cal protein highlights what is not said—the potential energy function fails to fold β sheet structures. This problem is also present in all atom simulations and other knowledge-based potential energy functions. Attempts to solve this problem have developed multibody cummulant expansions of the interaction between amino acids in a chain (Liwo et al., 2001; Eastwood et al., 2003).

The origin of this multibody interaction potential could be found in solvent-mediated interactions (Papoian et al., 2004). Explicit solvent simulations of protein folding equilibrium on protein A (Garcia and Onuchic, 2003) showed that protein desolvation, helix formation, and folding occur cooperatively and in synchronization. Although challenges remain, clear progress has been made toward the ultimate goal of protein folding prediction.

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Variations on the ABC

The single-chain twin-cassette ABC-ATPase RLI resembles other ABC nucleotide binding domains (Karcher et al., 2005, this issue of *Structure*), but seems to convey its mechanochemical ATP-dependent signals through a novel "hinge" region and/or an N-terminal FeS cluster, at sites where other ABC-ATPases have regulatory domains.

ATPases from the ATP binding cassette (ABC) superfamily are involved in important cellular processes in all kingdoms of life. The best known members are the transmembrane ABC transporters that are responsible for the active uptake and efflux of substances across cell and organelle membranes. Other ABC-ATPases are water soluble and function in, for example, DNA repair processes (MutS and Rad50 proteins), chromosome condensation (SMC), or ribosome biogenesis (RLI). The biomedical relevance of ABC-ATPases is high, as functional defects and aberrant expression levels are associated with a variety of human diseases including cystic fibrosis, cancer predisposition syndromes, and multidrug resistance of tumor cells.

ABC-ATPases form dimers of two nucleotide binding domains (NBD) that interact with additional substrate binding and regulatory domains. Each NBD consists of two lobes, in which lobe I contains the conserved Walker A and B motifs in a RecA-like fold, and lobe II is a helical domain that contains a so-called signature motif, specific for the ABC-ATPase family, and a structurally diverse region (SDR).

The NBD dimer has two composite nucleotide binding sites. ATP binding induces a movement of the two NBDs toward each other in such a way that the signature loop of one NBD interacts with the nucleotide that is bound by the Walker motifs on the other NBD. While in some proteins, NBDs dimerize only when bound to ATP, and dissociate upon ATP hydrolysis, additional structural elements in other members of the family ensure that the two NBDs are associated at all times. In either case, there are substantial movements upon ATP binding.

These ATP-dependent conformational changes are propagated toward other regions in the ABC protein and mediate signal transduction or performance of work, such as channel opening in the transporters or a change in interaction with DNA by MutS. In most cases, the ATP hydrolysis step itself is seen as a resetting of the structure for new cycles. Structural models for a variety of single ATP binding domains and NBD homodimers are available, including several that show the ATP-dependent conformational changes (reviewed in Hopfner and Tainer, 2003; Higgins and Linton, 2004).

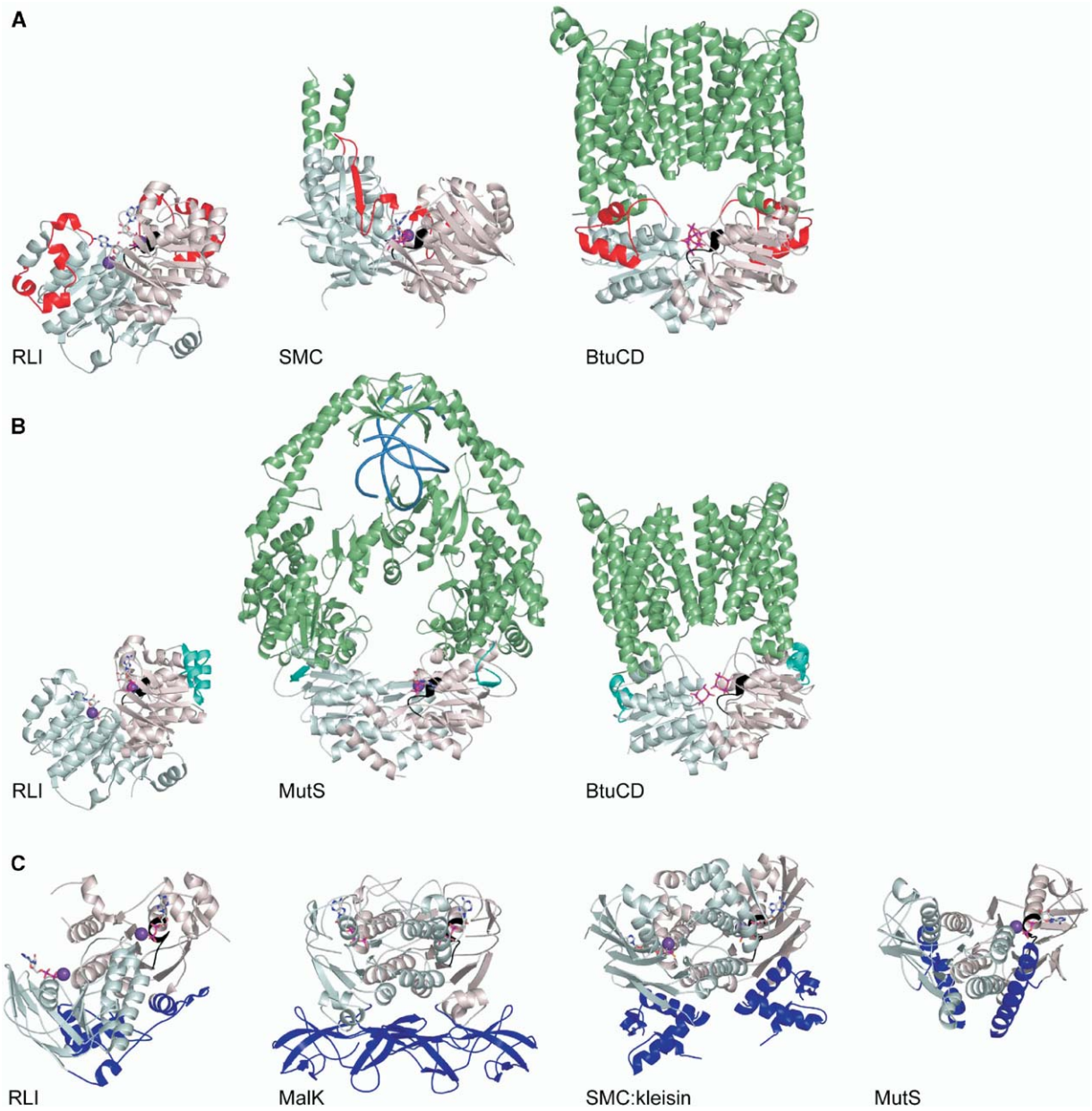

Figure 1. Possible Interaction Regions of ABC-ATPases

Comparison of RLI structure with other ABC-ATPases showing the two NBD cassettes (NBD1, light pink; NBD2, light blue) with bound nucleotide in ball-and-stick representation and magnesium as a purple sphere. Proteins are superposed and aligned onto their NBD1 Walker A motifs (depicted in black).

(A) RLI does not interact with substrate binding domains via its structurally diverse region (SDR). The structurally diverse regions (Schmitt et al., 2003) in ABC-ATPases are located between the Q loop and the signature loop on each NBD and are colored red. In RLI this region is nonessential. In SMC, the SDR interacts with the base of the coiled-coil domain (green). In the ABC transporter BtuCD, the SDR contacts the membrane spanning subunits (green).

(B) The helical insertion in RLI resembles interaction regions in BtuCD and MutS with substrate binding domains. The helix-loop-helix motif in RLI and corresponding regions in DNA repair protein MutS and the transporter BtuCD are indicated in cyan. In RLI, this motif may mediate interactions with the N-terminal FeS cluster domain. In MutS, the corresponding region connects to the long helix toward the DNA clamp domains (green). In BtuCD, a corresponding helical insertion interacts as well with the membrane spanning subunits.

(C) The RLI hinge region resembles the interaction site of MalK, SMC, and MutS NBD dimers with regulatory domains or subunits. In RLI, a novel hinge region (dark blue) interacts extensively with the NBD dimer interface. In the MalK transporter, this side of the dimer sandwich interacts with its regulatory domains, and the SMC protein with the kleisin regulatory subunit. In *E. coli* MutS, the (truncated) helix-turn-helix motif, involved in tetramerization, is located at approximately this site.

In this isssue, Karcher et al. (2005) describe the crystal structure of RLI, a conserved ABC-ATPase. RLI is an essential enzyme in ribosome biogenesis and formation of translation initiation components. In addition to the two ABC-type nucleotide binding domains, RLI contains a putative iron-sulfur cluster (FeS) binding domain, which is missing from the structure described in this issue, and a hinge domain that is tightly bound along the backside of the active site cleft.

RLI is a twin-cassette ABC-ATPase, meaning that its two NBDs are located on a single polypeptide chain. Nevertheless, the NBD1 and NBD2 in RLI are similarly arranged compared to other ABC proteins, with two ADP·Mg moieties sandwiched in composite active sites formed by Walker A and B motifs from one subunit and the signature motif of the opposing subunit (Figure 1). The distance between these motifs and the expected positions of the ATP γ-phosphate is approximately 11 Å, indicating a considerable conformational change upon ATP binding. In fact, the NBD arrangement resembles that of the vanadate bound NBDs of the ABC transporter BtuD (Locher et al., 2002) most closely, and requires a 40° rotation of one NBD toward the other in order to overlay with the ATP bound state of the dimer as seen in the archaeal MJ0796 transporter (Smith et al., 2002). This rotation is identical to the clamp-like motion that was described for the open and closed forms of the maltose transporter (MalK) (Chen et al., 2003).

In many ABC-ATPases, the NBDs communicate with associated domains through the SDR (Schmitt et al., 2003) (Figure 1A), probably by a movement that is initiated in the so-called Q loop. The membrane-spanning subunits in ABC transporters and the coiled-coils in the SMC/Rad50 subfamily interact with this SDR (Locher et al., 2002; Hopfner et al., 2000; Haering et al., 2004). In the RLI structure there are no domains interacting with the SDR, and amino acids in the SDR region are relatively poorly conserved. Furthermore, a mutational analysis shows that these amino acids are dispensable for yeast RLI function. Thus it seems clear that RLI does not use the SDR region for transmitting its signals.

So where do these ATP-dependent signals go? One option is a conserved helix-loop-helix insertion, located at the N terminus of the first NBD, that packs against the outside of the domain, adjacent to the truncated N terminus. The authors propose that this motif could mediate interactions of NBD1 with the FeS domain. They show that the latter domain is essential for RLI function. Similar insertions have been seen in other ABC-ATPases, where they interact with the transmembrane domain in BtuCD or connect the NBDs with DNA binding domains in MutS (Figure 1B) (Locher et al., 2002; Lamers et al., 2000).

A second option for ATP-dependent movements is in the hinge region, which is unique for the RLI structure. The hinge domain is tightly imbedded along the NBD1:NBD2 interface on the opposite side of the active site cleft. The large contact area with the NBD dimer is probably responsible for keeping the NBDs together in the absence of ATP. In their yeast mutagenesis analysis, the authors confirmed that the hinge region, and in particular its surface arginine patch, is essential for RLI function. Although the detailed positioning of the hinge domains is unique, that general area of the NBD surface presents the interaction sites of the MalK NBD dimer with its regulatory subunits, of the SMC dimer with the regulatory kleisin subunits, and the position of the C-terminal region in MutS that is responsible for its tetramerization (Figure 1C) (Chen et al., 2003; Haering et al., 2004; Lamers et al., 2000).

Apparently ABC-ATPases can use various levers to convey their signals. So far, however, the choice seems to be limited to three major regions that are important for transmission of the ABC-ATPase mechanochemical work. Additional structural investigation of complete ABC-ATPases and complexes with their effectors will be required to reveal whether these sites are the main locations for signal transduction or if ABC proteins have evolved additional interactions sites that resemble the wild variability of effector sites that is found for the small GTPases (Vetter and Wittinghofer, 2001).

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