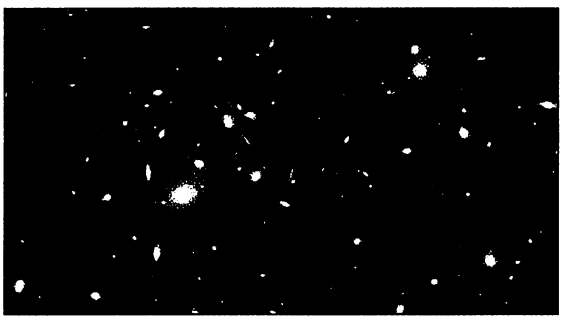
2218 (see the second figure). This cluster has received considerable attention because its large concentration of mass acts as a gravitational lens that strongly bends the light from more distant galaxies behind it (*8*). Abell 2218 is one of the densest concentrations of galaxies in the local universe, containing many hundreds of galaxies within the central 3×10^6 light years. For comparison, the Local Group of galaxies to which the Milky Way belongs contains only three major galaxies within the same radius.

Abell 2218 may therefore seem like a natural target for simultaneously detecting many galaxies through their 21-cm emission within the large field of view of the radio telescope used by Zwaan *et al.* Their observation of the cluster field was sufficiently long to allow atomic gas masses as small as those of our own Milky Way to be detected. But instead of detecting the hundreds of galaxies in the cluster core, only a single galaxy was seen, some 6×10^6 light years from the core.

The surprising absence of other 21-cm detections in the Abell 2218 cluster and its surroundings provides important insights into the physical processes that shape galaxies in such a dense cluster environment. Unlike most galaxies, which are relatively isolated and evolve relatively slowly with time, galaxies in the vicinity of a dense cluster evolve very fast. Galaxy interactions and mergers are much more likely when the density of galaxies is increased. Such interactions can disrupt the stability of galaxy disks, resulting in rapid processing of the atomic gas reservoir into new stars and in the loss of stars and gas from the parent galaxies.



Cluster surprises. This image from the Hubble Space Telescope shows the dense cluster of galaxies in Abel 2218. Zwaan *et al.* provide important insights into how galaxies behave in such a cluster.

The detritus of past galaxy interactions forms a dense medium, which settles into the gravitational potential well at the center of the cluster. Any galaxy passing through this dense intergalactic medium is doomed to a rapid demise, at least as far as its gas is concerned. The large relative velocity of such a galaxy compared with the intergalactic medium results in strong compression of the galactic gas that is tightly gravitationally bound to the galaxy and complete stripping from the galaxy of the gas that is not strongly bound to it. The galaxy detected by Zwaan *et al.* is the only one in the field of view that has not yet been stripped of gas by the cluster.

Earlier indications for the efficiency of these processes were seen in 21-cm observations of the galaxy cluster Abell 2670 at the smaller redshift of 0.08 (*9*). We will likely need to study clusters at redshifts of at least 1 or 2 to witness the assembly of galaxy clusters and the early evolution of galaxies. This is one of the primary motivations for considering construction of the "Square Kilometer Array" (SKA), an internationally funded radio telescope with two orders of magnitude greater sensitivity than is now available (*10*). With some good fortune, we can hope to make this next step in much less than the 50 years it has taken us to advance from the Milky Way to Abell 2218.

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PERSPECTIVES: STRUCTURAL BIOLOGY

The xyz of ABC Transporters

Christopher F. Higgins and Kenneth J. Linton

A cell must selectively translocate molecules across its plasma membrane to maintain the chemical composition of its cytoplasm distinct from that of the surrounding milieu. The most intriguing, and, arguably, the most important membrane proteins for this purpose are the ABC (ATP-binding cassette) transporters. These proteins, found in all species, use the energy of ATP hydrolysis to translocate specific substrates across cellular membranes. The chemical nature of the substrates handled by ABC transporters is extremely diverse—from inorganic ions to sugars and large polypeptides—yet ABC transporters are highly conserved. Mutations in the genes encoding many of the 48 or so ABC transporters of human cells are associated with diseases such as cystic fibrosis, adrenoleukodystrophy, Tangier disease, and obstetric cholestasis. Overexpression of certain ABC transporters is the most frequent cause of resistance to cytotoxic agents including antibiotics, antifungals, herbicides, and anticancer drugs.

Despite years of work on both bacterial and mammalian ABC transporters, particularly the multidrug resistance transporter P-glycoprotein, it is still not clear exactly how these proteins work. Enter Chang and Roth (*1*) on page 1793 of this issue with the first high-resolution structure of a complete ABC transporter, the MsbA lipid A transporter of the bacterium *Escherichia coli*. Theirs is a remarkable achievement, given the difficulties inherent in working with these membrane proteins. To obtain this structure they overexpressed and purified more than 20 bacterial ABC transporters, and tested a phenomenal 96,000 crystallization conditions with 20 different detergents.

ABC transporters comprise four "core" domains (*2*). Two transmembrane domains (TMDs) form a pathway across the membrane through which solutes move. These domains consist of multiple membrane-spanning segments (putative α-helices) and contain the substrate binding sites. The other two domains are highly conserved nucleotide-binding domains (NBDs), which are located at the cytoplasmic face of the membrane and couple ATP hydrolysis to substrate translocation. ABC transporters are conventional enzymes that undergo a conformation change in response to ATP binding and hydrolysis. This change alters both the affinity and orientation of the substrate binding sites. X-ray structures of isolated NBDs (*3*), and a low-resolution structure of intact P-glycoprotein (*4*) have provided many insights but have failed to reveal exactly how ABC transporters translocate substrates across cellular membranes.

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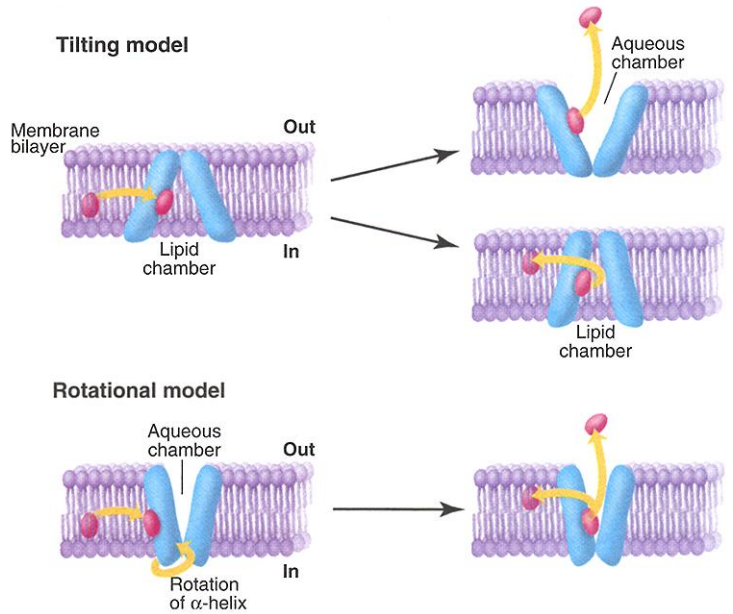
The Chang and Roth structure of the MsbA monomer reveals a plethora of important details (*1*). Although some of the conclusions are unsurprising, in that they are consistent with biochemical data, it is comforting to see them established beyond doubt. Perhaps most importantly, the data demonstrate that the pathway through which solute crosses the membrane bilayer is composed solely of the six membrane–spanning segments of each TMD, and that these segments are indeed α-helices. The structures of the NBDs (where resolved) closely resemble the structures previously determined for isolated NBDs. The NBDs are cytoplasmic and, as predicted from the biochemistry of P-glycoprotein (*5*), seem unlikely to contribute to the transmembrane pathway itself or to the "gate" that opens and closes this pathway. An intriguing new insight is that the TMDs and NBDs appear to be linked by a short bridge composed of intracellular TMD loops—this bridge is positioned to transduce signals between the NBD (via its signature motif) and its cognate TMD.

The monomer, however, is only half the story. MsbA is a "half-transporter" with only one NBD and one TMD. The complete transporter is presumed to be a homodimer composed of the two monomers. As expected, the crystal structure of MsbA does indeed reveal a dimer. The principal dimer interface is between the transmembrane α-helices within the outer leaflet of the membrane bilayer. This results in a large and rather unusual chamber within the membrane (or, rather, where the membrane is predicted to lie because the crystal structure was obtained in the absence of lipid). This chamber is formed between two angled blocks of six α-helices that correspond to the two TMDs and is closed at the extracellular face of the membrane (and, hence, does not appear to form a pathway across the entire bilayer). However, the chamber has two large side entrances between the TMDs that would be accessible from the inner leaflet of the bilayer. This chamber is likely to be lipid-filled to avoid an energetically unfavorable interface between the hydrocarbon chains of the phospholipids and water. These side entrances are consistent with evidence that P-glycoprotein (and presumably also lipid transporters such as MsbA) extracts substrate from the inner leaflet of the lipid bilayer (the flippase model) (*6*).

Because the nature of the dimer interface is crucial to the overall structure of the transmembrane pathway, it is perhaps appropriate to ask: Does the dimer in the crystals reflect the biologically relevant interface in the native protein? In favor is the fact that MsbA was purified as a dimer before crystallization, and that this was the only dimer interface observed despite each unit crystal consisting of eight monomers. Against is the fact that the dimer structure is not easily reconciled with existing data. First, although the MsbA structure is similar to low-resolution structures of two eukaryotic ABC multidrug transporters, P-glycoprotein and MRP1 (*4*, *7*), a key difference is that the chamber of MsbA is closed at the extracellular face of the membrane, yet the chambers of P-glycoprotein and MRP1 appear to have large extracellular openings. Furthermore, extensive cross-linking studies have identified α-helices that are adjacent to each other in P-glycoprotein but distant in the MsbA structure (*8*). Second, it is not immediately apparent how the MsbA structure can be extrapolated to other ABC transporters. Although many ABC transporters translocate hydrophobic substrates, many others handle highly hydrophilic substrates including sugars and inorganic ions. How such solutes could pass across the membrane through anything other than an aqueous pathway is not clear. An extreme example is CFTR (the cystic fibrosis transmembrane regulator), which unquestionably forms an aqueous pathway across the membrane through which chloride ions travel. Even for drug transporters closely related to MsbA, such as LmrA, there is biochemical evidence that the drug is extracted from the inner leaflet of the bilayer and translocated directly into the aqueous phase (*9*), implying that the chamber has an opening to the extracellular environment.

There are three ways in which these apparent discrepancies might be resolved. The first, and perhaps most likely, possibility is that any apparent inconsistencies are simply a reflection of the dynamic nature of ABC transporters. Second, despite their apparent evolutionary relatedness, it is possible that the transmembrane pathways (and therefore mechanisms) of different ABC transporters are very different. Thus, MsbA may not necessarily be a good model for other ABC transporters. This possibility, however, will not find favor with those who believe in the conservation of biological structure and function. It is also hard to reconcile with, for example, the clear evolutionary relatedness between MRP1 (which transports drugs and hydrophobic molecules), MRP5 (which transports cGMP), and CFTR (which is a chloride channel), despite the fact that their substrates are completely different. Third, we cannot rule out the possibility that the MsbA dimer interface is not the biologically relevant interface—purified MsbA was not shown to be active, and the protein was crystallized in the absence of lipids (required to maintain the activity of some other ABC transporters).



Solute translocation across cell membranes by ABC transporters. To transport a solute across a cell membrane (in this case in the outward direction), a substrate binding site must be alternately exposed to the intracellular and extracellular milieu. In the case of the bacterial lipid A transporter, MsbA, and multidrug transporters such as P-glycoprotein, the intracellular milieu is the inner leaflet of the membrane bilayer. The extracellular milieu is likely to be the aqueous phase, although for some ABC transporters (perhaps including MsbA) the substrate and lipid may be entirely shielded from water. There are two models for how a substrate binding site may be reorientated between the lipid and aqueous environments. **(Top)** In the tilting model, substrate enters the chamber from the inner leaflet of the bilayer and, following a conformation change in the transporter induced by ATP binding and hydrolysis, is released into the extracellular aqueous phase. In a variation of this model suggested for MsbA, the lipid-filled chamber does not open to the aqueous phase but reorientates so that the substrate is directed towards the outer leaflet of the bilayer. **(Bottom)** In the rotational model, rotation of the transmembrane α-helices of the ABC transporter reorientates the substrate binding site from the inner leaflet of the lipid bilayer to the aqueous chamber where the solute is released and either exits the cell or partitions back into the outer leaflet of the bilayer.

Of course, the structure of a protein is simply a means for getting at how the protein works. Two "big" questions keep researchers busy. First, how might ATP hydrolysis by the NBDs be coupled to substrate transport? There is strong biochemical evidence that the two NBDs of ABC transporters work in an alternating catalytic cycle, yet because only part of the NBD is resolved in the MsbA structure, it is not yet possible to ascertain whether the two NBDs interact directly with each other and, if they do, which amino acid residues are involved. Even more important is how the binding and hydrolysis of ATP by the NBDs is coupled to the conformational changes in the TMDs that mediate substrate translocation. The MsbA structure identifies amino acids in the intracellular loops of the TMDs that form a bridge that is likely to transduce conformational changes from the NBDs to the TMDs. However, the snapshot MsbA structure alone (in the absence of ATP and substrate) cannot answer these mechanistic questions.

The second "big" question is the nature of the transmembrane pathway, the substrate binding sites, and the conformational changes induced during the transport cycle. Chang and Roth propose an elegant and plausible model for lipid A transport by MsbA: Lipid A enters the chamber from the inner leaflet of the bilayer and, after an ATP-induced conformational change, is exposed to charged residues that create an unfavorable environment such that lipid A is "flipped" into the upper part of the chamber and ultimately into the outer leaflet of the bilayer (see the figure). However, as the authors themselves point out, this model cannot explain how many other ABC transporters operate.

Our two-dimensional crystallographic and biochemical studies of P-glycoprotein trapped at different stages in the transport cycle add another twist to the story (*10*). Unexpectedly, the membrane-spanning α-helices of the TMDs undergo a remarkable reorganization during the transport cycle. The most dramatic structural changes accompany binding of ATP to the NBDs, an event that results in loss of drug-binding affinity. Thus, the energy of ATP binding (rather than ATP hydrolysis) may provide the initial energy for translocation of substrate. Subsequent ATP hydrolysis and ADP/Pi release returns the transporter to its original configuration through at least one additional conformational intermediate. This reorganization appears to be due to lateral repacking of the α-helices within the plane of the membrane. One model for reorientating the substrate binding site is rotation of the transmembrane α-helices within the membrane (see the figure). An alternative model is helix "tilting"; of course, reorientation may depend on a combination of both. Crucially, the exceptional flexibility of the TMDs of ABC transporters may reconcile otherwise apparently incompatible data.

Although the structure of an ABC transporter is a necessary prerequisite, it is only one step in our efforts to unravel and understand the complex dynamic and vectorial processes that enable these fascinating proteins to translocate solutes across cellular membranes.

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PERSPECTIVES: APOPTOSIS

Till Death Us Do Part

Abigail Hunt and Gerard Evan

The wonder of multicellular organisms is that each individual cell seems to know what to do, where to be, and how to behave. Such remarkable self-organization relies in part on the surprising alacrity of cells to commit suicide, a process termed apoptosis, should they stray or be misplaced from their normal somatic compartment and so become deprived of the requisite social signals needed for their survival. Nowhere is this phenomenon more evident than in epithelial cells, which derive much of their positional information from their association with their neighbors and with the extracellular matrix. Deprived of such associations, epithelial cells typically undergo detachment-induced apoptosis, or anoikis. Such spontaneous suicide effectively confines epithelial tissues to their correct somatic compartments, ensuring the expeditious deletion of cells misplaced during development or through injury, and potently restraining the emergence of invasive malignancies. Accordingly, inactivation of anoikis is a critical step in the progression of epithelial cancers to an invasive and metastatic form. On page 1829 of this issue, Puthalakath *et al.* (*1*) show that detachment of epithelial cells from their extracellular matrix detonates an apoptotic bomb by triggering the release of the pro-apoptotic protein Bmf from the myosin V motor complex of the actin cytoskeleton.

Key players in the determination of cell survival and death are members of the Bcl-2/Bax protein family (*2*). Bcl-2/Bax family members fall into three general classes. Some, like Bcl-2 and its close homolog Bcl-x_L, suppress apoptosis and render a cell (at least temporarily) more resilient to a wide variety of lethal assaults including radiation, metabolic poisoning, growth factor deprivation, and even heat. They accomplish this not by mitigating the damage incurred by such insults, but rather by curbing the cell's suicidal response to the damage.

Other family members, such as Bax and Bak, closely resemble Bcl-2/Bcl-x_L in structure and share three of their four signature Bcl-2 homology (BH) regions. However, instead of suppressing apoptosis, they promote it, dimerizing with Bcl-2 and Bcl-x_L and antagonizing the protective activity of these proteins. More distant relatives are the "BH3-only" proteins whose apparent exclusive role is to promote apoptosis. The emerging consensus is that many of the diverse pathways that regulate cell survival and cell death have, as their terminal effector, one or more BH3-only proteins (see the figure). For example, the BH3-only proteins Noxa and PUMA are transcriptional targets of the p53 tumor suppressor protein, which is induced in response to DNA damage and promotes apoptosis (*3, 4*). The BH3-only protein Bid is cleaved in response to signaling through the Fas, tumor necrosis factor (TNF), or TRAIL death receptors; cleavage converts Bid to its active, proapoptotic form tBid (*5*). The lethal action of the BH3-only protein Bad is forestalled only as long as survival factor signaling through the serine-threonine kinase Akt/PKB pathway keeps it phosphorylated and sequestered by the cytosolic 14-3-3 proteins (*6*). The abiding image is one in which our cells are peppered with unexploded BH3 bombs, their fuses primed and, in some cases, lit, only to be quenched by survival signals from neighboring cells.

In their new work, Puthalakath *et al.* identify a BH3-only protein, Bmf, which appears to act as a detachment sentinel during anoikis. Identified initially through its interaction with the anti-apoptotic Bcl-2 family member Mcl-1, Bmf is, like its

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