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From scarcity to abundance: Adaptive strategies in adenosine triphosphate-binding cassette transporter function

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Adenosine triphosphate-binding cassette (ABC) transporters form one of the most ancient and functionally diverse protein superfamilies, mediating the translocation of substrates that span an expansive range of sizes, chemistries, and physiological roles. This diversity poses a challenge to unifying their mechanisms within a single conceptual framework. In this review, we examine recent advances that demonstrate how substrate properties, energetic constraints, and evolutionary pressures shape the molecular design and operating principles of ABC transporters. We discuss emerging insights into substrate recognition and selectivity in exporters and importers, revisit the physiological relevance of so-called 'futile' adenosine triphosphate (ATP) hydrolysis, and explore the role of stoichiometry as a regulatory and evolutionary variable. Together, these perspectives highlight common design principles that link molecular architecture to the functional demands of transport across the ABC family, offering broader insights for how protein systems adapt structure and energetics to diverse cellular challenges.

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Unmatched diversity of substrates

All living organisms devote a substantial portion of their genomes to encoding active transport systems—approximately 3–4% in humans, 7–8% in plants, and 8–9% in bacteria. These transporters cluster into roughly 10–11 major families, defined by sequence similarity and evolutionary origin [1]. Some exhibit a high degree of specialization, mediating the movement of a narrow range of substrates: F-, V-, and A-type adenosine triphosphate (ATP)ases primarily translocate protons or sodium ions, while P-type pumps are dedicated to inorganic cations such as Ca^{2+}, Na^{+}, K^{+}, Zn^{2+}, and Cu^{+}. Others, however, have evolved notable versatility. The solute carrier (SLC) superfamily, for instance, accommodates a wide array of substrates—including sugars, amino acids, neurotransmitters, nucleosides, vitamins, and organic ions—illustrating the adaptability of secondary active transport mechanisms [2].

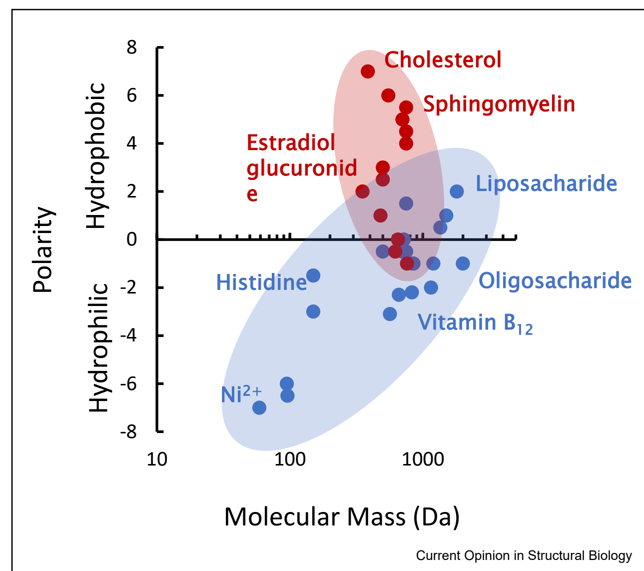
At the pinnacle of substrate diversity are the ATP-binding cassette (ABC) transporters, capable of recognizing and translocating virtually every major class of biomolecule from essential nutrients to toxic xenobiotics including antibiotics and chemotherapeutic agents. Their substrates differ dramatically in size, spanning roughly four orders of magnitude (Figure 1), from ions below 100 Da to macromolecules approaching 1 MDa [3]. Their substrates also vary in chemical nature and physiological function: ABC transporters handle ions, lipids, peptides, nucleotides, metabolites, and complex polymers (Figure 1), coupling transport to processes as varied as nutrient uptake, lipid trafficking, peptide secretion, and drug efflux. Importantly, they catalyze both import and export, maintaining both the acquisition of indispensable molecules and the removal of harmful ones.

Given the remarkable diversity in substrate size, chemical nature, and physiological context, it is little wonder that early attempts to formulate a unifying mechanistic model for ABC transporters proved unsuccessful. Rather, as we outline below, the diversity of mechanisms observed across the ABC family reflects an evolutionary response—one shaped by the size, chemistry, and functional breadth of the substrates they handle.

Exporters: Not so promiscuous after all

In ABC exporters, substrate recognition occurs within the transmembrane domains (TMDs). For years, their

Figure 1



An unmatched diversity of substrates. Shown are examples of transport substrates of ABC exporters (red) and importers (blue), plotted according to their molecular mass and polarity. Polarity values correspond to the logarithm of the octanol–water partition coefficient (log P). As illustrated, the physicochemical space of importer substrates is considerably more diverse than that of the exporters. ABC, adenosine triphosphate-binding cassette.

apparent promiscuity obscured identification of physiological substrates, leaving the relevance of many exported compounds uncertain. However, recent structural and biochemical studies have now defined the physiological substrates of several key systems with high confidence [4–9].

The physiological substrates of ABC exporters range from amphiphilic lipids and bile acids to polar nucleotides and peptides. Beyond these, exporters also expel numerous non-physiological compounds, earning a reputation for promiscuity. However, closer inspection reveals a recurring physicochemical pattern: most substrates—physiological or not—are medium-sized (200–1000 Da), amphipathic molecules enriched locally within membrane leaflets, bile canaliculi, or lipid droplets [10–12].

Similarities in substrate size, amphiphilicity (Figure 1), and localization explain the mechanistic convergence among exporters. All use large, flexible, predominantly hydrophobic, partially solvent-exposed cavities that favor transient, low-affinity binding, prevent trapping of amphiphilic molecules, and ensure rapid turnover. Because these substrates are abundant and often membrane-embedded, tight binding would be unnecessary, energetically costly, and detrimental to efficient transport.

Remarkably, the substrate-binding affinities of ABC exporters fall within a relatively narrow range—typically in the low-to mid-micromolar interval [13–16]. This convergence reflects an evolutionary optimization that couples transport efficiency to the physiological availability of substrates. Although exporters act on chemically distinct molecules, their intracellular concentrations also tend to lie within the micromolar range [17]. The close correspondence between binding affinity and substrate abundance (Figure 2a) ensures that transport operates efficiently under steady-state conditions, yet can scale proportionally when substrate levels increase, thereby maintaining homeostasis without risking cargo trapping or incurring the energetic cost associated with excessively tight binding and slow release. The physicochemical similarity between ABC-exporter substrates and natural lipids appears to play an important role in transport thermodynamics. Recent studies show that, in the outward-facing conformation, endogenous membrane lipids can enter the substrate-binding site, where they compete with bound cargo for transporter interactions and thereby facilitate its release [18].

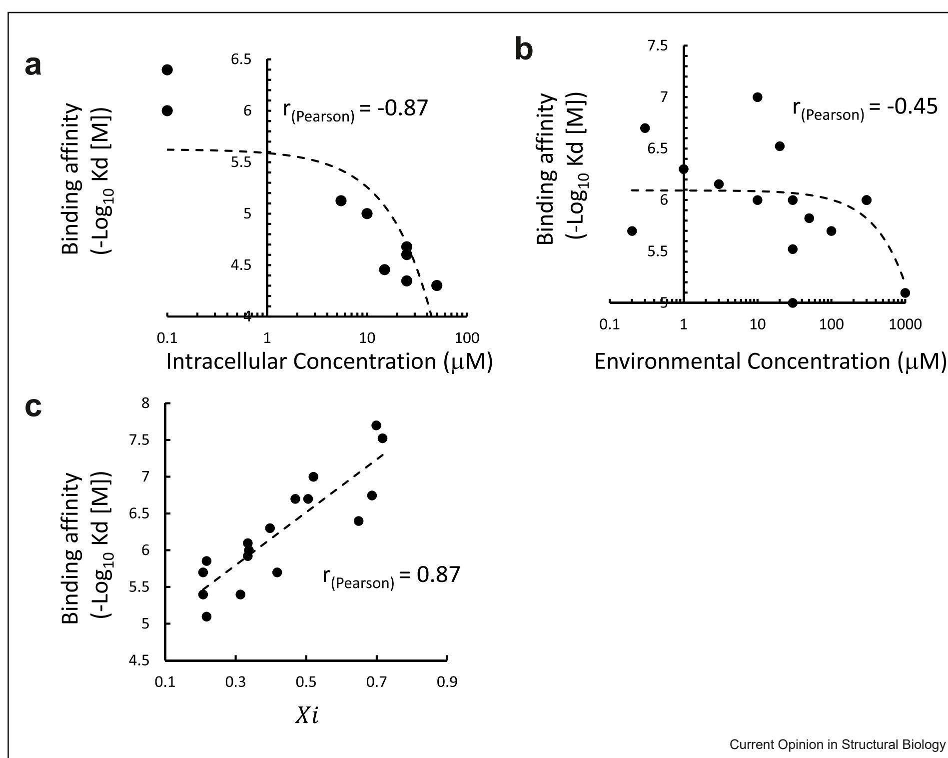
Although ABC exporters recognize substrates directly within their transmembrane domains, growing evidence indicates that accessory domains and regulatory segments can contribute to substrate recruitment and tuning of transport activity. Recent work on ABCC family members, for example, has shown that the regulatory (R) domain of ABCC2 participates in substrate engagement and modulates transport efficiency and specificity [19]. Such accessory elements likely provide an additional layer of control that fine-tunes exporter function under conditions of high local substrate availability, complementing the broadly permissive, amphipathic binding cavities that characterize this class of transporters.

Importers: First encounters

Substrate recognition by ABC importers is more demanding than in exporters as importers must discriminate among a vast array of environmentally available, chemically diverse molecules, whereas exporters act on a narrower set of intracellular amphiphiles. Importer substrates vary widely in size—from small ions (~60 Da, e.g., Ni^{2+}) to macromolecules exceeding 2000 Da such as oligosaccharides (Figure 1)—and occur across environmental concentrations spanning several orders of magnitude. These challenges drove the evolution of the defining feature of ABC importers: highly specialized substrate-binding proteins (SBPs), whose structural adaptability underpins their exceptional selectivity and sensitivity.

For exporters, intracellular substrate concentration emerged as the dominant determinant of substrate-

Figure 2



Correspondence between binding affinities and substrate concentrations. Correlation between the binding affinity of ABC exporters (**a**) or importers' SBPs (**b**) with the intracellular or environmental concentrations of their substrates, respectively. For exporters, a strong correlation is observed: higher binding affinities correspond to lower intracellular substrate concentrations, whereas lower binding affinities correspond to higher intracellular substrate concentrations. In contrast, for importers, binding affinities show only a very weak association with environmental substrate concentrations. (**c**) Correlation between the binding affinities of 17 SBPs for amino acids (Table S2) and the composite parameter *X*, which incorporates the energetic cost of biosynthesis, its environmental availability, its chemical stability and ease of reversible binding, and its functional indispensability. In all panels, the Pearson coefficient (r) is also shown. ABC, adenosine triphosphate-binding cassette; SBPs, substrate-binding proteins.

binding affinity (Figure 2A). We next asked whether the same relationship holds for importers. Examining the relatively homogeneous class of biogenic amino acids, we found that for the subset of 17 out of the 20 amino acids for which reliable and comparable SBP binding-affinity data are available (Table S1), environmental concentrations and binding affinities show only a weak correlation (Pearson r = −0.45; Figure 2B).

What other factors shape the diversification of substrate affinities in ABC importers? One candidate is biosynthetic cost: metabolically expensive amino acids are expected to select for tighter binding than inexpensive ones. Incorporating biosynthetic cost (*i.e.*, ATP required for synthesis) together with environmental concentration improved the model's predictive power, but the overall correlation remained modest (Pearson r = −0.59).

Building on previous analyses of transporter evolution and metabolic efficiency [20–23], we expanded the model by introducing two additional parameters: chemical manageability, reflecting the ease with which a substrate can be safely handled and reversibly bound (i.e., its chemical reactivity, redox sensitivity, and compatibility with aqueous binding pockets), and functional uniqueness, capturing the extent to which an amino acid performs irreplaceable biochemical roles as inferred from evolutionary substitution matrices [24,25].

Next, we assigned weight ranges for these four evolutionary constraints, relying on studies of metabolic efficiency, nutrient limitation, and environmental availability, chemical binding constraints in SBPs, and amino-acid interchangeability [20–25], and defined a composite parameter, hereafter referred to as X, calculated as:

$$X_i = w_C Cost'_i + w_s \mathrm{Scarcity}'_i + w_M Manageability'_i + w_U Uniqueness'_i$$

where Cost′ is the normalized energetic cost of biosynthesis, Scarcity′ represents the relative environmental availability of the substrate, Manageability′ reflects its chemical stability and ease of reversible binding, and

Uniqueness′ quantifies its functional indispensability within metabolism [26–32]. The coefficients w_C, w_S, w_M, and w_U represent their respective evolutionary weights, summing to unity (see methods for full details).

Optimization of these weights within biologically plausible bounds (see methods), constrained by comparative studies of evolutionary selection pressures and nutrient transport [20–22], yielded a strong correlation between the composite parameter and experimentally measured SBP affinities (Pearson $r = 0.87$, Figure 2C). This result indicates that the combined influence of these four factors explains a substantial portion of the evolutionary forces shaping substrate-binding affinities in ABC importers and provides a robust predictive framework for substrate recognition.

Wasting adenosine triphosphate: Myth or reality?

From an evolutionary perspective, one would expect ABC transporters to consume energy—through ATP hydrolysis—primarily when there is work to be done, namely when substrate is available. This expectation would manifest as low basal ATPase activity with strong substrate-dependent stimulation. Such behavior is indeed observed for some importers including the maltose transporter ($MalFGK_2$) and the osmoregulated OpuA systems [33,34], as well as for a number of heterodimeric exporters with asymmetric nucleotide-binding domains, exemplified by the sterol exporter ABCG5/ABCG8 and the transporter associated with antigen processing (TAP) [35,36]. In these heteromeric systems, the presence of a degenerate nucleotide-binding site is thought to act as a kinetic brake on the ATPase cycle, contributing to lower basal ATP hydrolysis and reducing energetically wasteful turnover in the absence of substrate (For more details on the role of asymmetric Nucleotide Binding Domains [37,38]).

However, many, if not most, ABC transporters hydrolyze ATP even in the absence of substrate [39–41]. This basal ATPase activity has been documented in exporters and importers across bacteria, plants, and mammals [42,43], and can be surprisingly high with little or no substrate stimulation [39,40,44,45]. The phenomenon is experimentally robust, reproduced in purified, reconstituted, and in situ systems [43,45].

Whether this apparent ATP ‘waste’ reflects physiology or artifact remains debated. Skeptics argue that continuous substrate presence—particularly for lipid exporters and flippases—may mask futile hydrolysis [46,47]. While plausible for some systems, this explanation does not apply universally. Several vitamin and transition-metal importers exhibit among the highest basal ATPase rates with minimal substrate stimulation [39,40], although substrates such as vitamin B_{12}, manganese, or zinc are not constitutively available.

How much of a problem could such waste really be? We suggest that the problem of this apparent waste of ATP is, in fact, not a problem at all. Consider, for instance, a bacterial cell. With basal ATPase rates ranging from 0.2 to 4.3 s^{-1}, the most ‘wasteful’ *E. coli* ABC transporters are BtuCD, FhuBC, MsbA, $LptB_2FGC$, LolCDE, and MacB [39,47–50]. Their cellular abundance spans from only a few copies per cell (BtuCD, FhuBC) to a few hundred ($LptB_2FGC$, MsbA) [51]. Combining these proteomic and biochemical data shows that, in total, the ensemble of ABC transporters in *E. coli* hydrolyzes roughly 2×10^6 ATP molecules per cell cycle (≈ 20 min). Although this may sound substantial, it represents only about 0.02% of the $\sim 1.1 \times 10^{10}$ ATP molecules consumed by the cell during the same period—an amount that is, for all practical purposes, negligible.

A similar picture emerges in eukaryotic cells. Human HEK293T cells express an impressive array of roughly 35 ABC transporters, spanning all major subfamilies and cellular compartments [52]. As in bacteria, their cellular copy numbers vary widely—from a few hundred molecules per cell for low-abundance organellar transporters such as ABCB8 or ABCD1 to several thousand for plasma-membrane exporters including ABCC1, ABCG2, and ABCA2 [52]. Many of these transporters, particularly ABCB1, ABCC1, and ABCG2, exhibit relatively high basal ATPase activity in the absence of transported substrate, with reported turnover rates of 0.2–10 s^{-1} [45,53,54]. Combining these proteomic and kinetic data indicates that the entire ABC transporter complement in HEK293T cells hydrolyzes on the order of 5×10^4 to 1×10^5 ATP molecules per second. Over a 24-h cell cycle, this amounts to roughly 10^9 ATP molecules—less than 0.03% of the total cellular ATP budget of $\sim 3 \times 10^{13}$ ATP molecules.

These calculations show that basal ATP hydrolysis by ABC transporters is negligible relative to total ATP turnover and exerts no meaningful evolutionary pressure.

Stoichiometry as a design principle

Many physiological functions are executed not by a single polypeptide but by assemblies of multiple proteins. This division of labor offers clear advantages: smaller subunits are easier to synthesize and maintain, modular components can evolve or be regulated independently, and, most importantly, multi-protein architectures enable stoichiometric flexibility, avoiding the rigid 1:1 constraints of single-chain systems.

Stoichiometric flexibility is largely irrelevant for ABC exporters, whose architecture is constrained by a fixed 2:2 stoichiometry of nucleotide-binding and

transmembrane domains, leaving little room for variation. In contrast, it is highly relevant for ABC importers in Gram-negative bacteria and for some Gram-positive import systems.

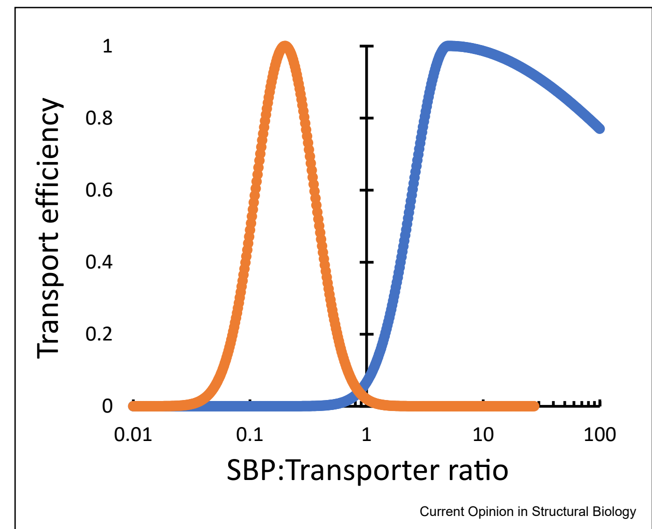
In recent work, we developed methods for proteomic quantification of ABC transporters [51], enabling comprehensive characterization of the *E. coli* 'ABC proteome.' We observed that in import systems for abundant nutrients (peptides, amino acids, carbohydrates), SBPs are present in large excess over their cognate transporters. In contrast, for scarce biomolecules (vitamins, siderophores), this ratio is reversed, with transporters outnumbering SBPs.

An excess of SBP over its transporter is the intuitive stoichiometry as more binding proteins enhance substrate capture and delivery. The opposite configuration—transporters outnumbering SBPs—is therefore unexpected. While SBP-rich stoichiometry is mechanistically essential for importing abundant nutrients as shown for the *E. coli* maltose transporter [55,56], we asked whether the reverse arrangement might be advantageous for scarce substrates. Manipulating the SBP-to-transporter ratio in the vitamin B_{12} system revealed that under substrate limitation, SBP excess **inhibits uptake** [51].

The contrasting SBP—transporter stoichiometries of ABC importers appear to reflect two fundamentally different operating regimes (Figure 3). For abundant nutrients, high SBP abundance enhances substrate capture and delivery, sustaining high-transport flux despite transient SBP-transporter interactions. By contrast, importers responsible for the uptake of scarce micronutrients such as vitamin B_{12} or siderophores function under extreme substrate limitation, where SBPs exhibit very high affinities and undergo minimal conformational change upon binding [57]. This 'lock-and-key' design prevents the transporter from discriminating between loaded and unloaded SBPs, allowing both to dock with comparable affinity. When SBPs are overproduced under such conditions, substrate scarcity results in a large population of unloaded SBPs. These substrate-free molecules compete with the few loaded ones for access to the transporter and, owing to their extremely slow dissociation rates [58,59], effectively clog the system. Consistent with this mechanism, increasing the SBP-transporter ratio in the vitamin B_{12} system inhibits transport [51].

In such systems, SBP dissociation does not rely on substrate-induced conformational differences but is instead driven by ATP-dependent cycling of the transporter, which transiently perturbs the SBP—transporter interaction surface. When transporters are present in excess, this promotes rapid rebinding to alternative transporter molecules, allowing SBPs to effectively 'hop' between transporters with an effectively negligible net free-energy difference.

Figure 3



Different substrate-binding protein **(SBP)-transporter strategies for import of abundant and scarce nutrients.** Schematic depiction of transport efficiency (normalized to unity) as a function of the stoichiometric ratio between the SBP and its cognate transporter. For import systems handling scarce nutrients (orange curve), SBPs bind their substrates through a 'lock-and-key' mechanism involving minimal conformational change. This design prevents the transporter from distinguishing between loaded and unloaded SBPs, allowing both to dock with comparable affinity. Under such conditions, an excess of SBPs leads to a substantial population of unloaded carriers that compete with substrate-loaded SBPs for transporter binding, thereby clogging the system. In contrast, for import systems handling abundant nutrients (blue curve), this limitation is avoided because [1] SBPs undergo a large conformational change upon substrate binding, enabling the transporter to discriminate against unloaded SBPs, and [2] the high substrate availability ensures that only a small fraction of SBPs remain unloaded.

Thus, two opposite stoichiometric strategies appear to have evolved: SBP-rich assemblies optimized for rapid acquisition of abundant substrates, and transporter-rich architectures tuned for efficient import of scarce molecules, where excessive SBP levels become counterproductive (Figure 3). These stoichiometric ratios balance substrate capture with transport throughput, embodying distinct solutions to the fundamental trade-off between flux and precision in cellular nutrient uptake.

A substrate-centric perspective: Conclusions and outlook

Evolutionary analysis indicate that ABC importers and exporters diverged early from a common ancestral ATP-binding cassette module, with the extensive mechanistic and transmembrane-domain diversity observed among ABC importers arising later as independent innovations grafted onto the conserved NBD core [60].

In this work, we outline a **substrate-centric perspective** that provides a conceptual framework for understanding how mechanistic diversity evolved across the ABC transporter family. At its center lies a simple guiding question: **how scarce or abundant is the cargo?**

The main principles of this perspective—summarized in Box I—are as follows.

Both primordial and contemporary ABC importers encounter substrates spanning a broad range of environmental concentrations. For clarity, we group these substrates into two coarse categories:

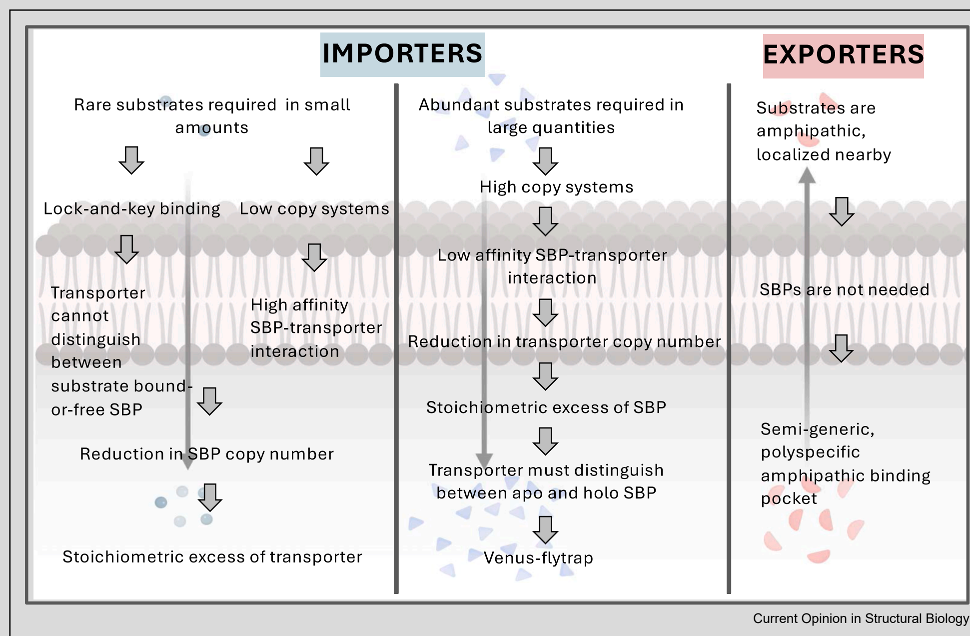
(1) **Scarce, essential compounds** required only in small quantities (e.g., vitamins, siderophores), and
(2) **Abundant nutrients** required in large quantities (e.g., amino acids, carbohydrates).

Scarce yet essential substrates impose two characteristic evolutionary pressures. First, because the total required flux of such molecules is low, cells benefit from maintaining **low copy numbers** of the corresponding import systems [51]—avoiding the costs of producing and sustaining multiple transporter units that would rarely be utilized. Second, high specificity and efficiency become paramount. Accordingly, ABC importers adapted to substrate scarcity by evolving binding mechanisms that rely on **near-perfect geometric complementarity**, essentially a lock-and-key mode of molecular recognition [57] that minimizes futile encounters and maximizes capture fidelity.

These adaptations drove further mechanistic speciation: First, because import systems for scarce substrates were maintained at **low cellular copy numbers**, selective pressure favored the evolution of **high-affinity interactions between the SBP and the transporter**.

Second, the lock-and-key mode of molecular recognition—which relies on a rigid, geometrically complementary binding site—entails only minimal conformational change upon ligand binding. Once an SBP evolves to capture its ligand through subtle, highly localized interactions, the **overall SBP conformation presented to the transporter becomes nearly indistinguishable** in the substrate-free and substrate-loaded states. This structural similarity imposes an inherent ambiguity: the transporter cannot easily 'sense' whether

Box I. Evolution of divergent mechanisms in the ABC transporter superfamily.



Shown are the key events that drove mechanistic diversification among importers of rare versus abundant substrates, as well as exporters. See the accompanying text for a detailed explanation.

the SBP carries cargo. Consequently, these systems rely more heavily on **SBP-mediated gating than on any transporter-based verification of substrate occupancy.**

Consistent with this view, single-molecule studies [61] have shown that in ABC import systems specialized for scarce substrates, the conformational change induced in the transporter upon SBP docking is **identical whether or not the SBP is loaded with substrate**.

Such systems were faced with a serious problem: **if the transporter cannot distinguish between substrate-free and substrate-loaded SBP, how can non-productive interactions be avoided?** This challenge is especially acute in systems for scarce substrates, where the **high-affinity SBP–transporter interaction** means that once an apo SBP binds, it is likely to remain associated for an extended period. A persistent, non-productive complex would **block access for substrate-loaded SBPs**, effectively **clogging the transport cycle** and starving the cell of an essential nutrient.

Thus, some mechanisms had to evolve to prevent futile SBP engagement. Bacteria unable to solve this problem would have suffered severe fitness costs and, in evolutionary terms, **those lineages simply disappeared**.

The surviving lineages adopted a seemingly counterintuitive solution: reducing SBP copy number while maintaining transporter levels, thereby solving the problem in three mutually reinforcing ways [51].

First, lowering SBP abundance increases the likelihood that each SBP is substrate-saturated, ensuring that most SBP–transporter encounters are productive. Second, because transporters outnumber SBPs, system clogging is avoided: even if an apo SBP binds, it cannot monopolize the transporter pool. Finally, transporter excess promotes SBP recycling, enabling SBPs to 'hop' between docking sites, reducing futile residence time and enhancing productive throughput. Together, these constraints define the mechanistic blueprint of importers specialized for rare substrates.

Abundant substrates that are required in large quantities are handled very differently. To sustain the high metabolic fluxes needed for growth, the **copy numbers of these import systems must be correspondingly high** as confirmed by our recent proteomic survey [51]. Under these conditions, there is **no selective pressure to maintain an energetically costly, high-affinity SBP–transporter interaction**. Accordingly, such systems are characterized by transient **SBP–transporter interactions. The high turnover rates** of the transport cycle fundamentally change the economic balance. Because each transporter can process substrate rapidly, the **penalty for maintaining fewer transporter copies is relatively small**. In contrast, the energetic burden of producing large numbers of transporters—complex, membrane-embedded, and biosynthetically expensive proteins—is substantial. Thus, natural selection favors a configuration in which **transporter copy number is reduced just enough to meet flux demands**, allowing the cell to **avoid unnecessary expenditure on these costly components** while still achieving high overall throughput.

This strategy naturally produces a **stoichiometric excess of SBP over transporter**, the mirror image of the architecture found in systems for scarce substrates. Under these conditions, however, a new vulnerability emerges: **substrate-free SBPs can now outnumber available transporter molecules**, raising the risk that apo SBPs will occupy transporters non-productively and impede flux. To avoid such clogging, the transporter must be able to **discriminate between the apo and holo states of the SBP**—a requirement fundamentally incompatible with the rigid lock-and-key mechanism used for scarce substrates.

The evolutionary solution was the emergence of the **Venus flytrap–type SBP**, in which ligand binding triggers a large, hinge-like conformational change that closes the binding cleft. This dramatic structural shift allows the transporter to recognize and preferentially engage only the **substrate-loaded SBP**, thereby preventing futile interactions with the apo form. In this way, the mechanistic divide between lock-and-key and Venus flytrap SBPs can be understood as a direct evolutionary consequence of **substrate abundance and system stoichiometry**.

Exporters constitute a third category with distinct evolutionary pressures that gave rise to their own mechanistic blueprint. Unlike importers, they handle **intracellular substrates**, whose concentrations are typically higher, more localized, and tightly regulated by metabolic pathways. These conditions reduce the need for SBPs and simplify substrate recognition. Exporters could therefore evolve **broad, semi-generic binding vestibules** capable of accommodating diverse substrates that share common physicochemical traits (Figure 1)—ranging from endogenous lipids and peptides to xenobiotics, toxins, and antibiotics.

Because exporters do not rely on SBPs and need not capture dilute substrates, they faced far fewer constraints on strict specificity. Instead, their evolution was shaped by the selective advantages of **polyspecificity**, enabling a single exporter to mediate both physiological transport and cellular defense against numerous harmful compounds. This functional flexibility likely contributed to the extensive expansion of exporter families across bacteria and eukaryotes. Indeed, among the 49 human ABC exporters, the physiological substrate is confidently known for only a minority, underscoring how

broad substrate recognition—and often striking functional ambiguity—became a hallmark of this class.

The evolutionary trajectory of exporters thus reflects pressures favoring **versatility and robustness**, rather than the precision capture and substrate-verification constraints that dominate the evolution of ABC importers.

Looking ahead, defining the **physiologically relevant substrates** of both bacterial and eukaryotic ABC transporters should remain a central focus of future research. As argued throughout this work, substrate identity is fundamental to understanding transport mechanism, architecture, and evolutionary constraints. For importers, systems dedicated to **scarce substrates** remain comparatively understudied. Although the vitamin B_{12} transporter stands as a well-characterized model, many others—particularly those mediating **siderophore uptake** (e.g., *Fhu*, *Fep*, *Fec*)—require substantial additional work to resolve their mechanistic details.

Moreover, for exporters, the challenge ahead is arguably even greater: **identifying genuine physiological substrates** *in vivo*, distinguishing them from artefactual or pharmacological substrates, and understanding the regulatory logic that determines which molecules are prioritized for export. Addressing these questions will be essential for explaining the remarkable diversity and biomedical relevance of these transporters across all domains of life.

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Box I was created using BioRender.com.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbi.2026.103249.

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