2T[98]). Motifs are annotated. The magnesium ion (green) and selected water molecules (red spheres) are also shown.

Figure 4. Transactivation of NTP hydrolysis in protein-protein interfaces

Schematic comparison of NTPs sandwiched between protein-protein interfaces reveal common principles of transactivation of NTP hydrolysis: ordering of loops in the interface that

position the catalytic water as well as providing positive charges “in trans” that stabilize the transition-state (lysines “K” in RecA, signature helix “S” dipole in ABC ATPases, arginine finger “R” in the GTPase activating protein RhoGAP).

Figure 5. Chemo-mechanical coupling and allosteric control

a) Comparison of the AMPPNP bound ABC transporter Sav1866 (PDB accession 2ONJ) with b) the DNA repair protein Rad50 in complex with ATPγS and DNA (PDB accession 5DAC). In each panel, two orientations of the NBDs are shown. In the ABC transporter, the conformation of the TMDs is coupled to the position and conformation of the NBDs via the coupling helix (magenta). A similar type of allosteric coupling by DNA is seen in the DNA repair protein Rad50. The DNA strands bind to equivalent sites on NBDs than the coupling-helix of ABC transporters.

c) Scheme for the conformational coupling between the distance and orientation of the NBDs with those of the TMDs. This coupling forms the basis for allosteric activation and the NTP hydrolysis driven powerstroke.

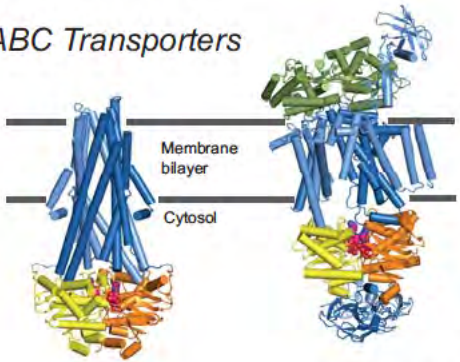
d) Equivalent scheme for chromosome-associated ABC proteins such as Rad50. Here DNA binding is linked to NTP-dependent NBD dimer formation and NTP hydrolysis.

Figure 6. Comparative mechanisms of transport and chromosome-associated ABC proteins.

a) Proposed conformational cycle of the maltose importer. Maltose binding protein (green) bound to maltose (green sphere) bind the trans side of the ABC transporter and induce a pre-translocation conformation, enabling a further switch to an outward-facing conformation through ATP-dependent NBD engagement. Maltose is released through a change in maltose binding protein and binds to its site in the TMDs. ATP hydrolysis induces a post-hydrolysis state that leads to maltose release at the cis side.

b) Equivalent scheme for the DNA repair enzyme Rad50 in complex with the nuclease (nuc) Mre11. ATP binding to Rad50 induces a closed state that results in a DNA binding conformation. In this conformation, however, the nuclease sites of Mre11 are buried and inaccessible for the DNA. A further state, possibly promoted by ATP hydrolysis, must bring nuclease and DNA together (see text).

a) *ABC Transporters*



Sav1866
ABC exporter

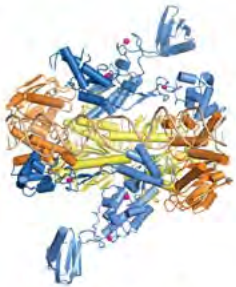
MalEFGK2
ABC importer

b) *Translation ABC proteins*

ABCE1 ribosome
splitting factor

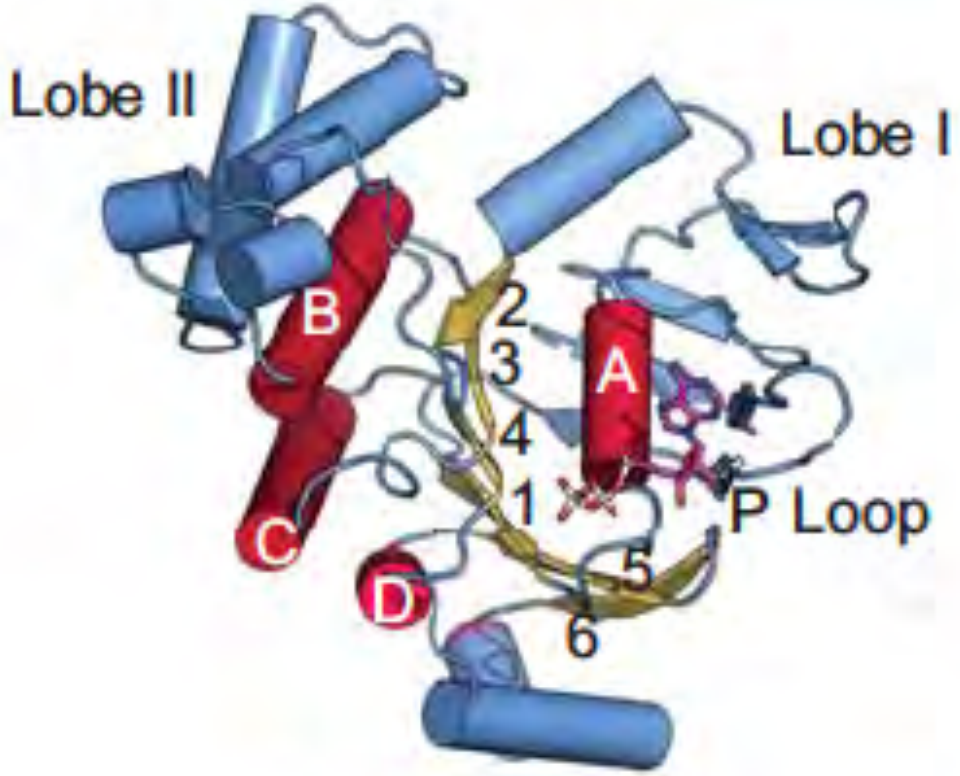
c) *DNA repair ABC proteins*

MutS DNA
mismatch sensor

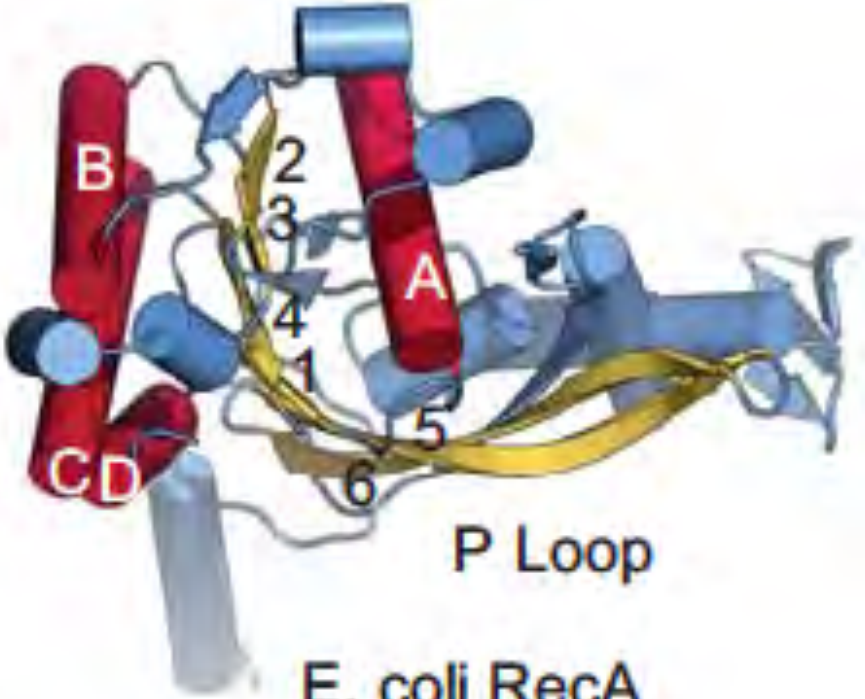


UvrA DNA
damage sensor

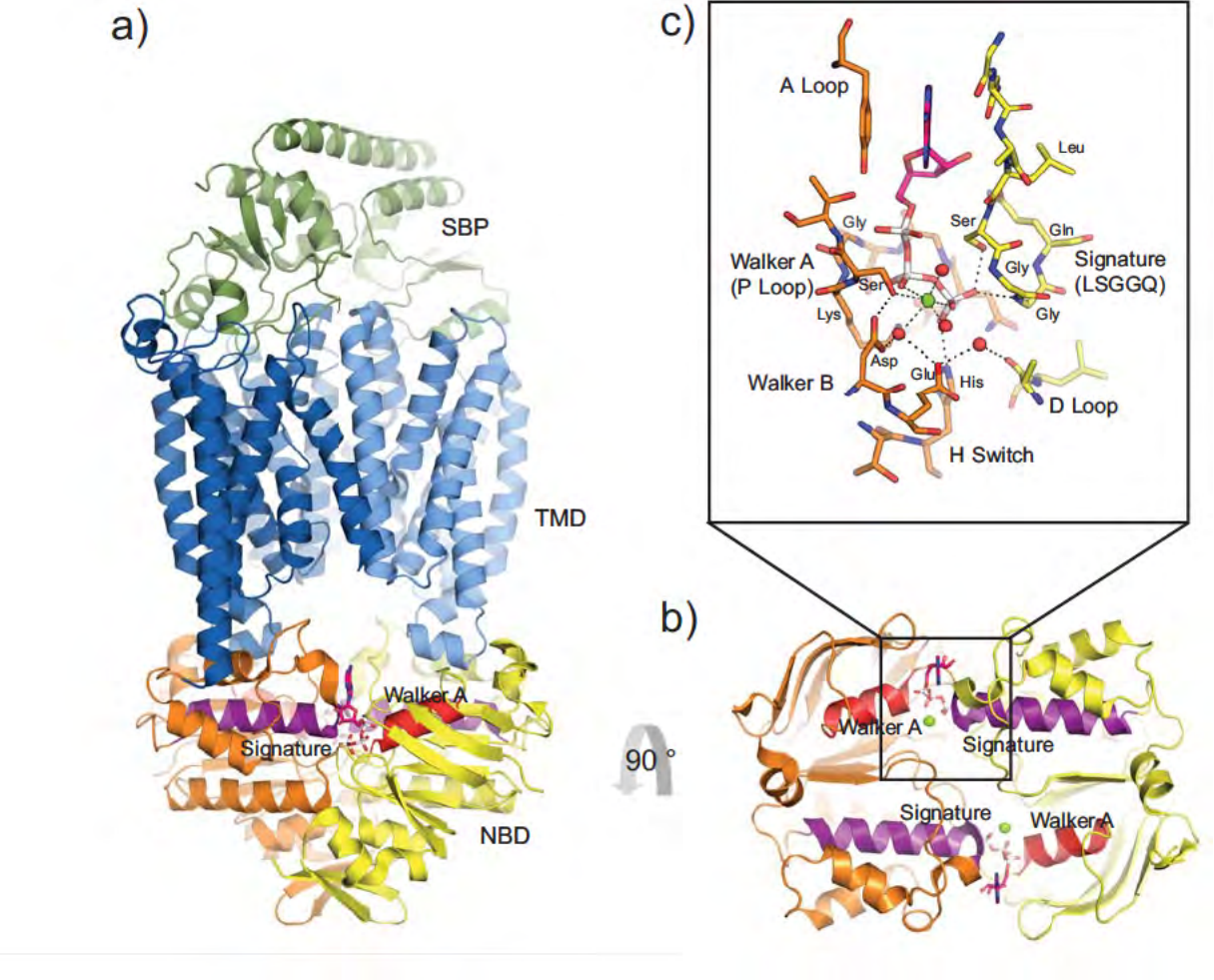
Mre11-Rad50
DNA double-strand break
repair nuclease

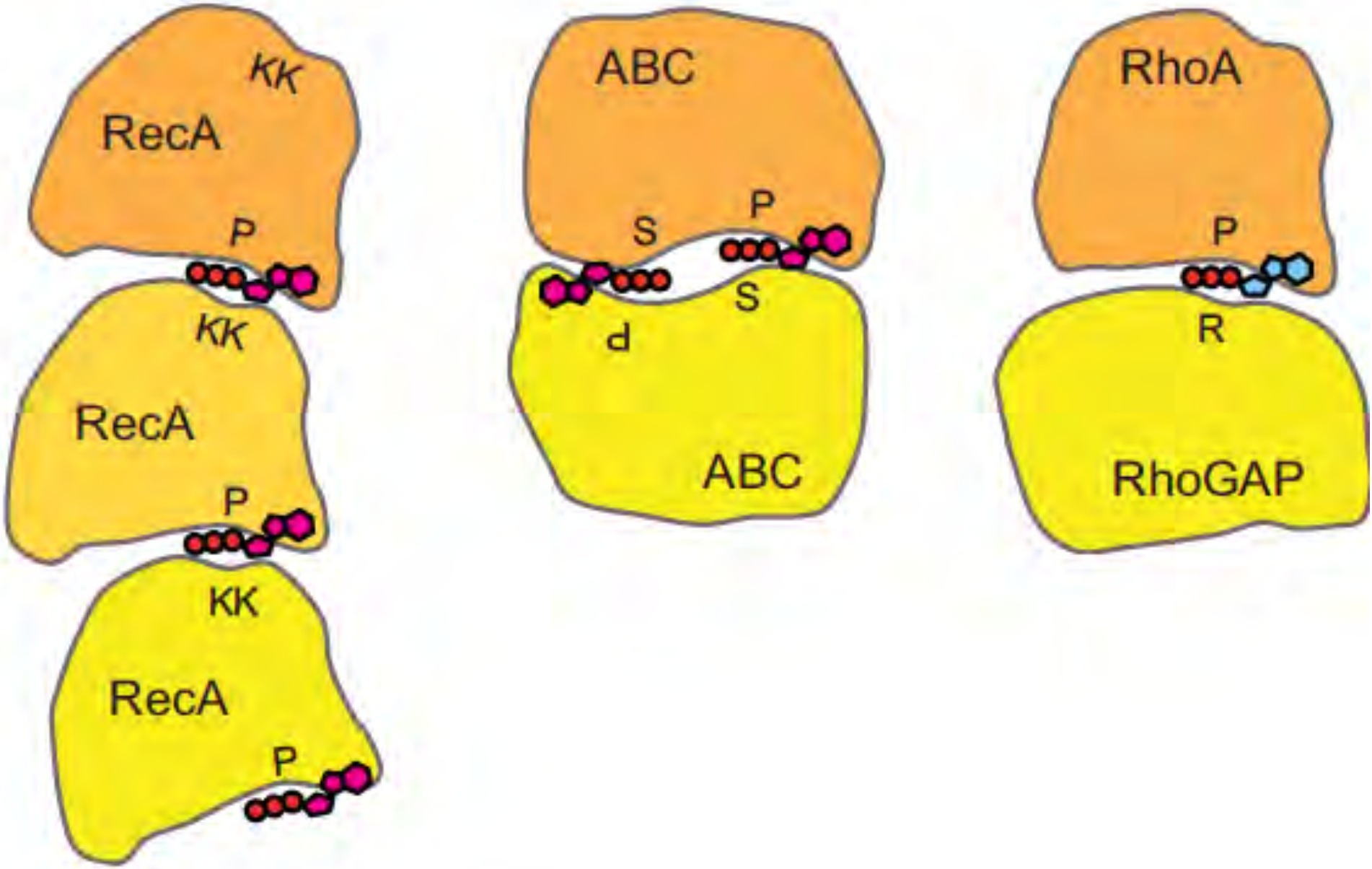


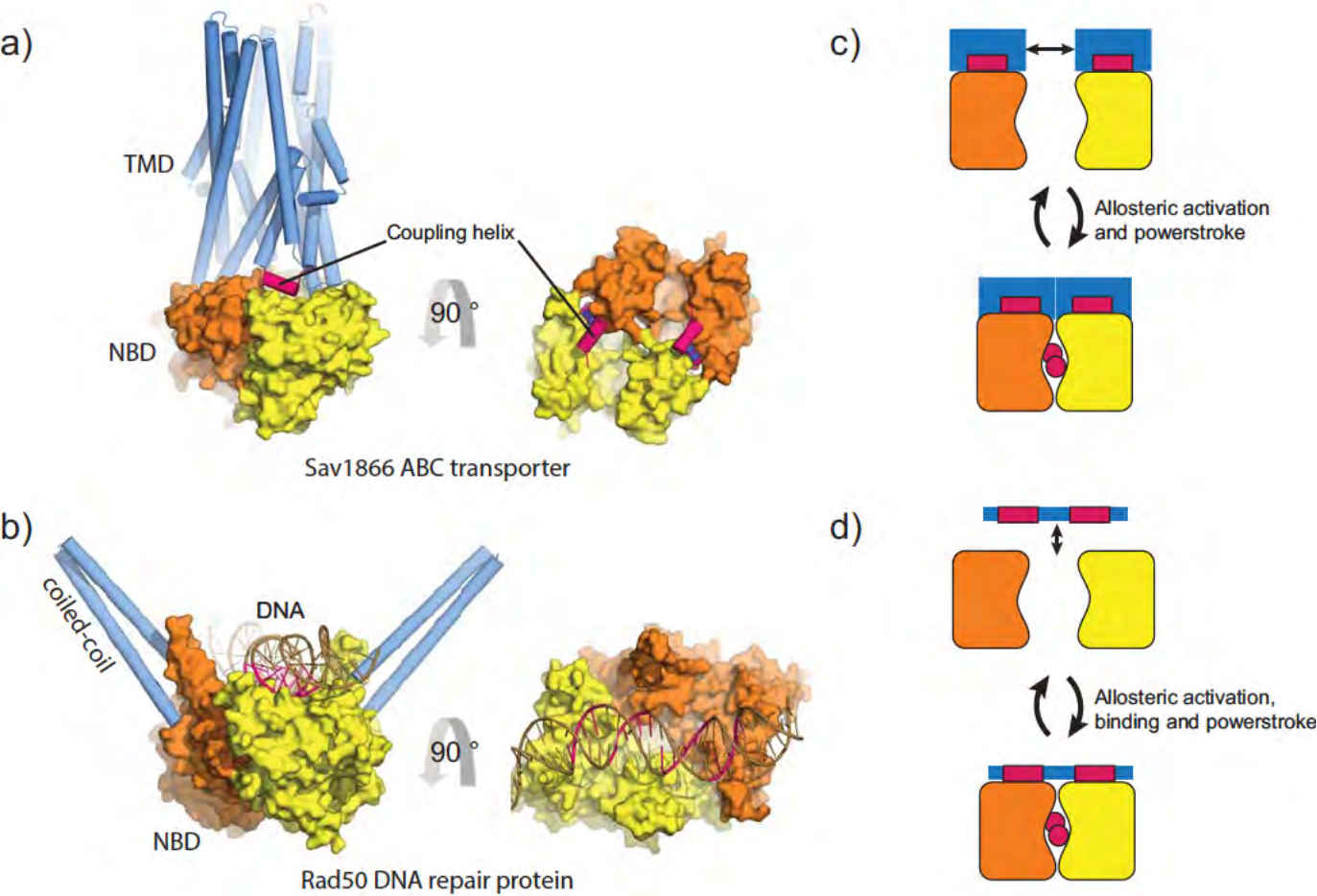
ATP binding cassette (HisP)

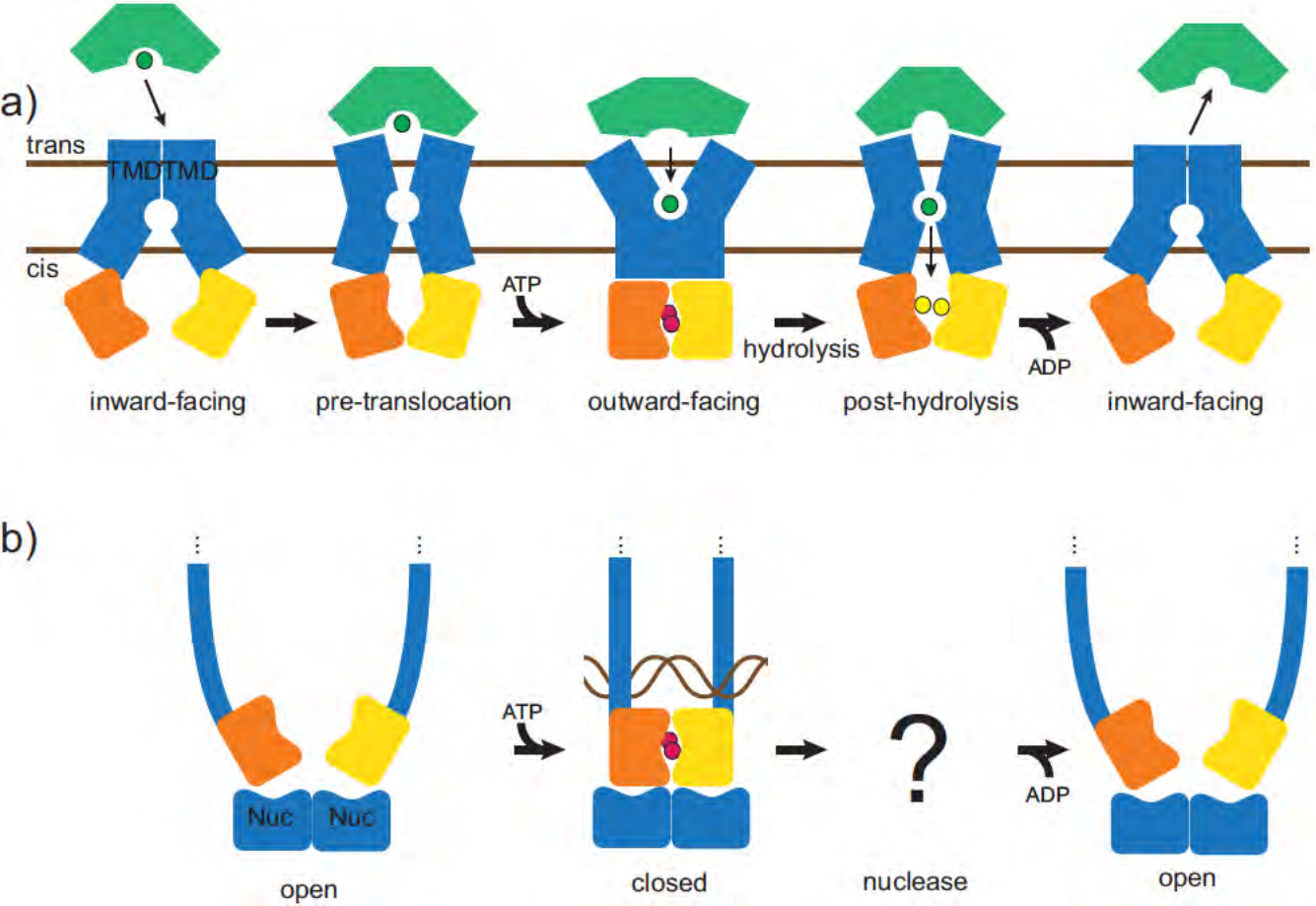


E. coli RecA


a)
SBP
TMD
Walker A
Signature
NBD
90 °
b)
Walker A
Signature
Signature
Walker A
c)
A Loop
Leu
Gly
Ser
Gln
Walker A
(P Loop)
Ser
Gly
Signature
(LSGGQ)
Lys
Gly
Asp
Glu
His
Walker B
D Loop
H Switch


KK
RecA
P
KK
RecA
P
KK
RecA
P
ABC
S
P
S
d
ABC
RhoA
P
R
RhoGAP


a)
TMD
Coupling helix
NBD
90 °
Sav1866 ABC transporter
b)
coiled-coil
DNA
NBD
90 °
Rad50 DNA repair protein
c)
Allosteric activation
and powerstroke
d)
Allosteric activation,
binding and powerstroke


a)
trans
TMD
TMD
cis
ATP
hydrolysis
ADP
inward-facing
pre-translocation
outward-facing
post-hydrolysis
inward-facing
b)
Nuc
Nuc
ATP
?
ADP
open
closed
nuclease
open