The human ATP-binding cassette (ABC) transporter superfamily

Michael Dean,[1],* Yannick Hamon,[†] and Giovanna Chimini[†]

Human Genetics Section, Laboratory of Genomic Diversity,* National Cancer Institute-Frederick, Bldg. 560, Rm. 21-18, Frederick, MD 21702; and Centre d'Immunologie INSERM/CNRS de Marseille-Luminy,[†] Marseille, 13288 Cedex 09, France

Abstract The transport of specific molecules across lipid membranes is an essential function of all living organisms and a large number of specific transporters have evolved to carry out this function. The largest transporter gene family is the ATP-binding cassette (ABC) transporter superfamily. These proteins translocate a wide variety of substrates including sugars, amino acids, metal ions, peptides, and proteins, and a large number of hydrophobic compounds and metabolites across extra- and intracellular membranes. ABC genes are essential for many processes in the cell, and mutations in these genes cause or contribute to several human genetic disorders including cystic fibrosis, neurological disease, retinal degeneration, cholesterol and bile transport defects, anemia, and drug response. Characterization of eukaryotic genomes has allowed the complete identification of all the ABC genes in the yeast *Saccharomyces cerevisiae*, *Drosophila*, and *C. elegans* genomes. To date, there are 48 characterized human ABC genes. The genes can be divided into seven distinct subfamilies, based on organization of domains and amino acid homology. Many ABC genes play a role in the maintenance of the lipid bilayer and in the transport of fatty acids and sterols within the body. Here, we review the current knowledge of the human ABC genes, their role in inherited disease, and understanding of the topology of these genes within the membrane.—Dean, M., Y. Hamon, and G. Chimini. **The human ATP-binding cassette (ABC) transporter superfamily.** *J. Lipid Res.* 2001. **42:** 1007–1017.

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ATP-BINDING CASSETTE (ABC) PROTEIN AND GENE ORGANIZATION

The ABC proteins bind ATP and use the energy to drive the transport of various molecules across the plasma membrane as well as intracellular membranes of the endoplasmic reticulum (ER), peroxisome, and mitochondria (1–3). ABC transporters contain a pair of ATP-binding domains, also known as nucleotide binding folds (NBF), and two sets of transmembrane (TM) domains, typically containing six membrane-spanning α-helices. The NBF contain three conserved domains: Walker A and B domains, found in all ATP-binding proteins, and a signature (C)

motif, located just upstream of the Walker B site (4). The C domain is specific to ABC transporters and distinguishes them from other ATP-binding proteins. The prototype ABC protein contains two NBF and two TM domains, with the NBF located in the cytoplasm. The molecules pump substrates in a single direction, typically out of the cytoplasm. For hydrophobic compounds, this movement is often from the inner leaf of the bilayer to the outer layer or to an acceptor molecule.

ABC genes are organized as either full transporters containing two TM and two NBF or as half transporters containing one of each domain (4). The half transporters assemble as either homodimers or heterodimers to create a functional transporter. The genes that encode ABC genes are widely dispersed in the genome and show a high degree of amino acid sequence identity among eukaryotes. Phylogenetic analysis has allowed the gene superfamily to be divided into seven subfamilies, and six of these subfamilies are found in both mammalian and the *S. cerevisiae* genome.

The human ABC gene subfamilies

Table 1 displays a list of all 48 known human ABC genes. As the human genome sequence is not complete, it is possible that one to three additional genes may be described (5, 6). There are surprisingly few ABC pseudogenes, with only 7 to 10 described to date. We will first provide an overview of the ABCB to ABCF subfamilies of human transporters, and then provide a more thorough discussion on ABCA and ABCG subfamilies.

TABLE 1. List of human ABC genes, chromosomal location, and function

Symbol	Alias	Location	Mouse Location	Expression	Function
ABCA1	ABC1	9q31.1	4 23.1	Ubiquitous	Cholesterol efflux onto HDL
ABCA2	ABC2	9q34	2 12.6	Brain	Drug resistance
ABCA3	ABC3, ABCC	16p13.3		Lung	
ABCA4	ABCR	1p22.1–p21	3 61.8	Rod photoreceptors	N-retinylidiene-PE efflux
ABCA5		17q24	11 69	Muscle, heart, testes	
ABCA6		17q24	11 69	Liver	
ABCA7		19p13.3	10 44	Spleen, thymus	
ABCA8		17q24	11 69	Ovary	
ABCA9		17q24	11 69	Heart	
ABCA10		17q24		Muscle, heart	
ABCA12		2q34		Stomach	
ABCA13		7p11–q11		Low in all tissues	
ABCB1	PGY1, MDR	7p21	5 1.0	Adrenal, kidney, brain	Multidrug resistance
ABCB2	TAP1	6p21	17 18.6	All cells	Peptide transport
ABCB3	TAP2	6p21	17 18.6	All cells	Peptide transport
ABCB4	PGY3	7q21.1	5 1.0	Liver	PC transport
ABCB5		7p14		Ubiquitous	
ABCB6	MTABC3	2q36		Mitochondria	Iron transport
ABCB7	ABC7	Xq12–q13	X 39	Mitochondria	Fe/S cluster transport
ABCB8	MABC1	7q36		Mitochondria	
ABCB9		12q24		Heart, brain	
ABCB10	MTABC2	1q42	8 67	Mitochondria	
ABCB11	SPGP	2q24	2 39	Liver	Bile salt transport
ABCC1	MRP1	16p13.1	16	Lung, testes, PBMC	Drug resistance
ABCC2	MRP2	10q24	19 43	Liver	Organic anion efflux
ABCC3	MRP3	17q21.3		Lung, intestine, liver	Drug resistance
ABCC4	MRP4	13q32		Prostate	Nucleoside transport
ABCC5	MRP5	3q27	16 14	Ubiquitous	Nucleoside transport
ABCC6	MRP6	16p13.1		Kidney, liver	
CFTR	ABCC7	7q31.2	6 3.1	Exocrine tissues	Chloride ion channel
ABCC8	SUR	11p15.1	7 41	Pancreas	Sulfonylurea receptor
ABCC9	SUR2	12p12.1	6 70	Heart, muscle	
ABCC10	MRP7	6p21		Low in all tissues	
ABCC11		16q11–q12		Low in all tissues	
ABCC12		16q11–q12		Low in all tissues	
ABCD1	ALD	Xq28	X 29.5	Peroxisomes	VLCFA transport regulation
ABCD2	ALDL1, ALDR	12q11–q12	15 E–F	Peroxisomes	
ABCD3	PXMP1, PMP70	1p22–p21	3 56.6	Peroxisomes	
ABCD4	PMP69, P70R	14q24.3	12 39	Peroxisomes	
ABCE1	OABP, RNS4I	4q31		Ovary, testes, spleen	Oligoadenylate binding protein
ABCF1	ABC50	6p21.33	17 20.5	Ubiquitous	
ABCF2		7q36	13 40	Ubiquitous	
ABCF3		3q25	16 22	Ubiquitous	
ABCG1	ABC8, White	21q22.3	17 A2–B	Ubiquitous	Cholesterol transport?
ABCG2	ABCP, MXR, BCRP	4q22	6 28–29	Placenta, intestine	Toxin efflux, drug resistance
ABCG4	White2	11q23	5 59	Liver	
ABCG5	White3	2p21	17	Liver, intestine	Sterol transport
ABCG8		2p21	17	Liver, intestine	Sterol transport

PBMC, peripheral blood mononuclear cells; VLCFA, very long chain fatty acids.

ABCB [MULTIDRUG RESISTANCE (MDR)/(TAP)]

The ABCB subfamily is composed of four full transporters and seven half transporters, and this is the only human subfamily to have both types of transporters. The *ABCB1* (*MDR/PGY1*) gene was discovered as a protein overexpressed in certain drug-resistant tumor cell lines. Cells that overexpress this protein display MDR and are resistant to or transport a wide variety of hydrophobic compounds including colchicine, doxorubicin, adriamycin, vinblastine, digoxin, saquinivir, and paclitaxel. *ABCB1* is expressed primarily in the liver and blood brain barrier, and is thought to be involved in protecting cells from toxic agents. The gene is duplicated in mice. Animals lacking both genes unfortunately display a very limited pheno-

type and are still viable and fertile. However, they have been very useful models to identify and characterize other drug resistance genes.

Secretion of cholesterol, phospholipids (PL), and other compounds into the bile is critical for normal bile function including the excretion of cholesterol and other sterols and the absorption of fat-soluble vitamins. The ABCB4 and B11 proteins are both located in the liver and participate in the secretion of phophatidylcholine (PC) and bile salts, respectively (7). Mutations in ABCB4 and ABCB11 are responsible for several forms of progressive familial intrahepatic cholestasis (PFIC). The PFIC are autosomal recessive liver disorders, characterized by early onset of cholestasis and liver failure, and are a major cause of liver transplants in children (8). Defects in *ABCB4* are

responsible for PFIC3 (9, 10), and are associated with intrahepatic cholestasis of pregnancy (11). Mutations in the *ABCB11* gene are found in patients with PFIC2 (12).

The process of antigen recognition by the class I histocompatibility genes involves the digestion of cellular and foreign proteins into short peptides and their transport into the ER where they form complexes with class I proteins and are expressed on the cell surface. The ABCB2 and B3 (TAP) genes are half transporters that form a heterodimer to transport these peptides into the ER. Rare families with defects in these genes display profound immune suppression, as they lack this essential portion of the immune recognition process. Altered alleles in the TAP genes in the rat are associated with restricted ability to present certain peptides (13).

The remaining ABCB subfamily half transporters are expressed in the lysosome (ABCB9) or the mitochondria (ABCB6, B7, B8, and B10). One of the mitochondrial genes (ABCB7) is located on the X-chromosome and mutations in this gene are responsible for X-linked sideroblastic anemia and ataxia (XLSA/A) phenotype (14). The human ABCB9 gene can complement the yeast ortholog of ABCB7, Atm1. This gene plays a role in mitochondrial iron homeostasis and in the biogenesis of cytosolic Fe/S proteins (15).

ABCC [CYSTIC FIBROSIS TM CONDUCTANCE REGULATOR (CFTR)/MULTIDRUG RESISTANCE PROTEIN (MRP)]

The ABCC subfamily contains 12 full transporters that perform functions in ion transport, toxin secretion, and signal transduction. Cystic fibrosis (CF) is an inherited multisystemic disorder characterized by anormalities in exocrine gland function consequent to loss of function of the CFTR transporter. The CFTR protein is unique among ABC proteins in that it is a cAMP-regulated chloride ion channel (16). CF is very common in Caucasian populations (1/900 to 1/2,500 births) but quite rare in African and Asian populations. One common mutation, a deletion of three base pairs (ΔF508) accounts for 50–80% of the alleles and arose after Caucasians separated from the other racial groups. At least two other alleles are also elevated in specific populations. The W1282X allele is the most common mutation in the Ashkenazi Jewish population, and the 1677delTA allele is found at a high frequency in Georgians, as well as in Turkish and Bulgarian populations. The high frequency of CFTR alleles could be explained by selection of an advantageous phenotype in the heterozygotes. Resistance to bacterial toxins such as cholera and *E. coli* has been proposed and partially supported by experimental data (17). CFTR has also been proposed as a receptor in epithelial cells for internalization of *P. aeruginosa* and *S. typhi* (18). This CFTR-mediated clearing function of bacterial pathogens could underlie the biology of CF lung disease and be the basis for the heterozygote advantage for carriers of mutant alleles of CFTR.

Patients with two nonfunctional CFTR alleles display severe disease with inadequate secretion of pancreatic enzymes leading to nutritional deficiencies, bacterial infections of the lung, and obstruction of the vas deferens leading to male infertility. Patients that possess one partially functional allele have a milder disease and retain residual pancreatic function (19); individuals with one of more very mild alleles display only the infertility phenotype (congenital absence of the vas deferens). Thus, there is a spectrum of disease severity that correlates with the residual function of CFTR (20, 21).

The ABCC8 gene was identified as the locus for familial persistent hyperinsulinemic hypoglycemia of infancy, an autosomal recessive disorder characterized by unregulated insulin secretion (22). Subsequent work demonstrated that the *ABCC8* gene is a high affinity receptor for the drug sulfonylurea. Sulfonylureas are widely used to increase insulin secretion in patients with non-insulin-dependent diabetes. These drugs bind to the ABCC8 and the closely related ABCC9 protein, and inhibit the KIR6 potassium channel. A polymorphism in the *ABCC8* gene has been associated with insulin response in Mexican American subjects (23) and type II diabetes in French Canadians (24), but not in a Scandinavian cohort (25).

The remaining ABCC genes are nine MRP-related genes. ABCC1 (MRP1) was identified as a multidrug resistance gene and demonstrated to transport glutathione conjugates of many toxic compounds. ABCC2 and C3 also transport conjugates to glutathionine and other organic anions. Similar to ABCB1, ABCC1 transports and confers resistance to a wide variety of toxic substrates, but is not essential for growth or development. ABCC1 can also transport leukotriene C4, a potent chemotactic factor controlling dendritic cell migration from peripheral tissues to lymph nodes (26).

The rat *Abcc2* gene was found to have a frame-shift mutation in the TR− strain that is defective in canalicular multispecific organic anion transport (27). The TR− rat has a similar phenotype to patients with Dubin-Johnson syndrome, and ABCC2 is, in fact, mutated in Dubin-Johnson syndrome patients (28). The ABCC2 protein is expressed on the canalicular side of the hepatocyte and mediates organic anion transport.

The positional cloning of the pseudoxanthoma elasticum (PXE) gene revealed that mutations in the ABCC6 gene are responsible for this connective tissue disorder (29). PXE is characterized by calcified deposits in elastin fibers, and results in arterial hemorrhage as well as in bleeding in the gastrointestinal track and retina. The ABCC6 gene is principally expressed in kidney and liver, sites not affected by PXE. This suggests that ABCC6 may have an indirect role in the disorder such as in the transport or removal of a toxic metabolite to which connective tissue cells are sensitive. This surprising result is likely to lead to new understanding of the MRP-related genes.

The ABCC4, C5, C11, and C12 proteins are smaller than the other MRP1-like genes and lack an N-terminal domain (30) that is not required for transport function (31). The ABCC4 and C5 proteins have been shown to confer resistance to several nucleosides including 9-(2-phosphonylmethoxyethyl) adenine and purine analogs, and may play a role in cGMP secretion.

THE ABCD [ADRENOLEUKODYSTROPHY (ALD)] SUBFAMILY

This subfamily contains 4 genes that encode half transporters expressed exclusively in the peroxisome. One of the genes, ABCD1, is responsible for the X-linked form of ALD, a disorder characterized by neurodegeneration and adrenal deficiency, typically initiating in late childhood (32). The presentation of ALD is highly variable with adrenomyeloneuropathy, childhood ALD, and adult onset forms. However, there is no correlation between the phenotype of ALD and the genotype at the *ABCD1* locus. Cells from ALD patients are characterized by an accumulation of unbranched saturated fatty acids, but the exact role of ABCD1 in this process has yet to be elucidated. The functions of the other ABCD family genes have also not been worked out, but the marked sequence similarity (especially for ALDP-ABCD2) suggest that they may exert related functions in fatty acid metabolism. The in vitro demonstration of homo- or heterodimerization of the product of ABCD1 with either ALDRP or PMP70 suggest that different peroxisomal half transporter heterodimer combinations are involved in the import of specific fatty acids or other substrates. ABCD genes are under complex regulation at the transcriptional level, and being very tightly linked to cell lipid metabolism, it is not surprising that they share with the ABCA and ABCG subclasses the sensitivity to the peroxisome proliferator-activated receptor and retinoid X receptor family of nuclear receptors (33–35).

ABCE [OLIGOADENYLATE BINDING PROTEIN (OABP)] AND ABCF (GCN20)-NONMEMBRANE ABC PROTEINS

The ABCE and ABCF subfamilies are composed of genes that have ATP-binding domains that are closely related to those of the other ABC transporters, but these genes do not encode any TM domains. The ABCE subfamily contains a single member, the OABP, ABCE1. This protein recognizes oligoadenylate produced in response to certain viral infections. The ABCF genes each have a pair of NBF, and the best characterized member is the *S. cerevisiae* GCN20 gene. GCN20 is involved in the activation of the eIF-2 alpha kinase (36). A human homolog, ABCF1 is part of the ribosome complex and may play a similar role (37).

THE DIVERSITY OF HUMAN GENETIC DISEASE CAUSED BY ABC GENES

To date, there are a total of 14 ABC genes that are associated with genetic disorders. In fact, several ABC genes were originally identified as a result of cloning disease loci (38, 39). The functions of ABC genes are very diverse, so it is not surprising that the diseases for which they are responsible are also diverse. In addition, ABC genes are involved in complex processes that are difficult to study. For example, despite over 7 years of research, the molecular basis of X-linked ALD is still not known. ABC genes predominantly encode structural proteins and, as a result, all of the disorders are recessive.

ABC GENES IN MODEL ORGANISMS

The complete sequence of several model eukaryotic organisms has allowed the identification and partial characterization of the ABC genes in those species. There are 31 ABC genes in the yeast genome, and all of the subfamilies found in the human genome are represented except for the ABCA subfamily (40, 41). The ABCA family, however, seems to be represented mainly by half transporters in higher plants, although a limited number of genes encoding full ABCA transporters has been recorded (E. Dassa, personal communication). The ABCD-like genes in the yeast (Pxa1 and Pxa2) are also expressed in the peroxisome, and are involved in oxidation of very long-chain fatty acids (42). The STE6 gene is a yeast ABCB family gene that transports the yeast mating factor, a small modified peptide, out of cells (41, 43). The best studied yeast ABC genes are the drug resistance loci PDR5 and SNQ2 and the related *S. pombe* BFR1 and *C. albicans* CDR1 genes. These loci are ABCG-related genes that, unlike ABCG genes in any other organisms, are full transporters with a NBF-TM-NBF-TM structure. Overexpression of these genes confers resistance to cycloheximide, chloramphenicol, cerulenin, staurosporine, and sporidesmin. PMD1 confers resistance to an antifungal drug leptomycin B, and is an ABCB-related gene (44). The yeast YCF1 gene is an ABCC-type gene conferring resistance to high levels of cadmium (45). The ABCB-like gene, HMT1, can also confer cadmium resistance, apparently by participating in the sequestration by phytochelatins of metal ions into intracellular organelles.

An analysis of the *Drosophila* genome revealed the presence of 56 ABC genes. For the most part, *Drosophila* has similar numbers of the different subfamilies, except for the ABCG genes. In flies, there are 15 ABCG-like genes compared with 5 found in the human genome (39). Unfortunately, except for the *White, Brown,* and *Scarlet* loci, there is very little known about the normal function of *Drosophila* ABC genes. The availability of gene disruption technology in the fly should allow the systematic study of these genes.

ABCA and G classes as gatekeepers of cell and body homeostasis of sterols

Very recently, the ABCA and ABCG subclasses of mammalian ABC transporters have been implicated in the cellular homeostasis of PL and cholesterol (see below for a detailed description). In fact, the loss of function of ABCA1 prototype of the A subclass leads to the development of Tangier disease, one of the best studied models of reverse cholesterol transport (46). In this disease, the basic ABCA1-dependent defect is an impaired donation of cellular PL and cholesterol to the specific plasmatic acceptors that leads to a characteristic dislipidemic profile in affected individuals (47, 48).

Similarly, members of the ABCG subfamily, namely ABCG5 and G8 (49, 50), have been implicated in the genesis of sitosterolemia (51), a genetic disorder of lipid metabolism where hyperlipidemia results from impaired efflux of sitosterol and related compounds to the intestinal lumen and to the bile (52). This unambiguous genetic evidence links these classes of ABC genes to membrane trafficking of lipids, and points out their pivotal role as gatekeepers of cellular sterol content. No clear insight into the molecular mechanisms that they drive or on the nature of the substrates that they translocate across the membrane leaflets has been provided, as yet. Thus, we will briefly introduce here some basic information on the handling of lipids at the plasma membrane that may be relevant to the yet-to-be-established molecular function of ABCA and ABCG classes before reviewing the genetic complexity of both subclasses and the so-far-available data concerning their membrane topology.

The fluid mosaic of lipids at the plasma membrane

The basic components of biomembranes are lipids: amphipathic molecules that tend to spontaneously form a bi-molecular leaflet in aqueous solution. Intercalated in this fluid phospholipid bilayer are proteins, carbohydrates, and their complexes, whose organization provides the molecular support to the specialized metabolic tasks of each cellular membrane (**Fig. 1**).

At the level of a single membrane, however, the distribution of lipid moieties is not equal. Indeed, asymmetry in lipid architecture at the plasma membrane has long been known and, at first, thought to be a general and static property of plasma membranes (53, 54). Typically, the plasma membrane, which is the most studied example, contains 49% of cellular PL, 69% of sphingomyelin (SM), and 64% of cholesterol; its outer leaflet is predominantly formed by SM and most of the cell PC (e.g., in the
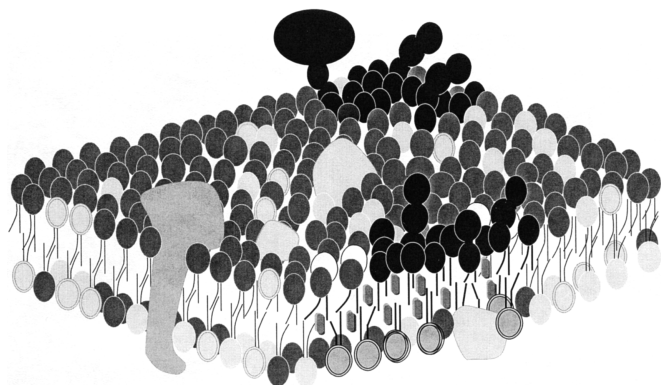
erythrocyte membrane, 65–75% of PC and more than 85% of the SM are located there), whereas the aminophospholipids, phosphatidylserine (PS) and, to a lesser extent, phosphatidylethanolamine (PE), are confined to the cytosolic face of the membrane (80–85% of PE and more than 95% of PS) (55). From their respective chemical structure (richness in long saturated acyl chains in the case of sphingolipids, and high content of unsaturated fatty acyl chains for aminophospholipids), we could expect an ordered outer leaflet facing the extracellular milieu, and a fluid less ordered inner leaflet favoring fusion events between the plasma membrane and intracellular vesicles. Cholesterol is a special case because it is actually embedded in the membrane interior, and estimations of its TM distribution, which varies widely in diverse cell types, gave discordant results (56, 57).

In addition to the asymmetrical distribution along the transverse axis, proteins and lipids are not freely diffusing in the plane of the membrane but, rather, they assemble in domains generating lateral heterogeneity (58). In the case of lipids, this is mainly a consequence of their intrinsic chemical properties. The best known lateral lipid domains are rafts, or detergent-insoluble glycosphingolipid-enriched domains, as identified biochemically by their resistance to detergent extraction. These consist of cholesterol and sphingolipid assemblies in the exoplasmic leaflet of the plasma membrane. Cholesterol rigidifies the packing of SM molecules and occupies the spaces delimited by their saturated carbon chains. Hydrogen bonding further strengthens the lateral association between the sterol ring and the ceramide backbone of SM moieties (58). These islands of tightly packed molecules are thought to form a separate liquid-ordered phase floating in the liquid-disordered phase of the membrane matrix. The preferential partition of cholesterol into the liquid-ordered phase is instrumental to the maintenance of distinct phases.

The size of rafts, estimated at 50 nm and containing ~3,500 SM molecules (59), may vary as a consequence of cell lipid concentration and external conditions; their modifications may eventually lead to raft coalescence and dispersion of the liquid-disordered phase, with consequent dramatic modifications of the biophysical properties of the membrane.

Conspicuous modifications of the biophysical properties of the membrane indeed occur continuously during the cell life span as a function of the cell cycle, cell growth, cell motility, or cell activation state (60). For simplicity, we may consider that at steady state conditions, molecular motors essentially serve to maintain the asymmetry, and drive an active translocation of lipid species, whose spontaneous movement across the leaflet is extremely difficult. As an example, the spontaneous transbilayer diffusion of PC, the most abundant membrane lipid, occurs at very slow $t1/2$ (days), both in artificial bilayer virtually devoid of inserted proteins and in erythrocytes, viral, or phagosomal membranes (55, 61). In these conditions, proteins can facilitate the movement of lipid across the bilayer simply by providing sliding surfaces for the lipid headgroups, but can also act as active translocators, consuming energy to

Fig. 1. The dynamic composition of membranes. Protein and carbohydrate moieties are embedded in a bilayer of lipids asymmetrically distributed in the two leaflets. Aminophospholipids (light gray) predominate in the inner leaflet and PC (dark gray) in the outer. In black are shown lipid moieties (glycosphingolipids and SM in the outer leaflet) and glycophosphatidyl inositol-anchored proteins that are preferentially distributed in detergent-insoluble domains or rafts. Cholesterol is shown as embedded in the bilayer. A protein kinase is anchored to the inner leaflet of rafts, which mainly contains glycerolipids (51).

generate transport. Many proteins have been suggested to play a role as lipid translocators (55, 62). The most studied activity is that of the aminophospholipid, translocase, responsible for the maintenance of the confined distribution of PE and PS in the inner leaflet. Other activities such as scramblase and/or specific floppases have been invoked to account for the dramatic perturbations of asymmetry occurring after Ca^{++} stimulation, during platelet activation, or after surface fusion of intracellular vesicles.

In addition to these activities, which have been discussed elsewhere (55, 62), members of the ABC family have long been considered candidate lipid transporters (1, 61, 63–65), although no precise definition of their activities has been achieved. Apart from the recent focus on ABCA and ABCG genes as sterol sensors, ABCC2 is the best known example, and its ability to translocate PC into the bile has been largely discussed (63, 66). More recently, MDR1 has also been proposed as an outward translocator of cholesterol (67). Although it is known that MDR1 activity is sensitive to the membrane cholesterol content and that MDR1 resides in cholesterol-enriched rafts (68), it is still unclear to what extent this transport effect is related to the drug pumping activity of the protein.

ABCA (ABC1)

This subfamily is composed of 12 full transporters (Table 1) that are split into two subgroups [ref. (69) and unpublished observations]. The first group (*ABCA1–A4, A7, A12, A13*) includes seven genes that map to six different chromosomes. The second group of ABCA genes (*ABCA5–A6, A8–A10*) is organized into a head-to-tail cluster on chromosome 17q24. This gene cluster is also found in the mouse genome. These genes are also distinguished from the ABCA1-like genes by having 37–38 exons, as opposed to the 50 exons in *ABCA1*. The expression pattern of the chromosome 17 genes is restricted with *ABCA5* and *ABCA10* expressed in skeletal muscle, *ABCA9* in the heart, *ABCA8* in the ovary, and *ABCA6* in the liver. No diseases map to the corresponding region of the mouse and human genomes, and the functions are as yet uncharacterized.

As shown in **Fig. 2**, the ABCA subfamily genes are dispersed in the genome, except for the cluster on chromosome 17. An alignment of the sequences and phylogenetic analysis demonstrates that the members of the chromosome 17 gene cluster form a distinct subgroup (**Fig. 3**). This is consistent with the genes that have arisen by gene duplication. Analysis of the splice sites of the genes shows that the chromosome 17 gene cluster members each have 38 introns, whereas the other ABCA genes have 50–51 introns. The location of the introns and the size of the exons are highly correlated among the chromosome 17 genes, again supporting a recent duplication. The mouse genome also has a cluster of ABCA subfamily genes related to the cluster on chromosome 17 (Table 1). In contrast, there are no such genes in the *Drosophila* or *C. elegans* genomes, suggesting that these genes arose after the separation of vertebrates from insects and worms.
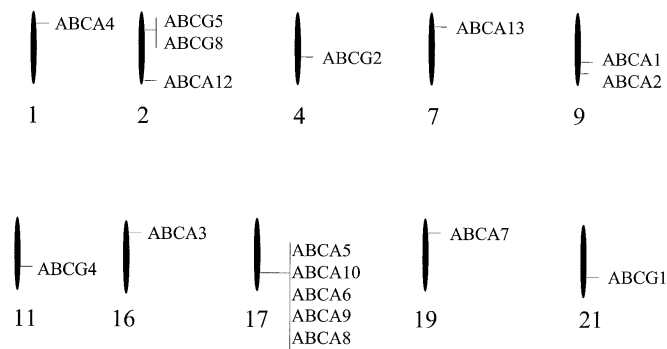

Fig. 2. The location of each of the ABCA and ABCG genes is displayed on the human chromosomes. Genes in clusters (ABCG5 and G8; ABCA5, A10, A6, A9, A8) are indicated by a vertical line.

Two of the genes belonging to the ABCA1 subgroup have been implicated in the development of genetic diseases. The ABCA1 protein is mutated in the recessive disorder Tangier disease [for a thorough discussion of diverse aspects of its biology, see refs. (47, 48, 52)]. ABCA1 controls the extrusion of membrane PL and cholesterol toward specific plasmatic acceptors, the apolipoproteins. It has been proposed that the ABCA-dependent step involves the flux of membrane PL, mostly PC, toward the lipid-poor nascent apolipoprotein particle, which now can accept cholesterol. The ABCA1-dependent homeostatic
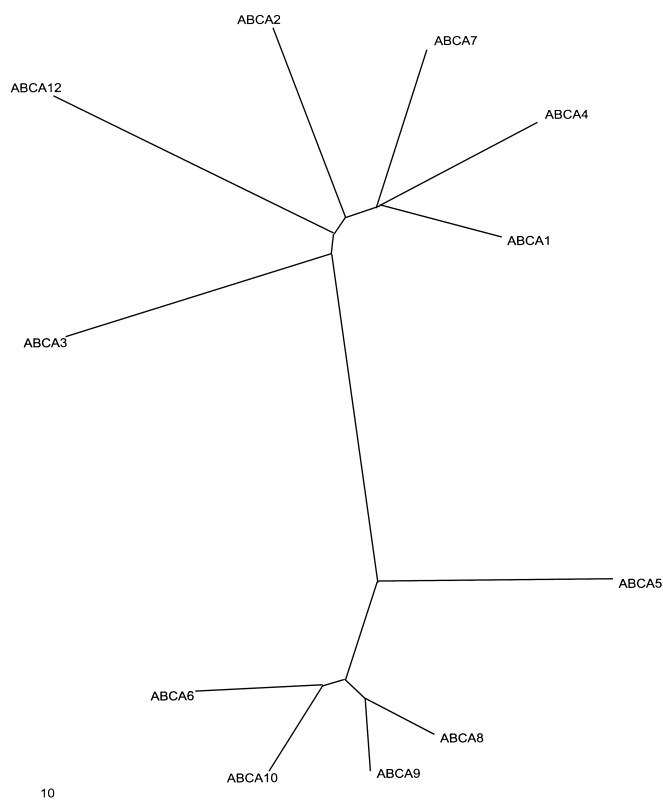

Fig. 3. A phylogenetic tree of the ABCA family genes is shown. The sequences were aligned with PILEUP and a common 2042-amino acid segment used to produce a neighbor-joining tree (106).

control of the lipid content of the membrane dramatically influences the plasticity and fluidity of the membrane itself and, as a result, affects the lateral mobility of membrane proteins and/or their association with membrane domains of special lipid composition. The proposed activity of ABCA1 as a facilitator of the engulfment of apoptotic bodies fits with this view (70). Indeed, mutations in *ced*-7, a putative ABCA1 ortholog, in *C. elegans* hampers optimal phagocytosis by precluding the redistribution of phagocyte receptors around the apoptotic particle (71).

The *ABCA4* gene was found to be highly expressed in rod photoreceptors, and maps to the region of chromosome 1p21 containing the gene for the Stargardt disease, a recessive childhood retinal degeneration syndrome. ABCA4 is mutated in Stargardt disease, as well as in some forms of recessive retinitis pigmentosum (RP), and the majority of recessive cone-rod dystrophy. The RP patients are homozygous for frame shift alleles and appear to represent the most severe phenotype. In contrast, Stargardt and cone-rod dystrophy patients almost always have at least one missense allele that could be partially functional. Retinol (vitamin A) derivatives produced in the photoreceptor outer segment disks must be transported to the cytoplasm to be further metabolized and transported out of the cell. ABCA4 is believed to mediate this transport by flipping outwardly modified PE. (72). Abca4 $-/-$ mice display increased all-*trans*-retinaldehyde following light exposure, elevated PE in the rod outer segments (ROS), and accumulation of these compounds (73). Retinoids stimulate the ATP hydrolysis of the ABCA4 protein in vitro, consistent with a role for these compounds as substrates (74). Individuals heterozygous for *ABCA4* mutations are increased in frequency in a late-onset retinal degeneration disorder, age-related macular degeneration (AMD). AMD patients display a loss of central vision after the age of 60 and the accumulation of pigmented retinoid compounds in the eye, similar to Stargardt disease. The causes of AMD are poorly understood, but involve a combination of genetic and environmental factors.

It is of interest in the context of lipid transport to note that photoreceptors represent an exquisite example of membrane dynamics and lipid composition (75).

Indeed, disk membranes are located in the interior of the ROS and arise from evaginations of the ROS plasma membrane. Nascent disks are progressively organized as a discontinuous stacked array of flattened membranous sacs and displaced toward the apical tip as additional new disks are formed. The transition from the base to the tip takes approximately 10 days, and maintains the ROS at constant length. The lipid composition of disk and plasma membranes is dramatically different and suggests a tremendous sorting of lipid constituents at the base of the ROS upon disk biogenesis. During the apical displacement of the disk, their cholesterol content decreases 5-fold, whereas fatty acid and PL composition is virtually unchanged. The loss of cholesterol is thought to take place by its exchange out of the PE-rich disk membrane into the PC-rich plasma membrane; the relative PE/PC ratio being instrumental to favor the movement of cholesterol toward

the plasma membrane during the disk life span. The proposed PE flippase activity of ABCR may thus fit well along a delicate sorting pathway of the lipid species across the ROS compartments.

The ABCA2 gene is highly expressed in oligodendrocytes in the brain (71); the ABCA7 gene highly expressed in the spleen and thymus (76, 77). The function of these genes, as well as ABCA12 and ACBA13, is not known, although it is tempting to speculate that they similarly participate in cellular lipid homeostasis in specialized environments. This is supported by the recent findings that both ABCA2 and ABCA7 share with ABCA1 a sterol dependent upregulation (77, 78).

THE ABCG (WHITE) HALF TRANSPORTERS

The human ABCG subfamily contains six half transporters that have an NBF at the N-terminus and a TM domain at the C-terminus: the reverse of the orientation of all other ABC genes. The *Drosophila White* locus was the first gene located by genetic mapping (79). The white protein forms a heterodimer with either of two other ABCG-related proteins, brown and scarlet, to transport guanine and tryptophan in the eye cells of the fly (80). These molecules are precursors of the fly eye pigments.

Surprisingly, there are only 5 ABCG genes in the human genome, whereas there are 15 in the *Drosophila* genome and 10 in yeast. Evolutionary analysis of the yeast genes shows that nearly all of them diverged a long time ago (**Fig. 4**). This is also evident in analysis of the position of
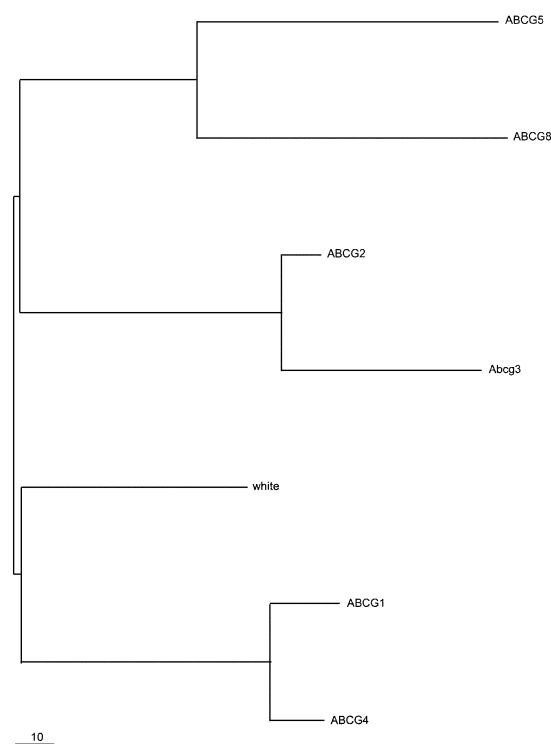

Fig. 4. A phylogenetic tree of the ABCG genes using a 672-amino acid segment of the genes.

the introns that shows that they are not conserved among the genes (Annilo et al., unpublished observations). The only exception is the ABCG1 and ABCG4 genes. This pair is closely related both in amino acid sequence and in having nearly identical intron location. ABCG1 is highly expressed in macrophages and is induced by cholesterol. ABCG4 is highly expressed in the brain. It will be interesting to see if these genes have related functions.

The *ABCG5* and *ABCG8* genes (49, 50) are located head-to-head on the human chromosome 2p15-p16, separated by a mere 200 bp. The genes are both mutated in families with sitosterolemia, a disorder characterized by defective transport of plant and fish sterols and cholesterol. Sitosterolemia patients display deficient sterol secretion from the intestine and the liver. This genetic evidence indicates that the two half transporters form a functional heterodimer. This is supported by the finding that the two genes are coordinately regulated by cholesterol. Perplexingly, the *ABCG5* gene is principally mutated in Asians; the *ABCG8* gene in Caucasians. This suggests that the proteins may form both hetero- and homodimers to transport the wide range of dietary sterols (compesterol, stigmasterol, avenosterol, sitosterol, cholesterol) encountered in the diet. The mammalian ABCG1 gene is also induced by cholesterol and is involved in cholesterol transport regulation (81).

The analysis of cell lines selected for high level resistance to mitoxantrone that do not overexpress ABCB1 or ABCC1 were instrumental in the identification of the *ABCG2* (*ABCP, MXR1, BCRP*) gene as a multidrug transporter (82–84). *ABCG2* can use anthracycline anticancer drugs, as well as topotecan, mitoxantrone, or doxorubicin as substrates. The ABCG2 gene is either amplified or rearranged by chromosomal translocations in resistant cell lines. Transfection of ABCG2 into cells confers resistance, consistent with its functioning as a homodimer. ABCG2 can also transport several dyes (rhodamine and Hoechst 33,462), and the gene is highly expressed in a subpopulation of hematopoetic stem cells (side population). The normal function of ABCG2 is not known; however, it is highly expressed in placental trophoblast cells, suggesting that it may pump toxic metabolites from the fetal to the maternal blood supply.

The Abcg3 gene is so far only found in the mouse and other rodent genomes. The gene is expressed in the spleen and thymus and has an ATP-binding domain that is missing several conserved residues in the Walker A and Signal domains (85).

Membrane topology: facts and speculations

Assessing unambiguously the membrane topology of polytopic proteins is known to be an issue of extreme difficulty, and ABC transporters have been no exception to the rule. As an example, it may be worth remembering the historical controversy regarding membrane topology of the prototypical ABC transporter, ABCB1 (MDR1). Its assessment brought along a number of contradictory results, only recently solved by the definitive observation of crystals (86–92).

In the case of the ABCA and ABCG classes, the topological exercise has just started. An early topological model of ABCA1, the prototype of the A class, was suggested based on the assessment of protease susceptibility of tagged forms of the transporter translated in vitro (93) and unpublished observations (Hamon). These experiments, although confirming that both the ATP-binding sites were located intracellularly, did not allow definitive conclusions to be formed on the exact number and topology of individual membrane spanners in the two symmetrical halves of the protein. In addition, the recent identification of the starting methionine (bp 84 in GBX75926) (94, 95), at a position conserved in and similar to that proposed for ABCA4 (96, 97), highlighted the presence of an N-terminal hydrophobic segment (AA 23–45; previously erroneously annotated as noncoding sequence in GB X75926) able to drive membrane insertion [ref. (98) and unpublished observations (Hamon)]. This new feature leads to the prediction of a large extracellular loop, confirmed experimentally, and modifies accordingly the topological model (**Fig. 5**). Systematic topological studies are currently in progress in several laboratories, and preliminary evidence suggests the presence of a second large extracellular loop after the so-called High Hydrophobic segment, which would now support its behavior as a in-to-out TM-spanning domain. However, the results obtained by epitope insertion approaches deserve a cautious interpretation. In fact, one can never exclude modifications of the delicate architecture of the transporter resulting from the insertion of even a
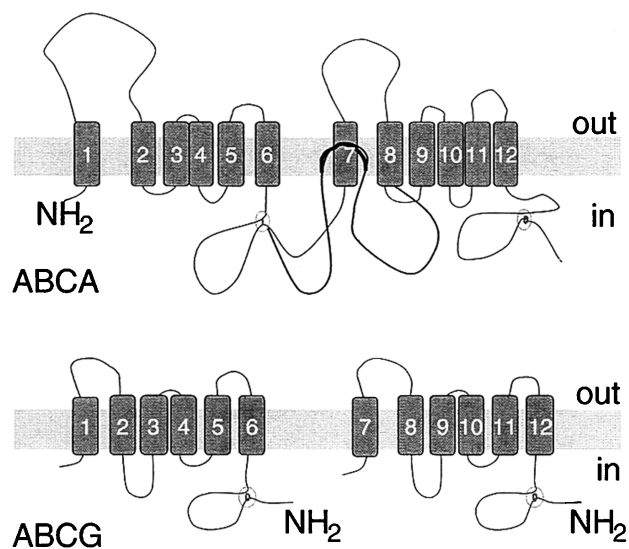

Fig. 5. A working model of the membrane topology for the ABCA and ABCG class of transporters. Experimental evidence has been provided for the assignment of the first TM-spanning domain and the extracellular loop at the N-terminal half of ABCA1 (98); preliminary evidence suggests that a similar loop is present on the second half (Hamon, unpublished). Similar predictions exist for ABCA4 (96, 97). The thickened line on the ABCA schematic shows an alternative topological model for the C-terminal set of spanners, taking into account a dynamic conformational change of the loop located after the high hydrophobic 1 (HH1) segment (93). For the ABCG family, the model is derived from computer-based predictions, and dimerization of half transporters is assumed.

short stretch of amino acids. On the other hand, extensive conformational changes may occur during the functional ATP cycle of the transporter, as suggested by the analysis of MDR crystals, and thus lead to a dynamic fluctuation of epitope accessibility (C. Higgins, personal communication). For the time being, however, the working model for the spatial arrangement of an ABCA1 monomer may be that of a TM pore limited at the bottom by the two closely opposed NBF (92), but also occluded on top by two loops protruding from each of the TM anchors, whose relative contacts are as yet undefined.

In the case of the ABCG class of genes, no topological approaches have yet been reported, and no information is available on their subcellular localization. ABCG2, however, has been localized at the plasma membrane (99). This is unusual among mammalian half transporters, whose functional dimers have so far been localized to internal membranes.

Although the ABCA and ABCG genes do not show any obvious structural similarity at first sight, they share a number of suggestive features. ABCA transporters are full-length four-domain proteins encoded by a single gene and bearing the most classical (TM/NBF/TM/NBF) arrangement, whereas ABCG genes are half transporters of the inverted NBF/TM type, related to the yeast PDR5-type full length transporters (40, 41). However, these structural differences may not be that crucial when surmising a hypothetical three-dimensional assembly that takes into account the predicted extracellular loop in the ABCG family. This is situated between the TM-spanning helices opposite to the NBF (5 and 6 in G nomenclature, which correspond to 1 and 2 or 7 and 8 in ABCA).

It is interesting to note that ABCA and ABCG genes are often co-expressed in pairs in distinct tissues or cell lineages (unpublished observations), that both classes of genes appear to be similarly regulated at the transcriptional level, and that they are both sensitive to the cellular lipid load (78, 81, 94, 95, 100–104). This is suggestive of a cooperative action of the two gene sets in the fine modulation of cell and body lipid homeostasis (105). Exactly how they exert tightly paired functions along similar pathways dedicated to the control of the extrusion of lipids from diverse cell types is still a matter of debate.🔳

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