Physiological Roles and Regulation of Mammalian Sulfate Transporters

DANIEL MARKOVICH

Department of Physiology and Pharmacology, University of Queensland, Brisbane, Queensland, Australia

I. Introduction

A. The sulfate ion, sulfate formation, and homeostasis
B. Sulfate conjugation
C. Sulfate movement through cells and across biomembranes

III. Sulfate Transport Systems

A. Sulfate transport in the kidney
B. Sulfate transport in the intestine
C. Sulfate transport in the liver
D. Sulfate transport in the lungs
E. Sulfate transport in erythrocytes
F. Sulfate transport in the brain
G. Sulfate transport in the placenta
H. Sulfate transport in the mammary gland
I. Sulfate transport in cultured cell lines
J. Sulfate transport in mitochondria
K. Lysosomal sulfate transport
L. Effects of pharmacological agents on sulfate homeostasis

IV. Cloning of Sulfate Transporters: The Molecular Biology Era

A. Na⁺-dependent sulfate transporters
B. Na⁺-independent sulfate transporters
C. Sulfate transport related proteins
D. Comparison of sulfate transporter structures

V. Regulation of Sulfate Transporters

A. Sulfate availability
B. Potassium availability
C. Metabolic acidosis/alkalosis
D. Vitamin D status
E. Thyroid hormone
F. Glucocorticoids/mineralocorticoids
G. Growth hormone
H. NSAIDs and prostaglandins
I. Cell membrane fluidity
J. Chronic renal failure
K. Pregnancy and postnatal growth
L. Posttranslational regulation
M. Heavy metals

VI. Pathophysiology of Sulfate Transporters

VII. Conclusion and Future Directions

Markovich, Daniel. Physiological Roles and Regulation of Mammalian Sulfate Transporters. Physiol Rev 81: 1499–1533, 2001.—All cells require inorganic sulfate for normal function. Sulfate is among the most important macronutrients in cells and is the fourth most abundant anion in human plasma (300 μM). Sulfate is the major sulfur source in many organisms, and because it is a hydrophilic anion that cannot passively cross the lipid bilayer of cell membranes, all cells require a mechanism for sulfate influx and efflux to ensure an optimal supply of sulfate in the body. The class of proteins involved in moving sulfate into or out of cells is called sulfate transporters. To date, numerous sulfate transporters have been identified in tissues and cells from many origins. These include the renal
sulfate transporters NaSi-1 and sat-1, the ubiquitously expressed diastrophic dysplasia sulfate transporter DTDST, the intestinal sulfate transporter DRA that is linked to congenital chloride diarrhea, and the erythrocyte anion exchanger AE1. These transporters have only been isolated in the last 10–15 years, and their physiological roles and contributions to body sulfate homeostasis are just now beginning to be determined. This review focuses on the structural and functional properties of mammalian sulfate transporters and highlights some of regulatory mechanisms that control their expression in vivo, under normal physiological and pathophysiological states.

I. INTRODUCTION

In the last decade, a tremendous amount of knowledge has been gathered on the processes by which cells import/export essential ions across their cellular membranes and how individual systems maintain the precise levels of ion concentrations for proper body function. One essential electrolyte is inorganic sulfate, an anion which is required by all organisms for life. Sulfate is required for proper cell growth and development of the organism. It is involved in a variety of important biological processes, including biosynthesis and detoxification via sulfation of many endogenous and exogenous compounds. Sulfate is required for cell matrix synthesis and for the maintenance of cell membranes. Despite its importance in cellular activities, up until 30 years ago it was considered an inert ion, whose precise function was unknown. Only recently have molecules been identified that facilitate cellular sulfate transport to/from the extracellular environment. Such transmembrane movement is of utmost importance, since without it, cells would not be able to regulate the contents of cellular sulfate required for many biological processes, nor would they be able to control sulfate homeostasis in the body. With the use of molecular biological techniques, many families of sulfate transporters, of both prokaryotic and eukaryotic origins, have been cloned in the past 10 years. As expected, sulfate transporters are not only restricted to mammals, but also exist in abundance in a variety of eukaryotic (e.g., birds, fish, amphibians, crustaceans, insects, plants and yeasts) and prokaryotic (i.e., bacteria) species (35, 142, 203, 237). Due to the breadth of this research field, this review focuses solely on the physiology and regulation of mammalian sulfate transporters, a topic which has not been thoroughly reviewed since the cloning of these novel proteins. The aim of this review is to provide a detailed report on the research of membrane sulfate transporters, with the rationale that a tremendous amount of information has been acquired, with over 100 original papers published in this field in the last 10 years.

This review is arranged into sections in chronological order of discovery on topics in sulfate transport physiology. Initially, the physiological role of the sulfate anion and its historical perspective is described in section II. This is followed by a review of early studies that first identified and characterized sulfate transport systems from various mammalian organs, cell lines, and intracellular compartments (sect. III). With the use of molecular and cell biological techniques, the structural identification and functional characterization of cloned sulfate transporters are depicted in section IV. Subsequently, the regulatory factors that control sulfate transporter expression in vivo and in vitro are elucidated in section V, and the pathophysiological conditions associated with defective sulfate transporters are described in section VI. Finally, future research trends are outlined in section VII, describing research areas which have not been extensively investigated, but are likely to be important for studying sulfate transporters in the coming years.

II. HISTORICAL PERSPECTIVE OF SULFATE

The sulfate ion in clinical medicine has been regarded as an end metabolite of cysteine and methionine, both sulfur-containing amino acids. Sulfate has been associated with an increase in body acidity and has been shown to lead to a drop in fluid osmolarity of body fluids (91, 222). Despite the fact that sulfate itself does not possess a catalytic function or a role in human energy metabolism, there is outstanding evidence to suggest that sulfate is not a metabolically inert molecule and that it plays a key function in life.

A. The Sulfate Ion, Sulfate Formation, and Homeostasis

The sulfate ion is the oxidized form of the 16th element of the Periodic Table, sulfur (S⁶⁺⁻) [Latin: sulfur], which is surrounded tetrahedrally by four oxygen molecules (O²⁻) forming the divalent anion SO₄²⁻. In nature, sulfate is an inorganic molecule belonging to the group VI oxyanions, which includes other structurally similar members such as selenate, molybdate, tungstate, and chromate. It is an important anion involved in many physiological processes, having numerous biosynthetic and pharmacological functions. Sulfate is involved in a variety of activation and detoxification processes of many endogenous (including glycosaminoglycans, cerebrosides, steroids, catecholamines) and exogenous (acetaminophen, isoproterenol, ibuprofen, salicylate, α-methylldopa) compounds (for extensive reviews, see Refs. 180–182). In the body, sulfate can be directly obtained from the diet (110, 238) or formed by oxidation of sulfur-containing amino
acids, cysteine and methionine, present in the diet (132, 209). The degree of contribution by either process toward total sulfate load is yet unknown. Outside the body, sulfate is formed by reaction of the sulfur atom in sulfite (S(4+)\textsuperscript{+}) with atmospheric oxygen. The sulfite ion (SO\textsubscript{3}\textsuperscript{2-}) is a precursor to the sulfate ion (SO\textsubscript{4}\textsuperscript{2-}) and is relatively reactive compared with sulfate. Most of the sulfite in the body is formed from hydrolysis of 3-sulfinylpyruvate by sulfite oxidase found within the intermembraneous space of liver mitochondria (for recent review, see Ref. 44). An early report documented rat liver mitochondria to be able to transport sulfite (48). Furthermore, sulfite was able to weakly cis-inhibit and trans-stimulate sulfate uptake into rabbit renal brush-border membrane vesicles (221), suggesting that it may not be transported by the same system as for the sulfate ion.

Body sulfate homeostasis was suggested to be in part maintained through renal clearance mechanisms (10, 15). The plasma SO\textsubscript{4}\textsuperscript{2-} levels in humans are generally maintained fairly constantly (±10%) over a 24-h period (165). However, after oral SO\textsubscript{4}\textsuperscript{2-} loading (i.e., high protein diets), plasma SO\textsubscript{4}\textsuperscript{2-} can increase up to twice normal levels, with excess SO\textsubscript{4}\textsuperscript{2-} quickly excreted within 12 h. In contrast, upon fasting, the majority of filtered SO\textsubscript{4}\textsuperscript{2-} is reabsorbed (165, 185). Renal handling of SO\textsubscript{4}\textsuperscript{2-} is altered in patients with chronic kidney disorders, there being a marked reduction in tubular SO\textsubscript{4}\textsuperscript{2-} reabsorption, that is sufficient to prevent an increase in plasma SO\textsubscript{4}\textsuperscript{2-} (170). At decreased serum sulfate concentrations, decreases in renal sulfate clearance are observed (144). Since sulfate is not extensively bound to serum proteins and the majority of filtered sulfate is reabsorbed, it has been suggested that sulfate is not secreted to any significant extent (15). This renal tubular-mediated process has been suggested to be the mechanism of sulfate homeostasis and is discussed in greater detail in sections iii and iv.

B. Sulfate Conjugation

One physiological importance of the sulfate anion is in the process of sulfate conjugation of various compounds. For conjugation, sulfate must be activated to form adenosine 3'-phosphate 5'-sulfatophosphate (PAPS), the “universal” sulfate donor. Various sulfotransferase enzymes catalyze the transfer of sulfate from PAPS to various compounds, and these enzymes are located in virtually all tissues of the body. Sulfation (also known as sulfonation) is an important step for the biotransformation and detoxification of xenobiotics (including analgesics, anti-inflammatory agents, and adrenergic stimulants/blockers), steroids, catecholamines, and bile acids (180–182). For a schematic view of the processes involved in the formation, activation, and conjugation of sulfate, see Figure 1. In addition to having a role in the detoxification of numerous toxic substrates (e.g., phenols, drugs, heavy metals), sulfate conjugation also serves a role in the biosynthetic pathway for the production of numerous biologically active substrates (including steroids, neurotransmitters, bile acids) (182). Sulfate is also essential for the biosynthesis of numerous structural components of membranes and tissues; it is involved in the formation of sulfated glycosaminoglycans (sGAG), major components of cartilage and other tissues, and in the formation of cerebroside sulfate, a constituent in the myelin membranes of the brain (55, 58). The degree of sulfation of various endogenous compounds can affect their physiological activities: increased sulfation of heparan sulfate

![Diagram](https://example.com/diagram.png)
and dermatan sulfate enhances their anticoagulant activities (19, 188). Furthermore, the importance of sulfation in growth and development has been demonstrated by the increased serum sulfate concentrations in the fetus, pregnant women, and children compared with adults (41, 43, 176, 244). Since sulfate conjugation is an extremely broad research area, with no direct links established to date to sulfate transporters per se, it is not covered in this review. For an excellent recent series of reviews on sulfation, see References 72, 83, 123, 265.

C. Sulfate Movement Through Cells and Across Biomembranes

Sulfate transport has been extensively studied both at the biomembrane and tissue levels, particularly in intestinal absorption, renal tubular reabsorption, and transport from the liquor compartment of the central nervous system (CNS) (50). Individual cells can obtain sulfate by three distinct mechanisms: 1) intracellular hydrolysis of sulfoconjugates, 2) oxidation of reduced organic sulfur, or 3) transport of sulfate from extracellular fluids into cells. The quantitative contribution of each process to total cellular sulfate levels is not precisely known, but it is believed that without the latter (membrane transport) step, cells would not be able to maintain a sufficiently high enough level of sulfate for all the cellular processes to function properly. It is this latter step that forms the focus of this review.

III. SULFATE TRANSPORT SYSTEMS

Since sulfate is a highly dissociated, divalent hydrophilic anion, it cannot pass freely through the phospholipid bilayer of plasma membranes and is therefore dependent on a transport system to allow movement into/out of active cells and cell organelles (such as mitochondria). Transport mechanisms in mammals are also needed for sulfate absorption from the gastrointestinal tract, reabsorption by the renal tubules, and excretion from the cerebrospinal fluid. The notion that sulfate transport through plasma membranes is mediated by a specific protein embedded in the lipid bilayer was proposed about 35 years ago (62). However, only in the last 10 years has structural information been available for proteins that are responsible for these transport mechanisms (see sect. iv). Since intracellular sulfate-containing nucleotides, such as adenosine 5′-phosphosulfate (APS) and PAPS, and the enzymes needed for sulfate activation (sulfotransferases) are present in all animal tissues and cells, all cells would be expected to be equipped with systems for the uptake of sulfate. Before the cloning of specific sulfate transporters, numerous approaches were used to determine and characterize sulfate transport systems in various tissues. These studies are documented below.

A. Sulfate Transport in the Kidney

The most widely studied organ in terms of sulfate transport, as with many other ion transport systems, has been the mammalian kidney. Early studies in dogs demonstrated that sulfate was freely filtered and extensively reabsorbed by the kidneys (84). Porous membranes such as the renal glomerular basement membrane were shown to freely filter sulfate (242). Stop-flow experiments proposed that the kidney proximal tubule was the major site of active sulfate reabsorption (98). With the use of turbidimetric analysis (16), serum sulfate concentrations in humans were measured to be 270 ± 20 μM (133). In dogs, normal plasma sulfate levels were measured to be 1–2 mM, with fractional excretion being ~10% (84); thus the majority of sulfate was reabsorbed by the kidneys. Tubular transport capacity for sulfate was calculated as ~120 μmol/100 ml filtrate in the dog (149). The active sulfate reabsorption process was shown to be capacity limited and saturable (144). Under physiological conditions, tubular SO₄²⁻ reabsorption works near the maximal rate (179), whereas if plasma SO₄²⁻ increases, the filtered load of SO₄²⁻ quickly exceeds the maximal tubular reabsorption and SO₄¹⁻ is excreted in the urine. Renal clearance of sulfate increases with increasing serum sulfate concentrations reaching approximately the glomerular filtration rate (GFR). At physiological serum sulfate concentration of 700–1,000 μM in the rat (133, 181), sulfate renal clearance was measured to be ~30% of the GFR.

In proximal tubular cells, sulfate transport systems have been extensively characterized on the luminal brush-border membrane (BBM) and the contraluminal basolateral membrane (BLM), by microperfusion studies in vivo (54, 252–258), with isolated tubules (24) and membrane vesicle studies (85, 150, 152, 197, 221, 251). The principle pathway for secondary active sulfate uptake is initiated across the BBM via sodium-coupled sulfate transport (Na⁺:SO₄²⁻ cotransport), driven by the luminal membrane Na⁺ gradient (8, 152, 221, 251). The influx of sulfate into the proximal tubular cell generates an outward sulfate gradient. Early studies demonstrated that exit of sulfate across the BLM occurs via an SO₄²⁻/HCO₃⁻ exchanger (24, 150, 197), which completes the process of transcellular sulfate reabsorption in the proximal tubule. The direct consequence of this process would be to drive HCO₃⁻ uptake into the proximal tubular cell across the BLM, thereby contributing to intracellular pH regulation (i.e., raising the pH). The same scenario would occur across the BBM: backflux of sulfate from the proximal tubular cell to the tubular lumen via a SO₄²⁻/HCO₃⁻ exchanger (117, 196, 245) for HCO₃⁻, would also drive HCO₃⁻ into the
cell, thereby raising intracellular pH. The gain of intracellular $\text{HCO}_3^-$ could be counteracted by the exit of $\text{HCO}_3^-$ from the cell by oxalate/$\text{HCO}_3^-$ exchanger involved in bidirectional transport of oxalate and $\text{HCO}_3^-$ on both the luminal and basolateral membranes of the proximal tubule (264).

1. BBM sulfate transport

Early uptake studies using radiotracer $[^{35}\text{S}]$sulfate in BBM vesicles (BBMV) demonstrated the presence of a $\text{Na}^+$-dependent sulfate transport system (221, 251, 253). In rat renal BBMV, the affinity ($K_m$) for this transport system was estimated to be 600 $\mu\text{M}$ for sulfate and 36 $\text{mM}$ for sodium, with a Hill coefficient ($n$) of $\approx 1.6$ (221). This transport system was kinetically characterized to be transporting two $\text{Na}^+$ with one $\text{SO}_4^{2-}$, a stoichiometry consistent with an electroneutral transport mechanism (221, 251). This electroneutral kinetic model was favored for many years, right up until the molecular isolation of this protein structure in 1993 (157), when a more detailed functional characterization demonstrated this protein to be electrogenic (28). Transport characterization of the cloned renal BBM $\text{Na}^+\text{-SO}_4^{2-}$ cotransporter (NaSi-1; Ref. 157) by electrophysiological recordings (28) demonstrated a kinetic model favoring the transport of three $\text{Na}^+$ for one $\text{SO}_4^{2-}$ (Fig. 2, see also sect. vA1). With regard to substrate specificity, the BBMV $\text{Na}^+\text{-SO}_4^{2-}$ cotransport activity was shown to be inhibited by thiosulfate, molybdate, and other oxyanions (including chromate and selenate), but not by phosphate or tungstate (253), suggesting that this transport system was unable to mediate phosphate transport. $\text{Na}^+\text{-SO}_4^{2-}$ cotransport was well characterized in BBMV from rat (8, 152) and rabbit (1, 221, 251) kidney cortex. The presence of a $\text{Na}^+$-dependent $\text{SO}_4^{2-}$ transport system was more recently demonstrated by injecting rabbit kidney cortex mRNA into Xenopus laevis oocytes (266) (see also sect. vA1).

In addition to the $\text{Na}^+\text{-SO}_4^{2-}$ cotransport system present on the BBM, there has been evidence to suggest that sulfate can be transported by an anion exchange (AE) mechanism across the luminal membrane in BBMVs from rat (196), bovine (245), and rabbit (117) kidney cortex. This transport system was shown to mediate an electroneutral anion exchange, with little or no interaction with $\text{Cl}^-$ and to be able to transport bicarbonate, oxalate, acetate, lactate, succinate, and $p$-aminohippurate (PAH), in addition to sulfate (117). The overall importance and contribution of this transport activity to sulfate reabsorption in proximal tubular cells is yet unknown, nor has this transport system been identified at the molecular level.

2. BLM sulfate transport

The proximal tubular BLM possesses an anion exchange sulfate transport system that transports sulfate out of the cell into the systemic circulation and exchanges sulfate with thiosulfate, hydroxyl ions, bicarbonate, oxalate, and a variety of organic ions (24, 150, 197). In rat renal BLM vesicles (BLMV), the sulfate anion exchanger shows specificity for the exchange of bicarbonate, hydroxyl, thiosulfate, and sulfate ions, but not chloride, phosphate, lactate, or PAH (88, 197). Furthermore, stilbene derivatives DIDS and SITS, characterized as inhibitors of anion exchangers (3, 8, 18, 24, 54, 197) (among other proteins), could inhibit sulfate uptake into BLMVs (8, 85, 88). This BLM transport system has been shown to have different transport kinetics and a different sensitivity to inhibitors than the BBM sulfate anion exchanger (54), suggesting that they are not encoded by the same protein. For example, the apparent inhibitory constant ($K_i$) for DIDS, probenecid, phenol red, and oxalate (among others) on sulfate uptake is over threefold higher for the BBM than for the BLM sulfate anion exchanger, suggesting that the BLM transporter is more sensitive to inhibition by these compounds than the BBM sulfate transporter (54). Furthermore, upon the cloning of the BLM sulfate anion exchanger (sat-1; Ref. 18), antibodies raised against this protein showed immunocytological staining only on the BLM (118), suggesting that this protein is structurally (in addition to functionally) different to the BBM sulfate anion exchanger (see sect. vB1).

Despite the identification of several different $\text{SO}_4^{2-}$ transport systems in renal proximal tubules, which of these plays the predominant role in sulfate homeostasis is still unclear. Since the active reabsorption process is dependent on a normal $\text{Na}^+\text{-K}^+$-ATPase activity in the proximal tubular cells (183), it has been suggested that a sodium gradient may be important in sulfate uptake (as compared with anion exchange) and that the $\text{Na}^+$-sulfate cotransporter on the BBM may play the predominant role (17). In the lower urinary tract (i.e., human ureteral epithelial cells), there is evidence of a DIDS-sensitive sulfate/chloride anion exchanger, but not a $\text{Na}^+$-dependent sul-
fate transporter (69), suggesting that active sulfate uptake may not be occurring in the lower urinary tract, but is restricted to the renal proximal tubule. For a model of the sulfate transport systems present in the renal proximal tubule, see Figure 2.

B. Sulfate Transport in the Intestine

The majority of sulfate absorption occurs in the latter part of the small intestine (ileum and jejunum) (183). Sulfate uptake has been studied mostly using membrane vesicles purified from mammalian small intestines. In pig jejunum BBMVs, there is evidence of sulfate transport by both a Na+-dependent SO42− cotransporter and a Na+-independent SO42− anion exchanger (269). Na+-dependent sulfate uptake was also demonstrated in BBMV isolated from rabbit (1) and rat (153) ileum, with similar transport properties to the renal BBM transport system. BBMV 35S[Sulfate uptake experiments measured the ileal Na+-sulfate cotransporter to have a K_m of 520 μM for sulfate in the presence of 100 mM sodium (1), which closely agreed with the sulfate affinity (K_m of 600 μM) of the renal BBMV Na+-sulfate cotransporter (221). The proposed kinetic model for this transporter was also suggested to be electroneutral, with two Na+ being transported with one sulfate ion (1). As with the renal BBMV Na+-sulfate cotransporter, this model was later proven to be incorrect (see sect. IV). In BBMV from rabbit ileum, there is evidence of a SO42−/OH− exchange system, which is stimulated by a pH gradient and inhibited by DIDS and SITS (223). A carrier-mediated Cl−/SO42− exchange system has been described in BLMV of the rabbit ileum (224) and rat jejunum (88), with characteristics similar to the renal BLM anion exchanger. The evidence gathered from membrane vesicle studies suggests that the mechanisms of sulfate transport in the small intestinal epithelium are identical to those in kidney proximal tubules. Structural isolation of the small intestinal sulfate transporter has demonstrated this indeed to be the case, with the rat ileal BBM Na+-SO42− cotransporter (195; see sect. IV) being identical to the renal BBM Na+-SO42− cotransporter (157).

C. Sulfate Transport in the Liver

Carrier-mediated hepatic sulfate uptake was measured in isolated perfused rat liver (22) and isolated hepatocytes (260). Sulfate uptake in hepatocytes was shown to occur by Na+-dependent and Na+-independent systems, with K_m values for sulfate of 2.3 and 33 mM and maximum velocity (V_max) values of 2.1 and 10 nmol·mg protein−1·min−1, respectively (260). Analysis of the Na+ dependency for the Na+-sulfate cotransporter system indicated an n value of 1.05, suggesting an equimolar stoichiometry for sodium and sulfate transport. Bicarbonate was shown to affect sulfate transport, with sulfate uptake increased by intracellular bicarbonate and competitively inhibited by extracellular bicarbonate (260). Sulfate transport in hepatocytes was further characterized in rat liver basolateral (sinusoidal) plasma membrane vesicles and was demonstrated to be mediated by an anion exchange pathway, through countertransport with intracellular hydroxyl ions (109). This sulfate uptake was saturable with increasing sulfate concentrations (K_m = 16.1 ± 3.9 mM), was stimulated by a pH gradient (pH 8.0 inside, pH 6.0 outside), and was inhibited by probenecid, DIDS, and nigericin (109). Subsequently, a sulfate/HCO3− anion exchange system was also identified in apical (canalicular) rat liver plasma membrane vesicles, which was stimulated by a bicarbonate gradient (50 mM in, 5 mM out, pH 8.0 and 8.0 producing a saturable sulfate transport, which could be inhibited by probenecid, acetazolamide, furosemide, DIDS, and SITS (166). Thus several sulfate transport systems exist in hepatocytes, for which the functional roles (i.e., possibly bile synthesis) need to be determined.

D. Sulfate Transport in the Lungs

There is evidence of an anion exchange DIDS/SITS-inhibitable sulfate transport system present on apical membrane vesicles of bovine tracheal epithelium (64). This sulfate transport system appears to be cis-inhibited and trans-stimulated by chloride, bicarbonate, thiosulfate, selenate, and molybdate, but not by phosphate or arsenate (64). The affinity of the transport system for sulfate was observed to be highest in low ionic strength media (K_m = 130 μM) and decreased in the presence of glucuronate (K_m = 680 μM). In agreement, bronchial epithelial cells were shown to transport sulfate by an anion exchanger that was inhibited by external chloride, DIDS, and SITS and was not stimulated by sodium (171). In human lung fibroblasts, sulfate transport was suggested to occur via several pathways: 1) a high-affinity SITS-sensitive pathway, 2) a low-affinity SITS-sensitive pathway, and 3) a SITS-insensitive pathway (68). At extracellular sulfate concentrations of <100 μM, the predominant pathway was the high-affinity SITS-sensitive component with a K_m of 34 ± 14 μM for sulfate. Under normal serum sulfate (100–500 μM) concentrations, the predominant pathway for sulfate influx was the SITS-sensitive, low-affinity pathway with a K_m of 1 mM for sulfate (68). The SITS-insensitive pathway, for which no K_m for sulfate transport could be determined, was detectable (to a lesser extent) only under serum sulfate concentrations. Arsenate and orthophosphate had no effect on sulfate influx, whereas bicarbonate, molybdate, vanadate, and thiosulfate inhibited sulfate influx but had no effect on sulfate efflux (68). Extracellular chloride was able to inhibit the influx of sulfate and stimulate the efflux of sulfate, suggesting that sulfate is transported across bron-
chial epithelial cells via an anion exchanger that exchanges chloride for sulfate (67, 68, 171).

E. Sulfate Transport in Erythrocytes

Sulfate transport has been extensively studied in the erythrocyte, where it occurs via an anion exchanger known as band 3 (124). Band 3 (130) constitutes the major integral membrane protein of the red blood cell (RBC) (124). Since erythrocytes have a high capacity for rapid exchange of anions, \( \text{SO}_4^{2-} \) exchange has been postulated to share a common transport mechanism with \( \text{Cl}^- \) and \( \text{HCO}_3^- \), a process which is electroneutral in RBCs (2, 3, 32). The primary function of the erythroid band 3 sulfate anion exchanger is to increase the capacity for plasma \( \text{CO}_2 \) transport by exchanging \( \text{HCO}_3^- \) generated by intracellular carbonic anhydrase for extracellular \( \text{Cl}^- \) (125). The physiological roles of the erythroid band 3 protein is discussed in greater detail in section ivB4.

F. Sulfate Transport in the Brain

The sulfate anion exchanger band 3 (or AE1; see sect. ivB4) was found to be expressed ubiquitously in all cells studied, including isolated neurons and neurons of the CNS (120). As in the erythrocyte, the function of band 3 in neurons has been postulated to be important for the exchange of \( \text{HCO}_3^- \) with \( \text{Cl}^- \) and sulfate (120). Despite this, very little information is available to date on the requirement for sulfate transport systems in neurons. Sulfation plays an important role in the CNS for the biosynthesis of sulfated proteoglycans that are involved in modulating cell interactions in developing nervous tissues (61). Neuronal heparan sulfate proteoglycans are involved in cell adhesion, neural crest migration, and neurite extension (60, 187), whereas chondroitin sulfate proteoglycans (of astrocyte origin) have been shown to inhibit neurite growth (86). Several findings have proposed that the brain may possess a sulfate transport system. Ventriculocisternal perfusion studies have demonstrated that a carrier mechanism for sulfate transport exists in the brain, capable of mediating sulfate transport from the cerebrospinal fluid (CSF) to plasma (50, 147). This proposed mechanism of sulfate transport was shown to be saturable, with the addition of unlabeled sulfate to the perfusate leading to significantly reduced \([^{35}S]\)sulfate clearance. In addition, sulfate transport was markedly reduced by the addition of thiosulfate, a sulfate analog that competed for the same transport mechanism. Since sulfate transport in the brain is important for maintaining CSF sulfate concentration (50, 147), it is likely that similar transport processes may also be required in the brain, where the import of sulfate into cells of the CNS would be needed. Recently, a rat brain sulfate transporter, which is sensitive to DIDS and oxalate, has been characterized in this laboratory (135), confirming that neuronal and/or glial cells contain a functional sulfate transporter (see sect. ivB1).

G. Sulfate Transport in the Placenta

Sulfate is an essential metabolite utilized by the developing fetus in the synthesis of sulfate mucopolysaccharides, proteins, and steroids (41). In the human placenta, \( \text{SO}_4^{2-} \) transport has been shown to be mediated by a DIDS-sensitive anion-exchange transport system (45). Human placental tissue slices showed concentrative sulfate uptake only in the absence of sodium and at low pH (45). BBMVs isolated from human placenta were shown to contain a sulfate transporter with broad specificities for other oxyanions including selenate, tungstate, molybdate, and chromate (57). Vesicles isolated from human placental trophoblasts expressed both apically and basolaterally located \( \text{SO}_4^{2-} \) transport systems, both being electroneutral with an approximate \( K_m \) of 2.5 mM for sulfate and DIDS sensitive (\( K_i \) for DIDS = 10 \( \mu \)M) (29). In placental tissue slices and placental BBMV, sulfate uptake was inhibited by salicylate, suggesting salicylate ingestion could compromise fetoplacental sulfate homeostasis (233). To date, there is no evidence of a \( \text{Na}^+ \)-dependent sulfate transport system in the human placenta. Sulfate transport properties in human placenta are in keeping with an anion exchange system, including the lack of stimulation by sodium, \( trans \)-stimulation by bicarbonate, and inhibition by SITS and DIDS (20, 21, 40, 57, 231), suggesting that a sulfate anion exchanger is most likely responsible for sulfate accumulation by the human fetus.

H. Sulfate Transport in the Mammary Gland

In lactating rat mammary glands, a DIDS-sensitive \( \text{SO}_4^{2-} \) anion exchange system was detected, which was \( trans \)-stimulated by chloride, iodide, and sulfate anions (230). Furthermore, selenate and other divalent anions (including molybdate and thiosulfate) were also able to \( trans \)-stimulate the efflux of radiolabeled sulfate from lactating rat mammary tissue slices (232). The effect of selenate on sulfate efflux was saturable with an apparent \( K_m \) of 270 \( \mu \)M (232). From this study, it was concluded that sulfate and selenate may share a pathway for transport in the lactating rat mammary gland (232). This sulfate transport system may be important for the metabolism of \( \text{SO}_4^{2-} \) by the mammary gland and may help in determining milk anion concentrations.

I. Sulfate Transport in Cultured Cell Lines

Anion-exchange carrier-mediated sulfate-transport systems, similar to band 3, have been characterized in a
variety of mammalian cell lines, including Ehrlich ascites tumor cells (141), Vero cells (189), human lung fibroblasts (68), Chinese hamster ovary (CHO) cells (65), and skin fibroblasts (66). All of these sulfate transport systems were shown to be mediated by an electroneutral anion exchange system and exhibited high affinity for chloride, bicarbonate, and sulfate. A recent study documented the presence of a sulfate anion exchange (AE) activity in Madin-Darby canine kidney (MDCK) cells (7), a renal epithelial cell line derived from the dog kidney tubule. Functional AE activity was demonstrated in MDCK cells by increased uptake of sulfate at pH 6.0 over pH 7.0 and the inhibition by DIDS and nonhalide anions including molybdate, oxalate, pyridoxal 5'-phosphate, but not phosphate (7). In Ehrlich ascites cells, sulfate flux was shown to be saturable, with a $K_m \approx 2 \text{ mM}$ for sulfate, in the absence of chloride (141). To date, there has only been one report of a Na+-coupled sulfate transporter in an established mammalian cell line, the OK-E cells, a clonal subline of the proximal tubular opossum kidney (OK) cells (250). Transport kinetics for sulfate interaction were determined: $K_m = 2.4 \pm 0.2 \text{ mM}$ and $V_{max} = 125 \pm 15 \text{ pmol/mg protein min}^{-1}$ for sulfate. Hill analysis demonstrated a Na+ dependence of sulfate transport with the following values: $n = 1.5$ and Michaelis constant $K_{Na} = 23 \text{ mM}$ (250). This transport mechanism was stimulated by an acid pH (lowering the pH from 7.4 to 6.4) but was inhibited by DIDS ($IC_{50}$ for DIDS = 0.9 $\mu$M), bicarbonate ($IC_{50}$ for $HCO_3^-$ = 7 mM), sulfite, thiosulfate, chromate, picrylsulfanooate, and ethancrynate (but not phosphate or amiloride). In the presence of extracellular chloride, the sulfate affinity for the carrier ($K_m = 500 \mu$M) was increased without affecting its $V_{max}$. A similar transport activity was observed in mouse renal BBM, although the sensitivity to DIDS ($IC_{50}$ for DIDS ~500 $\mu$M) and $HCO_3^-$ (~50 mM) was significantly lower than in OK-E cells (250). The sulfate transport system characterized in OK-E cells (250) is quite unique in its mechanism, since it is both a Na+-coupled system, with a slightly lower affinity (for sulfate) than the renal proximal tubular BBM Na+-SO_4^{2-} cotransporter and is extremely DIDS sensitive, a more common feature for sulfate anion exchangers, rather than Na+-SO_4^{2-} cotransporters which are insensitive to DIDS. Such an interesting (transport kinetic) system has not been observed in an in vivo system.

J. Sulfate Transport in Mitochondria

Mitochondria from rat kidney cortex and liver cells take up sulfate ions from their environment. This transport system was first described as a sulfate/dicarboxylate exchanger, which was also able to transport phosphate (48), suggesting a common transport mechanism for the uptake of sulfate, phosphate, and dicarboxylates into mitochondria. Furthermore, the mitochondrial sulfate transport activity was demonstrated to be mediated by a H+ symporter carrier (218). The kinetics of sulfate uptake were measured with a calculated $K_m$ value for sulfate of ~300 $\mu$M (49). In this study, a competitive relationship was demonstrated between the influxes of sulfate and malonate, whereas sulfate and phosphate showed a mixed mechanism of competition (49). Furthermore, mersalyl and bathophenanthroline showed similar inhibition patterns for sulfate and malonate transport, but not phosphate transport (49), suggesting that sulfate and malonate may bind to the same site whereas phosphate to a different site on the carrier. Early reports showed that parathyroid hormone could stimulate sulfate, phosphate, and arsenate uptake in mitochondrial transport system. Since the conversion of thiosulfate to sulfate in mammals is catalyzed by mitochondrial thiosulfate reductase and thiosulfate sulfotransferase enzymes (129), the physiological function of a mitochondrial sulfate transport system could be important for sulfur metabolism.

More recently, the rat mitochondrial dicarboxylate carrier (DIC) was cloned and showed the ability to exchange sulfate, thiosulfate, and succinate for malate and phosphate uptake in bacterial reconstituted proteoliposomes (76). Unlike the rat DIC, the bovine mitochondrial phosphate carrier (PIC) shows a more narrow substrate specificity, with a preference for high phosphate uptake when phosphate or arsenate is preloaded into liposomes, whereas sulfate and malate had almost no effect on mitochondrial phosphate uptake in reconstituted proteoliposomes (59). It is still unclear whether DIC or PIC encode mammalian mitochondrial sulfate transporters.

K. Lysosomal Sulfate Transport

Sulfate, being a by-product of the degradation of macromolecules such as glycosaminoglycans, exits lysosomes via a carrier-mediated pH-dependent sulfate transport process (127). This process was shown to be inhibited by DIDS, SITS, phenylglyoxal, 1,2-cyclohexanedione, niflumic acid, and dinitrofluorobenzene, suggesting the lysosomal sulfate transporter has functional similarities with the erythrocyte band 3 anion transporter. However, the potent band 3 inhibitor dipyriramole had no effect on lysosomal sulfate transport, suggesting that there may be some structural differences between the erythrocyte and lysosomal sulfate anion transporters. In an attempt to purify the lysosomal sulfate transporter, a method for reconstitution of transport in artificial membrane vesicles was developed (128). Proteoliposomes were prepared from Percoll density gradient-purified rat liver lysosomes and exhibited saturable sulfate transport with characteristics similar to those in lysosomal membranes, with a $K_m$.
value of 155 μM for sulfate, exhibiting a pH dependence and sensitivity to DIDS (128). ATP was shown to markedly stimulate sulfate uptake by rat liver lysosomes that were treated with N-ethylmaleimide, a blocker of the lysosomal proton-translocating ATPase (H+-ATPase), with maximal stimulation requiring millimolar concentrations of ATP and neutral pH (36). ATP-stimulated transport exhibited saturation kinetics with a $K_m$ of 175 μM for sulfate, identical to the $K_m$ for lysosomal sulfate uptake at pH 5.0, an ATP-independent process. Exposure of lysosomes to protein kinase A and protein kinase C inhibitors had no effect on the stimulation of sulfate transport by ATP, suggesting that the lysosomal sulfate transport protein does not require protein kinase A or C phosphorylation for expression (36). More recently, using thiol blocking agents, the role of sulphydryl groups for function of the lysosomal sulfate transport system was examined (37). Monothiol binding reagents $p$-hydroxymercaptozoic acid (p-HMB) and $p$-chloromercuribenzenzoic acid (p-CMB), dithiol binding reagents such as CuCl$_2$, the alkylating agent N-ethylmaleimide (NEM), and NADH all inhibited lysosomal sulfate transport (37). NEM exposure led to a sevenfold increase in $K_m$ (867 μM vs. control 126 μM) and a decrease in $V_{max}$ (99 pmol vs. control 129 pmol/unit β-hexosaminidase $^{-1}$ 30 s $^{-1}$) (37). Similarly, exposure to Cu$^{2+}$ led to an increase in $K_m$ for sulfate to 448 μM and a decrease in $V_{max}$ to 77 pmol/unit β-hexosaminidase $^{-1}$ 30 s $^{-1}$ (37). These data suggest sulphydryl groups may play a role in lysosomal sulfate transport through effects on substrate affinity. Despite extensive transport characterization, this protein remains yet to be cloned; however, sulphydryl binding may appear to be a strategy for the purification of this transporter.

L. Effects of Pharmacological Agents on Sulfate Homeostasis

Numerous studies have been aimed at elucidating the mechanisms by which body sulfate homeostasis is regulated. Various pharmacological agents have been shown to modulate serum sulfate levels in vivo. Probenecid, an inhibitor of renal organic anion secretion, increases renal sulfate clearance by ~1.8-fold and reduces serum sulfate levels by ~1.7-fold, without affecting serum levels or renal clearance of uric acid, magnesium, calcium, or phosphorus (53). Administration of a nonsteroidal anti-inflammatory drug (NSAID) salicylic acid (SA) to rats resulted in an approximately twofold increase in renal sulfate clearance (2.13 ± 0.74 vs. 1.09 ± 0.54 ml/min $^{-1}$ kg $^{-1}$ in control rats), which led to an approximately twofold decrease in serum sulfate levels (550 ± 120 vs. 1,040 ± 230 μM in controls) (175). SA has been shown to inhibit sulfate transport in the kidney, placenta, and erythrocytes (5). SA administration to rats inhibited sulfate transport in both renal BBMVs and BLVs: $K_i$ for SA values were calculated at ~20 mM for BBMV Na$^+$-sulfate cotransport and 1–2 mM for BLMV sulfate/bicarbonate exchange (52). Due to the lower $K_i$ for SA value measured in BLVs, the inhibitory effect of SA may be predominantly inhibition of the BLM sulfate transporter, which may (at least in part) be responsible for the SA-induced increases in renal sulfate clearance (52). Chronic aspirin administration to healthy human individuals caused a small but significant decrease in serum sulfate levels (after 8 days of administration), but unlike in rats, no apparent change in renal clearance of sulfate (or creatinine) was observed (172), suggesting chronic SA administration has little effect on sulfate homeostasis in humans.

Chronic administration of two other NSAIDs, naproxen and sulindac, to arthritic patients suffering from renal impairment led to a significant (10–25%) increase in serum sulfate concentration and a significant (20–33%) decrease in renal clearance of sulfate and creatinine, compared with younger subjects with normal renal function (174). Naproxen and sulindac produced no changes in renal clearance or serum levels of other electrolytes, including sodium, phosphate, potassium, magnesium, and calcium (174), suggesting their effects were specific on renal sulfate handling. The effect on renal function by naproxen and sulindac was less pronounced than by chronic SA administration in rats (175) and was postulated to be a consequence of mild renal impairment in older arthritic patients.

Acetaminophen (AA) administration to normal rats decreased serum free sulfate concentrations by sevenfold (from 1,070 ± 130 to 150 ± 10 μM), whereas it had no effect in rats with renal dysfunction or renal failure (143). In normal rats, AA is conjugated with sulfate forming AA sulfate, which is excreted by the kidneys, thereby depleting free sulfate in the body, which leads to the formation of other metabolites (i.e., acetaminophen glucuronide) and AA-induced hepatotoxicity (143). In rats with renal dysfunction/failure, retention of free sulfate occurs, which prevents the depletion of serum sulfate by AA, permitting sufficient sulfate availability for the biotransformation of AA to AA sulfate (143). When serum sulfate levels were raised to ~1.5 mM in rats (by sulfate infusion), AA had no effect on the high renal clearance of sulfate during hypersulfatemia (144). Injection of mice with N-acetylcysteine, a compound which decreases AA-induced toxicity, led to increases in serum sulfate concentrations and prevented the depletion of serum sulfate by AA (101). These studies demonstrate the importance of free sulfate levels in the body on the elimination of drugs subject to sulfate conjugation and the pronounced serum concentration dependence of renal sulfate clearance on sulfate homeostasis.
IV. CLONING OF SULFATE TRANSPORTERS:  
THE MOLECULAR BIOLOGY ERA  

A. Na⁺-Dependent Sulfate Transporters  

1. NaSi-1  

The first functional mammalian sulfate transporter was isolated in 1993 using the *Xenopus laevis* oocyte expression cloning system (157). Previous attempts to purify plasma membrane sulfate transporters using biochemical techniques were unsuccessful. Up until the early 1990s, sulfate transport systems had only been characterized using biochemical techniques such as membrane vesicular uptake assays as well as perfusion studies using radiolabeled [³⁵S]sulfate uptakes. In the late 1980s, the first plasma membrane transporter to be isolated using the *Xenopus* oocyte expression cloning system was the intestinal Na⁺-glucose cotransporter, SGLT-1 (97). The *Xenopus* oocyte expression system, which was pioneered by Gurdon et al. in the early 1970s (87), relies on the microinjection of poly(A)⁺ RNA (purified from any source of tissue) into the cytoplasm of *Xenopus* oocytes. Proteins are then translated, folded, modified, and sorted to their respective compartments (to the cell membrane for plasma membrane proteins) by the oocytes. The newly synthesized proteins can then be functionally characterized by either radiotracer uptake, electrophysiological measurements, or receptor binding assays. A cDNA library can then be generated from the poly(A)⁺ RNA with the functional activity, and the same functional assay is then used to isolate the cDNA(s) of interest by expression cloning. For a recent methodological review of this technique, see Reference 162. The beauty of this technique is that it does not rely on sequence information but rather a good functional assay to permit the initial expression and subsequent cloning of a novel protein. This powerful heterologous expression system was applied extensively in the 1990s for the isolation of novel membrane transporters, receptors, and ion channels (for reviews see Refs. 162, 235) and is still being used today in many laboratories, with varying degrees of success.  

For the expression of sulfate transporters, the *X. laevis* oocyte system was first implemented in 1990. Injection of poly(A)⁺ RNA from rabbit renal cortex into *Xenopus* oocytes led to a three- to fourfold stimulation of Na⁺-sulfate cotransport (above controls), which allowed transport kinetics to be determined: sulfate interaction, $K_m = 600 \pm 100 \ \mu M$ and $V_{max} = 37.3 \pm 2.2 \ pmoles\text{-oocyte}^{-1}\text{h}^{-1}$; Na⁺ interaction, $K_m = 33.8 \pm 3.4 \ mM$, $V_{max} = 26.1 \pm 2.3 \ pmoles\text{-oocyte}^{-1}\text{h}^{-1}$, and $n = 2.5 \pm 0.5$ (155). On the basis of this preliminary data, a rat kidney cortex cDNA library was constructed, and upon screening this library for Na⁺-sulfate uptake in *Xenopus* oocytes, a cDNA named NaSi-1 (for Na⁺-sulfate inorganic cotransporter-1) was isolated by expression cloning (157). NaSi-1 cRNA injected into *Xenopus* oocytes led to a three- and dose-dependent stimulation of Na⁺-dependent $SO_4^{2-}$ uptake, for which the following transport kinetics were determined: sulfate interaction, $K_m = 620 \pm 80 \ \mu M$, $V_{max} = 42.7 \ pmoles\text{-oocyte}^{-1}\text{min}^{-1}$; Na⁺ interaction, $K_m = 16.8 \pm 2.9 \ mM$, $V_{max} = 17.0 \pm 1.4 \ pmoles\text{-oocyte}^{-1}\text{h}^{-1}$, and $n = 1.8 \pm 0.4$ (157). These transport kinetics resemble closely the activity of the BBM Na⁺-$SO_4^{2-}$ cotransporter of the rat kidney proximal tubule (152, 184).  

NaSi-1 transport kinetics were then further characterized in *Xenopus* oocytes by electrophysiological recordings using current- and voltage-clamp techniques. Superfusion of NaSi-1-injected oocytes that were current-clamped with 1 mM sulfate led to a 12-mV depolarization of the cell membrane (28). In voltage-clamped NaSi-1-injected oocytes, sulfate induced an inward current that was dependent on both Na⁺ and sulfate concentrations, with a calculated $K_m$ for Na⁺ and sulfate of ~70 mM and 100 $\mu M$ (28), respectively, suggesting electrophysiological determination of NaSi-1 affinity for sulfate is somewhat higher than by radiotracer uptake studies (157). For Na⁺ interaction, the Hill coefficient was measured electrophysiologically to be 1 and 2.8 for sulfate and Na⁺, respectively (28), suggesting that three sodiums are transported per one sulfate ion. This was the first study to demonstrate that the renal Na⁺-$SO_4^{2-}$ cotransporter NaSi-1 was in fact not electroneutral (as previously thought), but rather an electrogenic cotransporter of sulfate and Na⁺, with a stoichiometry of 1:3, with one additional Na⁺ being responsible for a net positive charge entering the cell. Thiosulfate and selenate created similar currents as with sulfate perfused to NaSi-1-expressing oocytes, allowing for the determination of $K_m$ for thiosulfate (85 ± 50 $\mu M$) and selenate (580 ± 90 $\mu M$) (28). This was the first evidence to suggest that NaSi-1 can in fact transport thiosulfate and selenate, in addition to sulfate. To determine whether the three substrates bind to the same or different regions on the NaSi-1 protein, the compounds were added in combination to NaSi-1-injected oocytes. Sulfate was unable to induce an additive current when added to oocytes perfused with thiosulfate or selenate (28), suggesting that all three substrates were...
most likely transported on the same site of the NaSi-1 protein. Furthermore, perfusion of NaSi-1-injected oocytes with sulfate at various pH values (pH 6.3, 7.3, and 8.3) did not evoke different currents (28), suggesting NaSi-1 activity is not altered by changes in pH.

The NaSi-1 cDNA contains 2,239 bp [including a poly(A) tail] and encodes a protein of 595 amino acids (66.05 kDa), with the hydropathy profile suggesting a protein with at least eight transmembrane domains (TMDs) (157). In vitro translation of NaSi-1 cRNA in rabbit reticulocyte lysate resulted in a protein of expected size, and in the presence of microsomes, the protein size was slightly increased, suggesting possible glycosylation. Northern blot analysis showed two mRNA transcripts of 2.3 and 2.9 kb in kidney (more abundant in cortex than in papilla/medulla) and small intestinal mucosa of rats. NaSi-1 protein localization was confirmed to the BBM of rat renal proximal tubular cells by immunocytochemistry using a NaSi-1 peptide-specific polyclonal antibody (148). These experiments were the first to structurally characterize a membrane protein encoding a Na\(^{+}\)-SO\(_4\)\(^{2-}\) cotransport system in renal and small intestinal BBMs.

The lack of endogenous NaSi-1 protein expression in any mammalian renal or intestinal cell lines (Markovich, unpublished data) led to several studies being initiated at expressing NaSi-1 protein in a cell culture model, in addition to the existing Xenopus oocyte expression system. NaSi-1 cDNA was transfection into the MDCK cell line (199) and into the baculovirus-driven SF9 insect cells (80). In both systems, NaSi-1 protein expression was characterized functionally. NaSi-1 stably expressing MDCK cells led to a fourfold increase in Na\(^{+}\)-sulfate cotransport (compared with vector only transfected cells), which was predominantly expressed at the apical cell surface (199). This result suggested that the sorting behavior of NaSi-1 to the apical membrane is identical to that observed for the renal BBM proximal tubular Na\(^{+}\)-sulfate cotransporter in vivo (148, 152, 184). NaSi-1 expressing SF9 cells demonstrated a 60-fold higher Na\(^{+}\)-sulfate cotransport activity compared with noninfected cells (80). The calculated \(K_m\) for sulfate in NaSi-1 expressing SF9 cells was 300–400 \(\mu\)M, in close agreement with the values obtained in NaSi-1-expressing Xenopus oocytes (157) and the Na\(^{+}\)-sulfate cotransport activity in renal BBMVs (152, 184). With the use of a NaSi-1 polyclonal antibody, Western blotting and immunoprecipitation detected NaSi-1 proteins of 55–60 kDa in size in NaSi-1-expressing SF9 cells (80). Recently, this laboratory has generated OK cell lines stably expressing NaSi-1 (unpublished data), which will be used to study the sorting mechanisms and posttranslational regulation of the NaSi-1 protein. To identify the possible involvement of either (or both) phospholipase C (inositol trisphosphate/diacylglycerol) and adenylate cyclase (cAMP) signaling pathways on NaSi-1 function, this laboratory has recently tested pharmacological activators of protein kinase A (8-bromo-cAMP) and protein kinase C (sn-1,2-dioctanoylglycerol; DOG) on NaSi-1 expression in Xenopus oocytes. Our data showed that both 8-bromo-cAMP and DOG inhibited NaSi-1-induced sulfate transport activity in Xenopus oocytes (unpublished data), suggesting possible involvement of phosphorylation for protein function. For a recent review on the membrane trafficking of mammalian sulfate transporters, see Reference 154.

A) OTHER NASI-1 PROTEINS. Recently, this laboratory cloned two additional mammalian NaSi-1 orthologs: the mouse NaSi-1 (mNaSi-1) cDNA (9) and the human NaSi-1 (hNaSi-1) cDNA (136). These cDNAs encode proteins that are very similar both structurally and functionally to the rat NaSi-1 (designated from now as rNaSi-1), with the major differences outlined below. By comparison of the amino acid sequences using the ClustalW alignment program, mNaSi-1 shares 93.6% identity and 96% similarity with rNaSi-1, hNaSi-1 shares 82.9% identity and 88% similarity with rNaSi-1, and mNaSi-1 shares 81% identity and 87% similarity with hNaSi-1 (see Table 1). As with rNaSi-1, hNaSi-1 encodes a protein of 595 amino acids, whereas the mNaSi-1 protein is one amino acid shorter at 594 amino acids, with a glutamate missing at residue 314 of rNaSi-1 and hNaSi-1. All three proteins have a calculated molecular mass of 66.1 kDa, comprising 13 putative TMDs (see Fig. 3), predicted by the TopPred2 program (261). Slight differences are present in the locations of putative consensus sites on the three NaSi-1 homologs. Three putative N-glycosylation sites are present in both rNaSi-1 (at Asn\(^{140}\), Asn\(^{174}\) and Asn\(^{201}\)) and mNaSi-1 (Asn\(^{140}\), Asn\(^{174}\) and Asn\(^{200}\)) proteins, whereas hNaSi-1 has four putative N-glycosylation sites (at Asn\(^{140}\), Asn\(^{174}\), Asn\(^{207}\) and Asn\(^{291}\)). The significance of these sites on NaSi-1 function is presently being investigated. Preliminary data from this laboratory suggest that Asn\(^{140}\) and Asn\(^{291}\) on rNaSi-1 may be true glycosylation sites that are essential for protein function. Mutagenesis of these asparagines to serines led to a total loss of Na\(^{+}\)-sulfate cotransport activity in Xenopus oocytes (unpublished data), most likely due to defective trafficking of the protein to the plasma membrane, as demonstrated previously for N-glycosylation mutants of the renal Na\(^{+}\)-phosphate cotransporter NaPi-2 (96). Initially, using the Kyte and Doolittle algorithm, the rNaSi-1 protein was predicted to contain 8 TMDs (157); however, more recently using the TopPred2 program (261), the NaSi-1 proteins were suggested to contain 13 putative TMDs (Fig. 3). This 13-TMD model was confirmed by two other web-based programs, TMPred (103) and Sosui (99). In this model, there is a large intracellular loop between TMDs four and five, which was previously predicted to be an extracellular loop in the eight-TMD model, where two putative N-glycosylation sites (Asn\(^{140}\) and Asn\(^{174}\)) were present (157). Despite the fact that mutating the putative glycosylation site Asn\(^{140}\) (to serine)
in this large loop on the rNaSi-1 protein led to a loss of sulfate uptake in Xenopus oocytes, this result could be due to an alteration in protein folding-sorting and not due to a loss of glycosylation, as shown previously for the NaPi-2 protein (96). Furthermore, the requirement of the N-glycosylation sites for protein sorting and function needs to be determined by the expression of the mutated proteins in a mammalian renal proximal tubular cell line (e.g., OK cells or LLC-PK1 cells), to rule out that Xenopus oocytes have a different sorting process when compared with mammalian cells. Furthermore, all three NaSi-1 proteins contain numerous putative phosphorylation sites: (1) one cAMP/protein kinase A site (Thr240 on hNaSi-1, Thr405 on mNaSi-1, Thr406 on rNaSi-1); (2) several protein kinase C sites (rNaSi-1: Ser213, Thr218, Ser230, Thr232, and Thr243, mNaSi-1: Ser213, Thr218, and Ser230; hNaSi-1: Ser74, Thr209, Ser213, Thr230, Thr236, and Thr243); (3) one tyrosine kinase site (rNaSi-1 and hNaSi-1: Tyr39, none present on mNaSi-1); and (4) numerous putative casein kinase II and N-myristoylation sites. The functional significance of all of these putative sites still needs to be determined. Of particular interest is a 17-amino acid-long consensus sequence motif (TSFALFLLPVANPPNAV) called the "sodium:sulfate symporter family signature" (PROSITE PS01271) situated at amino acids 523 to 539 of rNaSi-1, which is highly conserved among the NaSi-1 proteins, as well as with other structurally related proteins, including SUT-1 (82) and the Na+-dicarboxylate cotransporters (190) (see below and Table 1). The significance of this motif is yet unknown.

**B) FUNCTIONAL CHARACTERIZATION.** Substrate specificities are shared among the three NaSi-1 protein homologs; however, slight alterations are observed in their substrate kinetics for Na+ and sulfate interactions (see Table 2). The affinities (Km) for sulfate are in close agreement for all three NaSi-1 proteins; however, the maximal transport velocity (Vmax) for sulfate uptake was shown to be four-fold higher in hNaSi-1 (136) than for either of the rodent NaSi-1 proteins (9, 157). This is intriguing since higher serum SO42− levels have been measured in rodents (~1 mM in rat and mouse) and nine other mammalian species (including guinea pig, rabbit, cat, sheep, goat, cow, pig, monkey, and horse) than in humans (~300 μM) (10, 16, 133). The lower levels of SO42− in humans were suggested to be due to slower rates of SO42− depletion by xenobiotic drugs that are metabolized to SO4− conjugates. Thus the expression of a higher capacity SO42− transporter (hNaSi-1) in the human kidney is quite advantageous, particularly when the serum SO42− levels are depleted by high doses of substrates for glutathione conjugation and sulfonation (192), and rapid repletion of SO42− is needed through tubular reabsorption. In fact, it has been demonstrated that upon severe depletion of endogenous SO42−, almost complete reabsorption

<table>
<thead>
<tr>
<th>Protein</th>
<th>Species</th>
<th>Function</th>
<th>Protein Size</th>
<th>Transmembrane Segments</th>
<th>Overall Identity, %</th>
<th>Symporter Signature Identity, %</th>
<th>GenBank Accession Number</th>
<th>Chromosome Location</th>
<th>HGNC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>rNaSi-1</td>
<td>Rat</td>
<td>Renal and intestinal Na+-sulfate cotransporter</td>
<td>595</td>
<td>13 100</td>
<td>100</td>
<td>100</td>
<td>L19102</td>
<td>ND</td>
<td>Slc13a1</td>
</tr>
<tr>
<td>mNaSi-1</td>
<td>Mouse</td>
<td>Renal and intestinal Na+-sulfate cotransporter</td>
<td>594</td>
<td>13 94</td>
<td>100</td>
<td>100</td>
<td>AF199365</td>
<td>6 4 cm</td>
<td>Slc13a1</td>
</tr>
<tr>
<td>hNaSi-1</td>
<td>Human</td>
<td>Renal Na+-sulfate cotransporter</td>
<td>595</td>
<td>13 83</td>
<td>100</td>
<td>100</td>
<td>AF260824</td>
<td>7q31-32</td>
<td>SLC13A1</td>
</tr>
<tr>
<td>hSUT-1</td>
<td>Human</td>
<td>High endothelial venule Na+-sulfate cotransporter</td>
<td>627</td>
<td>12 48</td>
<td>76.5</td>
<td>76.5</td>
<td>AF169901</td>
<td>7q35</td>
<td>SLC13A4</td>
</tr>
<tr>
<td>hNaDC-1</td>
<td>Human</td>
<td>Renal sodium-dicarboxylate cotransporter</td>
<td>592</td>
<td>13 45</td>
<td>82.4</td>
<td>82.4</td>
<td>U26290</td>
<td>17p11.1-17q1.1</td>
<td>SLC13A2</td>
</tr>
<tr>
<td>mNaDC-1</td>
<td>Mouse</td>
<td>Renal sodium-dicarboxylate cotransporter</td>
<td>586</td>
<td>13 42</td>
<td>76</td>
<td>76</td>
<td>AF201903</td>
<td>11 45 cm</td>
<td>Slc13a2</td>
</tr>
<tr>
<td>rbNaDC-1</td>
<td>Rabbit</td>
<td>Renal sodium-dicarboxylate cotransporter, identical to rSDCT2 (AF085674.1)</td>
<td>593</td>
<td>13 42</td>
<td>76.5</td>
<td>76.5</td>
<td>U12186</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
<tr>
<td>rNaDC-1</td>
<td>Rat</td>
<td>Renal sodium-dicarboxylate cotransporter, identical to rSDCT1 (AF058714.1)</td>
<td>587</td>
<td>13 42</td>
<td>70.6</td>
<td>70.6</td>
<td>AB001321</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
<tr>
<td>rNaDC3</td>
<td>Rat</td>
<td>Sodium-dicarboxylate cotransporter, identical to rSDCT2 (AF080451.1)</td>
<td>600</td>
<td>13 37</td>
<td>58.8</td>
<td>58.8</td>
<td>AF081825</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
<tr>
<td>rNaDC-2</td>
<td>Rat</td>
<td>Intestinal sodium-dicarboxylate cotransporter</td>
<td>587</td>
<td>12 35</td>
<td>76.5</td>
<td>76.5</td>
<td>U51153</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Species</th>
<th>Function</th>
<th>Protein Size</th>
<th>Transmembrane Segments</th>
<th>Overall Identity, %</th>
<th>Symporter Signature Identity, %</th>
<th>GenBank Accession Number</th>
<th>Chromosome Location</th>
<th>HGNC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>rSDCT1</td>
<td>Rat</td>
<td>Sodium-dicarboxylate cotransporter</td>
<td>592</td>
<td>13 45</td>
<td>82.4</td>
<td>82.4</td>
<td>U26290</td>
<td>17p11.1-17q1.1</td>
<td>SLC13A2</td>
</tr>
<tr>
<td>rSDCT2</td>
<td>Rat</td>
<td>Sodium-dicarboxylate cotransporter</td>
<td>593</td>
<td>13 42</td>
<td>76.5</td>
<td>76.5</td>
<td>U12186</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
<tr>
<td>rSDCT4</td>
<td>Rat</td>
<td>Sodium-dicarboxylate cotransporter</td>
<td>587</td>
<td>13 42</td>
<td>76.5</td>
<td>76.5</td>
<td>U51153</td>
<td>ND</td>
<td>Slc13a2</td>
</tr>
</tbody>
</table>

For transmembrane segments, determination of the secondary structure was performed using the TopPred2 program. Numbers refer to the number of transmembrane segments. Overall identity refers to identity to the full-length human NaSi-1 protein, as determined with the alignment program ClustalW. Symporter signature identity refers to identity to the "Na+-sulfate symporter family signature" motif (Prosite PS01271) found in the NaSi-1 proteins, as determined with the alignement program ClustalW. HGNC refers to Human Nomenclature Gene Committee approved name. ND, not determined.
of SO$_4^{2-}$ occurs in the human kidney (144). Since humans may be more susceptible to SO$_4^{2-}$ depletion (by many forms of exogenous substrates) and the rate-limiting step of renal SO$_4^{2-}$ reabsorption has been suggested to be the BBM Na$^+$-SO$_4^{2-}$ cotransporter (17), it is highly likely that hNaSi-1 plays a key role in total body SO$_4^{2-}$ homeostasis in humans.

As with the rodent NaSi-1 proteins, hNaSi-1-induced Na$^+$-SO$_4^{2-}$ cotransport in *Xenopus* oocytes was significantly inhibited by (1 mM) thiosulfate, selenate, molyb-

![Secondary structure model of the rNaSi-1 protein. Topology is based on the prediction by the TopPred2 program (261). The NH$_2$ terminus is intracellular, and the COOH terminus is extracellular. Putative consensus sites are indicated.](image)

**TABLE 2. Comparison of transport properties of the NaSi-1 protein family**

<table>
<thead>
<tr>
<th>Protein</th>
<th>$V_{	ext{max}}$ for SO$_4^{2-}$, pmol · oocyte$^{-1}$ · h$^{-1}$</th>
<th>$K_m$ for SO$_4^{2-}$, μM</th>
<th>$K_m$ for Na$^+$, mM</th>
<th>Hill Coefficient for Na$^+$</th>
<th>$K_m$ for S$_2$O$_3^{2-}$, μM</th>
<th>$K_m$ for SeO$_4^{2-}$, μM</th>
<th>Significant Inhibition of $^{35}$SO$_4^{2-}$ Uptake by (1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rNaSi-1</td>
<td>42.7 ± 2.0</td>
<td>2.0 ± 0.4</td>
<td>62.0 ± 8.0</td>
<td>31.9 ± 13</td>
<td>1.8 ± 0.4</td>
<td>0.3 ± 0.1</td>
<td>Yes</td>
</tr>
<tr>
<td>mNaSi-1</td>
<td>49.2 ± 4.1</td>
<td>4.1 ± 0.0</td>
<td>60.0 ± 6.0</td>
<td>13.1 ± 2.2</td>
<td>2.8 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td>Yes</td>
</tr>
<tr>
<td>hNaSi-1</td>
<td>179.5 ± 8.0</td>
<td>8.0 ± 0.4</td>
<td>60.0 ± 6.0</td>
<td>24.3 ± 2.5</td>
<td>2.4 ± 0.6</td>
<td>2.6 ± 0.6</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NT, not tested; $K_m$, Michaelis constant; $V_{	ext{max}}$, maximum velocity.
date, and tungstate (in order of potency), whereas phosphate, oxalate, cholate, probenecid, and DIDS had no significant effect (136). Interestingly, unlike the rodent NaSi-1 proteins, hNaSi-1-induced Na\(^{+}\)SO\(_{4}^{2-}\) cotransport in Xenopus oocytes was significantly inhibited by (1 mM) citrate and succinate; however, succinate was not transported in NaSi-1-expressing oocytes (136), suggesting that in the absence of sulfate in the media, dicarboxylates may competitively bind for the sulfate binding site on hNaSi-1 but themselves are unable to act as transport substrates. To test the extent of hNaSi-1 involvement in mediating Na\(^{+}\)SO\(_{4}^{2-}\) cotransport in the human kidney, hybrid depletion was performed using hNaSi-1-specific oligodeoxynucleotides (ODNs) annealed to human kidney mRNA. hNaSi-1 antisense ODNs prevented >90% of the induced Na\(^{+}\)SO\(_{4}^{2-}\) uptake by human kidney mRNA, whereas sense ODNs had no effect (136), suggesting that hNaSi-1 is the major functional Na\(^{+}\)SO\(_{4}^{2-}\) cotransporter in the human kidney.

A very detailed tissue distribution was determined for both mNaSi-1 and hNaSi-1 mRNA expression. By Northern blotting and RT-PCR analysis, out of 23 different tissues, mNaSi-1 was strongly detected in RNA from kidney, duodenum/jejunum, ileum, and colon, with lower levels observed in cecum, testis, adrenal, and adipose tissue (9). This result was in close agreement with the tissue distribution of rNaSi-1 (157). The physiological importance of the expression at low levels found in testis, adrenal, and adipose tissue remains to be determined. In contrast, hNaSi-1 mRNA was detected by Northern blotting and RT-PCR, exclusively in the kidney, suggesting that its tissue distribution in the human is restricted solely to the kidneys. This difference in mRNA expression could be due to differences in promoter sequences of the rodent and human NaSi-1 genes, accounting for cell-specific promoter activities. This unique feature would suggest that in humans, Na\(^{+}\)-sulfate cotransport by hNaSi-1 may not play an important role in intestinal sulfate absorption [as in rodents (1, 153, 195)], but rather that other sulfate transporter(s), such as DRA (see sect. nB2), may play an important role in sulfate absorption in the human gut. The difference in the pattern of NaSi-1 expression between rodents and humans is of great interest for the understanding of the regulation of sulfate homeostasis. This anomaly is presently being investigated.

Recently, the chromosomal localization of the mNaSi-1 gene (Nas1) and the hNaSi-1 gene (NAS1; SLC13A1) was performed by radiation hybrid mapping (9, 136). The data placed Nas1 on mouse chromosome 6 in the position between marker D6Mit170 (LOD score 20.8) and D6Mit380 (LOD score 11.2) (9). Nas1 was found to be 2.3 cR distal to D6Mit170, which has been assigned map positions of 4.4 cm (MIT) and 4.0 cm (MGD and Chromosome Committee). The Nas1 gene maps very close to the calcitonin receptor gene, which has been assigned a position of 4.5 cm (MGD and Chromosome Committee) in mouse. The NAS1 gene was mapped to human chromosome 7, close to marker SHGC-639, which was mapped to cytogenetic band 7q31–7q32 (136). Interestingly, other sulfate transporter genes, namely, DRA (243) and SUT-1 (82) (see below), were also mapped in close proximity to NAS1 on human chromosome 7, suggesting that this region may be important for SO\(_{4}^{2-}\) homeostasis. Further studies are needed to determine the significance of this chromosomal colocalization and whether these genes, encoding sulfate transporters, may be coordinately regulated in the body.

C) NAS1 GENE. Recently, this laboratory cloned the complete genomic structure of the mouse Nas1 gene, the first gene to be isolated encoding a mammalian Na\(^{+}\)-sulfate cotransporter (9). Nas1 is a single copy gene that spans ~75 kb, containing 15 exons (all within the coding region) and 14 introns. Using three different programs [TopPred2 (261), TM pred (103) and Sosusi (99)] to predict the secondary structure of the mNaSi-1 protein, all programs predicted the transmembrane domains to be encoded by separate exons, with the exception of transmembrane domains 10 and 11 being encoded by the same exon (exon 13). A 3,229-bp region of the Nas1 promoter has been cloned and sequenced (9). It is A+T rich (61%), with an atypical TATA sequence, TATTTAA, located 29 bp upstream of the transcription start site and a CAAT-box present at position −91 on the negative strand. The Nas1 promoter contains a number of potential cis-acting elements recognized by transcription factors that may play a role in the basal or chronic regulation of the Nas1 gene, including 2 AP-1 sites, 1 AP-2 site, 1 AP-4 site, two CAAT/ enhancer binding protein (C/EBP) binding sites, 3 Oct-1 sites, 3 NF-Y sites, 2 NFAT sites, and 10 GATA-1 binding sites. Consensus sequences for the binding of other transcription factors activated by mitogenic or differentiation signals (c-Ets-1, Sox-5, HNF4, USF, FREAC4, Pit-1) are also present. Five glucocorticoid responsive elements (GREs) are detected within the first 792 bp from the transcription start site. The distal region of Nas1 promoter (from −2549 to −2515) contains sequences bearing strong resemblance to the consensus sequence of thyroid responsive elements (TREs) and vitamin D responsive elements (VDRs). Recently, we have shown that the Nas1 promoter can drive basal transcription and is responsive to vitamin D in OK cells (9). Cotransfection of Nas1 promoter with the vitamin D receptor (VDR) and the human retinoic acid receptor (RXR) a into OK cells resulted in an 8.9-fold higher transcriptional activity above the basal Nas1 promoter activity (9). Furthermore, in the presence of 0.5 nM and 50 nM 1,25-dihydroxyvitamin D\(_{3}\), Nas1 promoter activity was further increased by 1.8- and 4.1-fold, respectively (9), suggesting that vitamin D and
VDR/RXR were able to transactivate the *NasI* promoter in OK cells (9). The significance of the other promoter consensus binding sites in relation to *NasI* function is presently being investigated.

2. **SUT-1**

Recently, another Na\(^{+}\)-sulfate cotransporter (SUT-1) has been cloned from human high endothelial venules (HEV) present in lymphoid organs and in chronically inflamed tissues (82). Functional characterization of HEV endothelial cells (HEVEC) revealed the presence of both DIDS-sensitive and DIDS-insensitive sulfate transporter systems (82). The DIDS-sensitive sulfate transporter in HEVEC was suggested to be DTDST (82) (see sect. vB3), an ubiquitously expressed Na\(^{+}\)-dependent and resistant to 1 mM DIDS transporter (93). To identify the DIDS-resistant sulfate transporter, a human HEVEC cDNA library was screened using a partial hNaSi-1 probe, resulting in the cloning of a 2.9-kb cDNA called SUT-1 (82). Injection of SUT-1 cRNA into *Xenopus* oocytes led to a >30-fold stimulation of sulfate uptake (compared with water-injected control oocytes), which was Na\(^{+}\) dependent and resistant to 1 mM DIDS (82). No transport kinetic data (*Km* and *Vmax*) were determined for SUT-1-induced uptake activity (82); thus it is not known whether SUT-1 encodes a low- or high-affinity sulfate transporter. By sequence comparison, human SUT-1 (hSUT-1) was found to share 48, 48, and 47% amino acid identities with rNaSi-1, mNaSi-1, and hNaSi-1, respectively (Table 1). Northern blotting detected two transcripts (a strong 2.9-kb and a weaker 4.5-kb signal) for SUT-1 mRNA, primarily in the placenta, with weaker signals in the testis and heart. The strong expression of SUT-1 in the placenta might suggest it could be involved in sulfate transport across the placental trophoblast, facilitating sulfate transfer from mother to fetus. The SUT-1 gene was localized between markers DTS500 and DTS509 on human chromosome 7q33, in close proximity to the hNaSi-1 gene. Unlike the predicted 13 TMDs for the NaSi-1 proteins, SUT-1 was predicted to contain 12 putative TMDs, with extracellular locations for both NH\(_2\) and COOH termini (82). The SUT-1 protein contains two putative *N*-glycosylation sites, Asn\(^{140}\) and Asn\(^{622}\), which correspond to the Asn\(^{140}\) and Asn\(^{501}\) on the NaSi-1 proteins. SUT-1 also possesses the sodium:sulfate symporter family signature motif (PROSITE PS01271) at amino acids 555–571 on the SUT-1 protein, sharing 76.5% amino acid identity with the NaSi-1 proteins (Table 1). By RT-PCR, SUT-1 mRNA was detected in HEVEC from human tonsils, at comparable levels with those detected in placental tissue. SUT-1 expression in HEVEC has been suggested to play an important role in sulfation of L-selectin counterreceptors in HEV (82); however, its precise functional role is still to be determined.

### B. Na\(^{+}\)-Independent Sulfate Transporters

1. **sat-1**

In 1994, a second distinct type of sulfate transporter protein was isolated using the *Xenopus* oocyte expression cloning system: the sulfate anion transporter-1 (sat-1) cloned from a rat liver cDNA library (18). Functional characterization in *Xenopus* oocytes revealed sat-1 to encode a Na\(^{+}\)-independent sulfate transporter, with a high affinity (*Km* = 136 µM) for sulfate, that was strongly inhibited by DIDS (*IC_{50} for DIDS = 28 µM*) and oxalate, but not by succinate or cholate (18). These properties correlated closely with the rat liver mRNA induced sulfate transport in oocytes (191) and the functional activities of the sulfate/bicarbonate exchanger in liver canalicular membrane vesicles (166). The cloned rat sat-1 cDNA was determined to be 3,726 bp in length, with an open reading frame encompassing 2,109 bp, a 5'-untranslated region of 367 bp, and a 3'-untranslated region of 1,250 bp. The coding region predicts a protein of 703 amino acids with a calculated molecular mass of 75.4 kDa. Hydrophobicity analysis of sat-1 protein suggests 12 putative TMDs (Fig. 4), with three potential *N*-glycosylation sites (Asn\(^{158}\), Asn\(^{163}\), and Asn\(^{387}\)), numerous putative phosphorylation sites (including several putative protein kinase C sites, 12 casein kinase II sites, and 1 tyrosine kinase site at Tyr\(^{526}\)), and 15 putative *N*-myristoylation sites. Northern blot analysis detected a single mRNA (3.8 kb) transcript for sat-1 very strongly in liver and kidney, with weaker signals in skeletal muscle and brain (18). The abundance of sat-1 mRNA in the kidney was identical to its expression in the liver (the tissue from which it was cloned), prompting us to examine the role of sat-1 in the kidney. Rat kidney mRNA when injected into *Xenopus* oocytes induced a Na\(^{+}\)-independent sulfate transport activity that was inhibitable by DIDS, probenecid, and phenol red (155). The degree of inhibition by these compounds was closely correlated with the inhibition pattern of the renal BLM sulfate anion exchanger (54), as well with sat-1 cRNA-induced activity in *Xenopus* oocytes (18). Furthermore, with the use of a hybrid depletion strategy, sat-1 antisense ODNs led to a complete abolition of the kidney mRNA-induced Na\(^{+}\)-independent sulfate transport activity (in *Xenopus* oocytes), confirming that sat-1 encodes the Na\(^{+}\)-independent sulfate transporter, whose function most closely correlates with the proximal tubular BLM sulfate/bicarbonate anion exchanger. This finding was later confirmed by immunocytochemistry using sat-1-specific peptide polyclonal antibodies showing the location of the sat-1 protein in the kidney to be restricted to the basolateral membrane of kidney proximal tubules (118).

Recently, sat-1 expression in the brain has been characterized (135). Injection of rat brain mRNA into *Xenopus* oocytes induced a saturable Na\(^{+}\)-independent sulfate up-
take, which was inhibited by DIDS (IC_{50} for DIDS = 150 ± 30 μM) and oxalate, with properties identical to sat-1 anion exchange activity. The involvement of a sat-1-like transporter responsible for the Si transport in rat brain was confirmed by hybrid depletion using sat-1 antisense ODNs, which completely abolished the brain mRNA-induced sulfate transport activity in *Xenopus* oocytes (135). Furthermore, in situ hybridization using sat-1 probes detected sat-1 mRNA expression most prominently in the hippocampus and cerebellum, in particular in the neuronal-rich cell layer, suggesting that sat-1 may play an important role in sulfate transport in neuronal or glial cells (135).

This laboratory recently isolated the mouse and human sat-1 homologs (named msat-1 and hsat-1, respectively) of the rat sat-1 cDNA (from now called rsat-1), using a homology screening approach (unpublished data). The msat-1 cDNA was determined to be 3,342 bp in length with 368 bp of 5' UTR and 1,233 bp 3' UTR. The hsat-1 cDNA is 3,673 bp in length, with 378 bp of 5' UTR and 1,189 bp 3' UTR. The msat-1 amino acid sequence translates into a protein of 704 amino acids, whereas hsat-1 contains 701 amino acids. As observed with the rodent NaSi-1 proteins (with mNaSi-1 having one additional amino acid more than rNaSi-1), msat-1 has one additional amino acid more than the rsat-1, a threonine at position 167 of rsat-1. The hsat-1 has two less amino acids than rsat-1. The msat-1 and hsat-1 proteins share 94 and 77% amino acid identities, respectively, with the rsat-1, suggesting that they belong to the same family of proteins.
(Table 3). The locations of the putative glycosylation, phosphorylation, and myristoylation sites are practically identical between rsat-1 and msat-1, with the exception of rsat-1 having one additional putative protein kinase C site (Thr\textsuperscript{280}) compared with msat-1 and hsat-1 lacking the putative N-glycosylation sites (Asn\textsuperscript{587}) present on both rodent sat-1 proteins. Interestingly, there are two consensus regions that are highly conserved between the two sat-1 proteins, as well as with other related proteins (see below). These are the “phosphopantetheine attachment site” (PROSITE PS00012) motif at residues 415–430 of rsat-1 (QMTSSYFANLY-FLMGT MSR) and the “sulfate transporter signature” (PROSITE PS01130) motif at residues 98–119 of both rsat-1 and msat-1 (PIYSLYTSFFANLIV). The significance of these motifs is yet unknown.

The mouse sat-1 gene (Sat1) has been localized to chromosome 5, mapping close 57.0 cM (38). The Sat1 genomic structure has three exons and two introns spanning about 5.6 kb in length (208). The first intron (∼1.1 kb) is located within the 5′-UTR and the second intron (694 bp in size) is located at position 593 bp within the coding region of msat-1 cDNA (unpublished data). Interestingly, Sat1 is located within another larger gene (on the opposite strand) encoding the enzyme α-l-iduronidase (IDUA) (38), which has been linked to mucopolysaccharidosis type I (MPS I), a lysosomal storage disorder resulting from defective lysosomal degradation of glycosaminoglycans. Of clinical importance are five mutations underlying MPS I which lie within exon II of IDUA in the segment that overlaps with Sat1 exon 3. The effects of IDUA mutations on sat-1 function and the role of sat-1 on IDUA expression are still unknown. The arrangement of such antisense overlapping transcripts is quite rare in eukaryotes, unlike in bacteria and viruses, where it is quite common due to their smaller, condensed genomes. The significance of this structural arrangement between IDUA and sat-1 genes is yet unknown but may be important for the regulation of their gene expression, as demonstrated by overlapping genes in bacteria (56, 217). The Sat1 promoter (∼3 kb) has been sequenced and was found to contain numerous putative consensus binding sites: AP-4, C/EBP, CP-2, Estrogen R, GC boxes, Oct-1, GATA, Sp-1, SRY, c-Ets-1, TCF-11, MZF-1, N-Myc, MyoD, RFX-1, and USF (unpublished data). The functional significance of these putative binding sites in relation to Sat1 gene expression is yet to be determined.

2. DRA/CLD

Several other proteins have been isolated sharing significant homology to sat-1. By subtractive hybridization using cDNA libraries from normal colon and adenocarcinoma tissues, a human cDNA that was downregulated in

---

**TABLE 3. The mammalian sulfate anion transporter family**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Species</th>
<th>Protein Function</th>
<th>Protein Size</th>
<th>Overall Identity, %</th>
<th>Sulfate Transporter Signature Identity, %</th>
<th>GenBank Accession Number</th>
<th>Chromosome Location</th>
<th>HGNC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>rsat-1</td>
<td>Rattus norvegicus</td>
<td>Sulfate anion transporter (sulfate/carbonate antiporter)</td>
<td>703</td>
<td>100</td>
<td>100</td>
<td>P45380</td>
<td>ND</td>
<td>Slc26a1</td>
</tr>
<tr>
<td>msat-1</td>
<td>Mus musculus</td>
<td>Sulfate anion transporter (sulfate/carbonate antiporter)</td>
<td>704</td>
<td>94</td>
<td>100</td>
<td>*</td>
<td>57 cm</td>
<td>Slc26a1</td>
</tr>
<tr>
<td>hsat-1</td>
<td>Homo sapiens</td>
<td>Sulfate anion transporter (sulfate/carbonate antiporter)</td>
<td>702</td>
<td>77</td>
<td>100</td>
<td>AF297659</td>
<td>4p16.3</td>
<td>SLC26A1</td>
</tr>
<tr>
<td>DTDSST</td>
<td>Homo sapiens</td>
<td>Sulfate transporter (diastrophic dysplasia protein)</td>
<td>739</td>
<td>44</td>
<td>77</td>
<td>P50443</td>
<td>5q31-q34</td>
<td>SLC26A2</td>
</tr>
<tr>
<td>dtdst</td>
<td>Rattus norvegicus</td>
<td>Sulfate transporter (diastrophic dysplasia protein)</td>
<td>739</td>
<td>44</td>
<td>81</td>
<td>O70531</td>
<td>ND</td>
<td>Slc26a2</td>
</tr>
<tr>
<td>dtdst (st-ob)</td>
<td>Mus musculus</td>
<td>Sulfate transporter (diastrophic dysplasia protein)</td>
<td>739</td>
<td>44</td>
<td>81</td>
<td>Q62273</td>
<td>ND</td>
<td>SLC26a2</td>
</tr>
<tr>
<td>PAT1</td>
<td>Homo sapiens</td>
<td>Putative anion transporter 1</td>
<td>738</td>
<td>33</td>
<td>59</td>
<td>AF270256.1</td>
<td>3p21</td>
<td>SLC26A6</td>
</tr>
<tr>
<td>PENDRIN</td>
<td>Homo sapiens</td>
<td>Sodium-independent chloride/iodide transporter</td>
<td>780</td>
<td>32</td>
<td>60</td>
<td>O43511</td>
<td>7q22-q31.1</td>
<td>SLC26A4</td>
</tr>
<tr>
<td>pendrin</td>
<td>Rattus norvegicus</td>
<td>Sodium-independent chloride/iodide transporter</td>
<td>780</td>
<td>31</td>
<td>54</td>
<td>AF167412.1</td>
<td>ND</td>
<td>Slc26a4</td>
</tr>
<tr>
<td>pendrin</td>
<td>Mus musculus</td>
<td>Sodium-independent chloride/iodide transporter</td>
<td>780</td>
<td>30</td>
<td>52</td>
<td>AF167411.1</td>
<td>12</td>
<td>Slc26a4</td>
</tr>
<tr>
<td>hDRA</td>
<td>Homo sapiens</td>
<td>Downregulated in adenoma (sulfate/chloride antiporter)</td>
<td>764</td>
<td>28</td>
<td>59</td>
<td>P40879</td>
<td>7q22-q31.1</td>
<td>SLC26A3</td>
</tr>
<tr>
<td>mDRA</td>
<td>Mus musculus</td>
<td>Downregulated in adenoma (sulfate/chloride antiporter)</td>
<td>757</td>
<td>27</td>
<td>52</td>
<td>AF136751</td>
<td>12</td>
<td>Slc26a3</td>
</tr>
</tbody>
</table>

Overall identity refers to identity to the full-length rat sat-1 protein, as determined with the alignment program ClustalW. Sulfate transporter signature identity refers to identity to the sulfate transporter signature (Prosite PS01271) found in rat sat-1 protein, as determined with the alignment program ClustalW. HGNC refers to Human Nomenclature Gene Committee approved name (http://www.gene.ucl.ac.uk/nomenclature). ND, not determined. * Sequence still to be submitted to GenBank.
adenomas (DRA) was isolated (226). DRA mRNA was found to be exclusively expressed in normal colon tissues, with its expression significantly decreased in colonic adenomas (polyps) and adenocarcinomas. The DRA gene was mapped to human chromosome 7q22-q31.1 (243). The predicted topology of this polypeptide was a 84.5-kDa protein, having either 10, 12, or 14 TMDs, with charged clusters of amino acids at its NH$_2$ and COOH termini, potential nuclear targeting motifs, an acidic transcriptional activation domain, and a homeobox domain (226). These putative domains in the DRA protein sequence may suggest it could interact with a transcription factor, which would be consistent with DRA having a role in tissue-specific gene expression and/or as a candidate tumor suppressor gene. With the use of the baculovirus expression system, human DRA was expressed in insect Sf9 cells where it was targeted to the cell membrane and led to greater than a threefold increase in sulfate uptake compared with control cells (30). The DRA protein expressed in Sf9 cells was a 78-kDa N-glycosylated form (30), which was in contrast to DRA expression in normal colon carcinomas or in transiently transfected monkey kidney COS-1 cells, which exhibit three polypeptides, a diffuse band at 95 kDa and a doublet at 75 kDa (30), suggesting that Sf9 cells may have an altered pattern of glycosylation compared with mammalian cells.

When expressed in _Xenopus_ oocytes, DRA was shown to function as a DIDS-sensitive Na$^+$-independent sulfate/oxalate transporter (236). By Northern blot analysis, a low level of DRA mRNA expression was detected in the mouse colon at birth, which increased in the first two postnatal weeks, whereas in the small intestine, there was a lower level of DRA expression, which remained constant in the postnatal period (236). In the differentiating human colon carcinoma cell line Caco-2, DRA mRNA was undetected in the preconfluent (undifferentiated) state but became highly expressed in the postconfluent (differentiated) state (236). Recently, using a combination of RT-PCR and RACE techniques, the mouse DRA (mDRA) cDNA homolog was cloned (167). When expressed in human embryonic kidney HEK293 cells, mDRA protein conferred Na$^+$-independent, electroneutral chloride/bicarbonate exchange activity. By Northern blot analysis, mDRA mRNA was detected at high levels in cecum and colon and at lower levels in small intestine (167). mDRA mRNA expression was upregulated in the colon of mice lacking the Na$^+$/H$^+$ exchanger NHE3 (167), suggesting that mDRA could act in concert with NHE3 to absorb NaCl in the colon. Human DRA (hDRA) mRNA was detected throughout the intestinal tract (duodenum, ileum, cecum, distal colon), but not in the esophagus or stomach (31). hDRA expression was restricted to the mucosal epithelium, in particular to the BBM of columnar epithelial cells (31). Consistent with its expression in the differentiated columnar epithelium of the adult human colon, mDRA was first expressed in the midgut of developing mouse embryos at _day 16.5_, corresponding with the time of differentiation of the epithelium of the small intestine (31). A recent study characterized the expression of DRA in extraintestinal normal epithelia and in intestinal inflammatory and neoplastic epithelia in vivo (90). In situ hybridization and immunohistochemistry showed DRA expression in eccrine sweat glands and seminal vesicles (90). Colon epithelial DRA mRNA expression in inflammatory bowel disease and ischemic colitis was found to be similar to that in normal colon epithelium, but the DRA protein was detected deeper in crypts, including the proliferative epithelial cells. As shown previously, the expression pattern of DRA in intestinal tumors was dependent on the differentiation state, with strong expression observed in epithelial polyps with no or minor dysplasia, whereas adenocarcinomas were completely devoid of DRA (90).

The DRA gene was subsequently found to be defective in patients with congenital chloride diarrhea (CLD; OMIM 214700) syndrome (178), a recessively inherited defect of intestinal chloride/bicarbonate exchange. CLD is a potentially fatal diarrhea characterized by a high chloride content (107, 121). The main clinical feature of CLD is prenatal onset of watery diarrhea, which in utero leads to polyhydramnios and often premature birth (121). Babies once born with CLD have distended abdomens; develop metabolic alkalosis, hyperbilirubinemia, and dehydration; and without treatment can die. Patients have severe electrolyte disturbances and can develop early renal complications, which can be treated by intravenous replacement (in newborns) or by oral replacement of lost fluid and electrolytes (in older children and adults) (107). The CLD locus was previously mapped to 7q3 (122), adjacent to the cystic fibrosis transmembrane regulator (CFTR) gene; however, by refined genetic and physical mapping, the DRA gene was implicated as a positional and functional candidate for CLD (104, 106). Due to its link with congenital chloride diarrhea, the DRA gene then in fact became known as the CLD gene.

The genomic organization of the CLD gene was found to span ~39 kb and is comprised of 21 exons (89). Comparison of the location of the predicted transmembrane domains to exon boundaries of CLD showed some correlation with the 14-TMD protein model, but not with the 10- or 12-TMD models. Analysis of the putative CLD promoter region (570 bp) showed putative TATA and CCAAT boxes and multiple transcription factor binding sites for AP-1 and GATA-1 (89). To date, over 20 different mutations have been identified in CLD patients, from various ethnic populations (121). For a recent review on the known CLD mutations and their contributions to congenital chloride diarrhea, see Reference 121. Many of these mutations are frameshifts leading to nonsense changes followed by a stop codon. The cellular defects arising from these muta-
tions have not been fully characterized. Recently, one study looked at two mutations (ΔV317 or C307W) found in 32 CLD patients from Finland (105) to determine whether these mutations may lead to a loss of protein function (178). *Xenopus* oocytes were injected with either wild-type or mutagenized (ΔV317 or C307W) DRA cRNA, followed by measurement of radiotracer Cl\(^-\) and SO\(_4^{2-}\) uptake. Both Cl\(^-\) and SO\(_4^{2-}\) were transported by wild-type DRA, whereas both transport activities were completely lost in the ΔV317 mutation, with no change observed for the C307W mutation (178). These results suggest that DRA is a functional chloride/sulfate transporter that was defective in all 32 CLD patients carrying the ΔV317 mutation, whereas the C307W mutation was shown to be a silent polymorphism (178).

3. *DTDST*

By positional cloning, a gene was identified on human chromosome 5q to be linked to an autosomal recessive osteochondrodysplasia called diastrophic dysplasia (DTD; OMIM 222600), with clinical features including dwarfism, spinal deformation, abnormalities of the joints (93), vocal tract (114), dentition (115), and craniofacial features (116). The human DTD gene is comprised of four exons and three introns spanning over 40 kb (39). Upon identification of the coding region, DTD was found to share 48 and 33% amino acid identity with rsat-1 and DRA, respectively (93). Due to its high sequence similarity with rsat-1 and the knowledge that rsat-1 encodes a sulfate transporter (18, 155), the exact function of the DTD gene was sought. Primary skin fibroblasts obtained from normal, carrier, and DTD patients (with mutations in the DTD gene) were cultured and assayed for sulfate uptake (93). Cells from normal and carrier samples showed saturable sulfate transport, whereas cells from the DTD patients showed a greatly diminished sulfate uptake (93), suggesting that the normal (wild-type) DTD gene encodes a functional sulfate transporter, which loses its transport activity when mutated (as in DTD patients). Thus the cDNA encoding the DTD gene became known as the diastrophic dysplasia sulfate transporter (DTDST) (93). Translation of DTDST cDNA produced a protein of 739 amino acids, with a predicted molecular mass of 82 kDa, containing 12 TMDs (based on hydrophobicity analysis) (93). DTDST mRNA expression was ubiquitous, detected by Northern blot analysis in all tissues of the body (93). Recently, the rat DTDST gene and cDNA (dtdst) were cloned, and its function was characterized in *Xenopus* oocytes (219). Rat dtdst was observed to function as a Na\(^+\)-independent sulfate transporter, whose activity was inhibited by chloride, unlike rsat-1 whose activity was not inhibited by chloride, but rather required extracellular chloride for sulfate transport (219). Furthermore, rat dtdst was sensitive to thiosulfate, oxalate, and DIDS, with a cis-inhibition pattern identical to both rsat-1 and DRA. Independently, a mouse cDNA was cloned encoding a sulfate transporter (named st-ob for sulfate transporter in osteoblasts) which was induced in osteoblast precursor cells in association with osteoblast differentiation (126). st-ob was isolated by subtraction hybridization, using immature mouse fibroblastic cells C3H10T1/2, which were either untreated or treated with bone morphogenetic protein-2 (BMP-2), which induced an osteoblast-like phenotype. st-ob was found to be induced by BMP-2 which is constantly expressed in osteoblastic cells (126). Upon translation of the cDNA sequence, the proposed mouse st-ob protein was found to share 80% amino acid identity with the human DTDST protein. Tissue distribution showed the st-ob mRNA to be strongly expressed in the thymus, testis, calvaria, and the osteoblastic MC3T3-E1 cells, having a reduced expression in the undifferentiated C3H10T1/2 cells. The expression of st-ob mRNA in C3H10T1/2 cells was shown to be increased by transforming growth factor-β1 (TGF-β1), retinoic acid, and dexamethasone, as well as BMP-2 (126). BMP-2 also led to a twofold increase in sulfate uptake in C3H10T1/2 cells compared with untreated (control) cells, suggesting that st-ob encodes a functional sulfate transporter, whose activity was upregulated by BMP-2. Since osteoblasts actively take up sulfate to synthesize proteoglycans (being major components of the extracellular matrix of bone and cartilage), the functional activity of st-ob/dtdst protein as a sulfate transporter may be important for osteoblastic differentiation.

At least 14 different mutations have been identified in the DTDST gene, which are responsible for a variety of recessively inherited chondrodysplasias, including diastrophic dysplasia (DTD; Ref. 93), atelosteogenesis type 2 (AO2; Ref. 94), and a lethal condition known as achondrogenesis IB (ACGIB; Ref. 241). For recent reviews on DTDST mutations and their contributions to these pathogenic states, see References 71, 241. In all three clinical conditions, the general phenotypic defect has been demonstrated to be a reduced sulfate transport leading to undersulfation of cartilage proteoglycans (94, 204–207). To further characterize the nature of several common DTD mutations and their clinical phenotypes, a recent study examined chondroitin sulfate proteoglycans from cartilage of 12 patients with known chondrodysplasias (206). The amount of nonsulfated disaccharides was found to be elevated in patients (when compared with normals), with the highest amount present in ACGIB patients (206), suggesting that undersulfation of chondroitin sulfate proteoglycans occurred in cartilage in vivo and that it was correlated with the clinical severity. Furthermore, by \[^{35}S\]sulfate and \[^{3}H\]glucosamine double-labeling of fibroblast cultures from patients with DTD, AO2, and ACGIB and controls, the uptake of extracellular sulfate was found to be reduced in all patients’ cells, with lowest values in ACGIB cells. However, disaccharide
analysis of chondroitin sulfate proteoglycans derived from these patients was found to be normally sulfated or only moderately undersulfated, suggesting that extracellular sulfate uptake was impaired, but fibroblasts were still able to replenish their reduced intracellular sulfate pools by oxidizing sulfur-containing compounds (i.e., cysteine and methionine) and thus partially rescuing proteoglycan sulfation under basal conditions (206). Clearly, further work is needed to determine the functional significance of the sulfate transport defects, due to specific mutations in the DTDST gene, and their contribution(s) to the observed clinical phenotypes.

4. AE1 (band 3)

Band 3, or the anion exchanger 1 (AE1), is one of the most extensively studied membrane transport proteins. Despite many reviews written about this protein (2, 34, 247), AE1 deserves to be mentioned in this review due to one of its physiological properties, being a plasma membrane sulfate transporter. In fact, AE1 is the most abundant integral protein of the RBC membrane, constituting ~25–30% of the total protein mass in the purified membrane, with ~1 million copies present per erythrocyte (247). The AE1 cDNA was cloned from the murine anemic spleen (130). AE1 has been shown to transport both monovalent (chloride and bicarbonate) and divalent (sulfate) anions (81). AE1 was shown to mediate rapid, tightly coupled, electroneutral transmembrane exchange of bicarbonate for chloride (102), which was highly sensitive to extracellular pH, with maximal activity at extracellular pH 5.0 (270). AE1 transport kinetics into human erythrocytes were estimated as $K_m$ of 4–5 mM and $V_{max}$ of 75 mmol/kg cell solid-1 min-1 for sulfate influx at pH 3.0 (79, 81). AE1 reconstituted into proteoliposomes led to a saturable sulfate uptake with a $K_m$ for sulfate of 9.2 ± 2.3 mM, which was strongly inhibited by (2.5 μM) DIDS (229). In SF9 cells transfected with the human AE1 (hAE1), the transport activities for Cl⁻ transport were determined: $K_m$ (Cl⁻) = 44 ± 10 mM and $V_{max}$ (Cl⁻) = 48.1 ± 5.7 mmol/min (51), with this activity inhibited by H₂DIDS ($K_{0.5} = 34 \mu M$) and extracellular sulfate ($K_{0.5} = 26 \text{ mM}$). For more extensive reviews on AE1 transport kinetic properties, see References 23, 79, 112, 193, 216.

AE1 performs two critical biological functions in erythrocytes: it maintains ionic homeostasis, by transporting Cl⁻ and HCO₃⁻, and it provides the mechanical stability to the erythroid membrane, serving as a critical attachment site of the peripheral membrane cytoskeleton (247). AE1 is primarily expressed in plasma membranes of erythrocytes, but also on the BLM of α-type intercalated cells of the kidney distal collecting tubule, which is involved in acid secretion. In both cell types, AE1 mediates the rapid, tightly coupled, electroneutral transmembrane exchange of bicarbonate for chloride, which is extremely pH dependent and highly sensitive to the disulfonic stilbenes DIDS and SITS (216). Erythroid AE1-mediated exchange of Cl⁻ for HCO₃⁻ serves to increase the total CO₂-carrying capacity of blood. With particular significance to this review, AE1 transport specificity spans across a wide range of oxyanions, including sulfate, selenate, selenite, and phosphate (81). The ability of AE1 to transport phosphate is quite intriguing, since none of the other known membrane sulfate transporters, with the exception of the mitochondrial sulfate transporter (48), is able to transport phosphate. Nevertheless, AE1 is still regarded to be the principal sulfate transporter of the erythrocyte plasma membrane, with [³⁵S]sulfate uptake being the assay of choice for measuring its functional activity.

a) AE1 PROTEIN STRUCTURE. AE1 encodes a plasma membrane glycoprotein with a molecular mass of ~97 kDa (130). It is composed of 911 amino acids, with two domains having distinct structure and functions. The first 403 residues constitute the NH₂-terminal cytoplasmic domain, which associates with a number of proteins, including ankyrin to anchor the erythrocyte cytoskeleton, and several peripheral membrane proteins, including protein 4.2 (247). Residues 404–911 constitute the COOH-terminal transmembrane domain, which mediates anion transport. The majority of the COOH-terminal portion constitutes the transmembrane region making up 14 membrane-spanning segments, with a substantial portion of α-helical structure. The oligomeric forms of AE1 in situ have been suggested to be mainly tetramers and dimers (247).

b) AE1 GENE. The AE1 gene maps to human chromosome 17q21 (234). The gene extends over 20 kb consisting of 20 exons separated by 19 introns (215). AE1 does not share significant homology (<20% amino acid identity) with the other above-mentioned sulfate transporters, belonging to a distinct gene family of proteins (Fig. 5). The AE1 gene has been shown to be transcribed in response to two promoters, an upstream promoter producing erythroid band 3 and a downstream promoter initiating transcription of the kidney-specific band 3 isoform (215). Several mutations in the AE1 gene have been found to be linked with familial distal tubular acidosis (25, 111, 113), an inherited condition associated with kidney stone formation in humans (for recent review, see Ref. 26). Furthermore, posttranslational changes in AE1 protein, including increased protein breakdown, decreased phosphorylation, and decreased sulfate transport activity, were observed in the brain of aged patients suffering from Alzheimer’s disease (46).

To assess the biological consequences of band 3 deficiency, recent studies have made targeted disruptions of the AE1 gene in mice, selectively inactivating the erythroid AE1 gene but not the kidney band 3. Disruption of the erythroid AE1 gene did not lead to death in utero; however, the majority of homozygous mice died within 2 wk after birth (239). The erythroid band 3 null mice (~/-) also showed retarded growth, spherocytic RBC morphology, and severe hemolytic anemia. Interestingly, the band 3 ~/- erythrocytes assembled a normal membrane skele-
ton, thereby challenging the hypothesis that the presence of band 3 is essential for the stable biogenesis of membrane skeleton (239). In another study, targeted inactivation of murine AE1 gene produced a hypercoagulable state in mice causing widespread thrombosis in vivo, with only 5–10% of mice surviving the neonatal period (92). Upon histopathological examination of 3 adult and 11 newborn AE1 null mice, all but 1 pup showed evidence of thrombosis. Other phenotypic characteristics included large thrombotic lesions in the heart, subcapsular necrotic areas in the liver, and large vein thrombi (92). The high mortality of band 3 null mice was suggested to be related to a hypercoagulable state, which appears to originate from changes in the phospholipid composition of the membrane leading to phosphatidylserine exposure on the outer leaflet (92). Due to the close phenotypic correlation of the AE1 null mouse phenotype with human RBC ovalocytosis prevalent in Southeast Asia (27, 259), the availability of AE1 \(^{-/-}\) mice offers a unique opportunity to investigate the role of erythroid band 3 in the regulation of RBC cell membrane-skeletal interactions, sulfate anion transport, and the process of invasion and growth of the malaria parasite into RBCs (239). A recent study in humans showed patients suffering from Southeast Asian ovalocytosis had sulfate transport diminished by \(>50\%\) (220). Furthermore, earlier studies identified patients with Southeast Asian ovalocytosis to have ovalocytes with a structurally and functionally abnormal band 3 protein (145), being in agreement with a decreased sulfate transport activity via the erythrocyte AE1.

C. Sulfate Transport Related Proteins

Recently, a gene (PDS) was identified by positional cloning (70) causing Pendred syndrome (PS; OMIM 274600), a recessively inherited disorder of syndromic deafness characterized by congenital sensorineural hear-
ing loss and thyroid goiter (for recent reviews, see Refs. 201, 268). Previous genetic linkage studies localized the gene to human chromosome 7q31 (initially named DFNB4) (6, 47). The PDS gene was found to be mutated in patients having PS (70), with more than 30 different mutations identified so far leading to PS (121). PDS produces a transcript of ~5 kb that is expressed primarily in the thyroid gland, with weaker signals in the kidney and brain (70). PDS encodes a putative 11-TMD protein designated pendrin, which shares significant amino acid identities with several Na$^{+}$-independent sulfate transport proteins, including DRA (45%), DTDST (32%), and sat-1 (29%) (70). On the basis of this homology and the presence of a structural motif, for which the significance is yet unknown. Clearly further work is needed to determine precisely why pendrin is unable to transport sulfate. More importantly, the contribution of PDS to thyroid and cochlear physiology and the pathophysiology of PS still needs to be resolved.

Another putative human sulfate transporter cDNA (accession number AF279265) was recently isolated (146). Its complete cDNA is 2,748 bp in length encoding a 738-amino acid protein that shares 33% identity with rsat-1 (Table 3). The function of this putative anion transporter (PAT1) is not yet known, but since it shares 59% amino acid identity (with rsat-1) within its sulfate transporter signature motif, for which the significance is yet unknown. Clearly further work is needed to determine precisely why pendrin is unable to transport sulfate. More importantly, the contribution of PDS to thyroid and cochlear physiology and the pathophysiology of PS still needs to be resolved.

A computational analysis of public protein databases reveals the presence of several families of mammalian sulfate transport proteins (Fig. 5). The phylogenetic tree dendogram suggests that the NaSi-I, SUT-1, and NaDC proteins share a common evolutionary branch and that evolutionary divergence was quite late among their individual protein members. The AE1 family of proteins, despite possessing quite divergent functional activities, sharing very little functional similarity with NaSi/NaDC families, seems to share some distant evolutionary identity with Na$^{+}$-coupled sulfate/dicarboxylate proteins. The sat-1 family of proteins shares a distant evolutionary origin with the DTDST proteins, whereas the DRA proteins share less sequence identity with sat-1, branching very early in evolution from the other sulfate transport proteins. The DRA proteins share a distant evolutionary origin with the PDS proteins, whereas the putative SO$_4^{2-}$ transporter (PAT1; yet to be functionally characterized) is structurally different from all other sulfate transporters within this phylogenetic tree (Fig. 5). Further protein structure/function information is required to determine the common evolutionary origins and the functional similarities between these families of mammalian sulfate transporters.

V. REGULATION OF SULFATE TRANSPORTERS

Very little is known about the factors that regulate the expression of sulfate transporters in vivo. Before the cloning of the sulfate transporters, a few factors/conditions (listed below) were suggested to be controlling membrane sulfate transport; however, some modes were quite controversial, and the precise mechanisms by which the regulation occurred were unclear. With the cloning of cDNAs/genes encoding sulfate transporters and the development of molecular biological techniques, it has become easier to identify and characterize specific factors that modulate sulfate transporters at the cellular and molecular levels. Recent data have demonstrated that the proximal tubular BBM Na$^{+}$-sulfate cotransporter NaSi-1 is a highly regulated protein in vivo under the control of various dietary/hormonal conditions. In addition, recent data from this laboratory suggest that the proximal tubular BLM sulfate anion transporter sat-1 may also be regulated posttranslationally (see below). For the other cloned sulfate transporters (DRA and DTDST), despite their definitive links to diseased states, less information is available on the factors or mechanisms that regulate their protein expression in vivo. For clarity, the modes of regulation have been subdivided (below) into individual factors/conditions, in no specific order.

A. Sulfate Availability

The most obvious place to look for a possible regulator of sulfate transporter expression is by studying its functional substrate (ligand), inorganic sulfate. Early studies showed that individuals with normal renal function maintained a fairly constant plasma sulfate level over a 24-h period; however, after ingestion of a protein-rich

Physiol Rev • VOL 81 • OCTOBER 2001 • www.prv.org
diet or a high oral sulfate load, plasma sulfate levels were elevated up to twofold over basal levels (165). Renal proximal tubular BBMV Na\(^{+}\)-SO\(_{4}\)\(^{2-}\) cotransport was shown to be reduced in guinea pigs (194) and rats (160) fed on a high-sulfate diet compared with control-sulfate diet. Rats fed on a high-sulfate diet showed a marked increase in the renal excretion of sulfate and a concomitant decrease in BBM Na\(^{+}\)-sulfate cotransport activity when compared with rats on a control-sulfate diet (160). The decrease in BBM Na\(^{+}\)-sulfate cotransport activity was associated with a decrease in BBM NaSi-1 protein abundance, as well as a decrease in expression of cortical NaSi-1 mRNA. Furthermore, cortical mRNA from rats fed on a high-sulfate diet led to reduced Na\(^{+}\)-sulfate cotransport activity (when injected into Xenopus oocytes) compared with mRNA isolated from control-sulfate diet rats (160). This study indicated that adaptation to a high-sulfate diet is accompanied by a decrease in renal cortical NaSi-1 mRNA abundance, which results in reduced expression of the NaSi-1 protein at the level of the proximal tubular BBM, confirming that dietary sulfate intake is an important factor in the regulation of renal proximal tubular Na\(^{+}\)-sulfate cotransport. In an analogous study, rats fed on a reduced sulfate diet (sulfate deprivation) containing negligible amounts of sulfate, cysteine, and methionine showed a reduced urinary sulfate excretion rate and decreased renal sulfate clearance, without any changes in serum sulfate concentrations (14). In rats fed on the reduced-sulfate diet, there was an increase in V\(_{\text{max}}\) (without affecting K\(_{\text{m}}\)) for BBM Na\(^{+}\)-sulfate cotransport and no changes in V\(_{\text{max}}\) or K\(_{\text{m}}\) for BLM sulfate transport, compared with control rats (14), suggesting that the reduced-sulfate diet regulated the activity of the BBM Na\(^{+}\)-SO\(_{4}\)\(^{2-}\) cotransporter, without affecting the basolateral membrane sulfate/anion exchanger (14). Sulfate deprivation led to no changes in membrane motional order or fluidity of the BBM or BLM, suggesting that changes in membrane fluidity do not lead to the observed changes in sulfate transport (14). Furthermore, rats treated for an extended period with a low-methionine diet showed increases in NaSi-1 protein and mRNA levels (212), suggesting that sulfate deprivation leads to an upregulation in the steady-state levels of the NaSi-1 transporter. These data strongly suggest that the renal proximal tubular BBMV Na\(^{+}\)-SO\(_{4}\)\(^{2-}\) cotransporter may play an important role in regulating circulatory SO\(_{4}\)\(^{2-}\) concentration.

B. Potassium Availability

Dietary potassium (K) affects renal reabsorption of citrate and sulfate (140, 161). Recently, the effect of changes in K intake on renal proximal tubular Na\(^{+}\)-sulfate cotransport was examined (161). Rats treated chronically (7 days) with a K-deficient diet had a significant decrease in serum sulfate levels and an increase in fractional excretion of ultrafilterable sulfate, which paralleled a significant decrease in BBM Na\(^{+}\)-sulfate cotransport activity (161). This is in total contrast to the effect of chronic K deficiency which stimulates rat renal BBM Na\(^{+}\)-citrate cotransport (140). The decrease in BBM Na\(^{+}\)-sulfate cotransport activity was associated with decreases in BBM NaSi-1 protein and renal cortical NaSi-1 mRNA abundance. In addition, in Xenopus oocytes injected with mRNA from kidney cortex slices of K-deficient rats, there was a significant reduction in the induced Na\(^{+}\)-sulfate cotransport, whereas there was no alteration in L-leucine uptake (control), suggesting that in K-deficient rats there is a specific decrease in functional mRNA encoding the NaSi-1 protein (161). These findings indicate that chronic K deficiency leads to a reduction in serum sulfate levels, an increase in fractional excretion of sulfate, and a reduction in renal sulfate reabsorption, by downregulating the expression of the proximal tubular NaSi-1 cotransporter protein and mRNA.

C. Metabolic Acidosis/Alkalosis

Changes in blood pH lead to alterations in renal sulfate reabsorption. An early study showed that under physiological concentrations, sulfate reabsorption is stimulated by an acid blood pH [pH 7.29, metabolic acidosis], whereas it is inhibited by an alkaline blood pH [pH 7.53, metabolic alkalosis] (78). A recent study demonstrated that rats subjected to metabolic acidosis showed a significant increase in the fractional excretion of sulfate, which was associated with a 2.4-fold decrease in BBM Na\(^{+}\)-sulfate cotransport activity (198). The reason for this discrepancy between the two studies is in their different experimental designs. The early study (78) used anesthetized rats infused with NH\(_{4}\)Cl for 2 h (metabolic acidosis), whereas the recent study (198) used conscious rats fed on an NH\(_{4}\)Cl-supplemented diet for 24 h before taking measurements. The recent study (198) also demonstrated that the decrease in Na\(^{+}\)-sulfate cotransport was correlated with a decrease in BBM NaSi-1 protein abundance and a decrease in cortical NaSi-1 mRNA expression. The inhibitory effect of metabolic acidosis on BBM Na\(^{+}\)-sulfate cotransport was also sustained in rats with chronic (10 days) metabolic acidosis. In addition, in Xenopus oocytes injected with mRNA from kidney cortex, there was a significant reduction in the induced Na\(^{+}\)-sulfate cotransport in rats with metabolic acidosis compared with control rats (198), suggesting that metabolic acidosis causes a decrease in the abundance of functional mRNA encoding the NaSi-1 cotransporter. These findings indicate that metabolic acidosis reduces sulfate reabsorption by causing a decrease in BBM Na\(^{+}\)-sulfate cotransport, by decreasing the expression of NaSi-1 protein and mRNA.
abundance in the kidney. This is in complete contrast to the effect of metabolic acidosis on the expression of the renal BBM Na\(^+\)-dicarboxylate cotransporter NaDC-1, for which both mRNA and protein were recently shown to be upregulated in the rat kidney (4). These findings suggest that metabolic acidosis may play an important role in sulfate homeostasis, by specifically downregulating renal sulfate reabsorption via the BBM Na\(^+\)-sulfate cotransporter NaSi-1.

D. Vitamin D Status

Vitamin D status has been shown to regulate renal sulfate reabsorption. Rats adapted to a vitamin D-deficient diet for 6 wk showed significantly reduced plasma sulfate levels and a threefold increase in renal fractional excretion of sulfate compared with control rats (73). Vitamin D-deficient rats showed a significant reduction in renal cortical BBMV Na\(^+\)-sulfate cotransport compared with control rats, which was due to a decrease in \(V_{\text{max}}\) but not \(K_{\text{m}}\) for sulfate uptake (73). This reduction in BBMV Na\(^+\)-sulfate uptake was associated with similar decreases in NaSi-1 protein (78 ± 3%) and NaSi-1 mRNA (73 ± 3%) levels, when compared with control rats (73). Vitamin D supplementation to vitamin D-deficient rats resulted in a return to normal plasma sulfate, fractional sulfate excretion, and NaSi-1 mRNA and protein contents (73). This modulation was shown to be the result of a direct effect of vitamin D, with no independent action of parathyroid hormone or calcium levels (73). Recently, this laboratory has shown that the Nas1 gene promoter contains several VDREs and that vitamin D can strongly increase (by nearly 9-fold) the transcription of the mouse Nas1 gene above basal levels (9). These results together demonstrate that vitamin D may be an important modulator of sulfate homeostasis by upregulating Nas1 gene expression and thereby stimulating renal BBM Na\(^+\)-sulfate cotransport in vivo.

E. Thyroid Hormone

Renal sulfate reabsorption has been shown to be modulated by thyroid hormone levels. Hypothyroid patients are known to have decreased serum sulfate concentrations; however, the mechanism involved in thyroid hormone-induced alterations of renal sulfate homeostasis is so far unknown. Thyroid hormone has been shown to increase Na\(^+\)-sulfate cotransport in renal BBMVs; triiodothyronine (T\(_3\)) administered in pharmacological doses significantly stimulated Na\(^+\)-sulfate cotransport in mouse renal BBMV compared with controls (249). Kinetic studies demonstrated that T\(_3\) increased the \(V_{\text{max}}\) by ~60% without changing the \(K_{\text{m}}\) for BBMV Na\(^+\)-sulfate cotransport compared with controls (249). A later study by another group contradicted this study, suggesting that thyroid hormone did not stimulate renal BBMV Na\(^+\)-sulfate cotransport in mice or rats (11), but found that T\(_3\) selectively upregulated renal BBMV Na\(^+\) -phosphate cotransport, without affecting Na\(^+\) -sulfate or Na\(^+\) -glucose cotransport in rats fed on a normal (0.7% P\(_i\)) or high-phosphate (1.4% P\(_i\)) diet (11). Interestingly, the high-phosphate diet actually led to an increase in renal BBMV Na\(^+\) -sulfate and Na\(^+\) -glucose cotransport, whereas it reduced Na\(^+\) -phosphate cotransport (11), with no explanation provided by the authors for a possible mechanism of this effect. Recently, the effect of thyroid hormone on renal sulfate transport was reinvestigated. Chemically induced hypothyroidism using 6-propyl-2-thiouracil (PTU) in rats led to significant decreases in serum sulfate concentrations, renal fractional sulfate reabsorption, and creatinine clearance (214). Renal BBMV Na\(^+\) -sulfate cotransport showed an almost twofold decrease in \(V_{\text{max}}\) (with no change in \(K_{\text{m}}\)), whereas no significant difference in \(V_{\text{max}}\) or \(K_{\text{m}}\) was observed in BLMV sulfate transport in hypothyroid rats compared with controls (214). Hypothyroidism did not alter the membrane motional order (fluidity) in BBM or BLM, indicating that changes in the membrane fluidity do not represent the mechanism for the altered renal sulfate transport. NaSi-1 mRNA and protein levels were significantly reduced in the kidney cortex from hypothyroid rats, suggesting that PTU-induced hypothyroidism decreases Na\(^+\) -sulfate cotransport by downregulating NaSi-1 mRNA and/or protein expression. Since the Nas1 promoter contains several TREs (9), this laboratory has recently tested whether thyroid hormone can affect Nas1 gene transcription. Preliminary data show that Nas1 promoter activity in OK cells is upregulated in the presence of T\(_3\) (unpublished data), providing evidence that thyroid hormone does indeed regulate Nas1 expression at the transcriptional level.

F. Glucocorticoids/Mineralocorticoids

Glucocorticoids/mineralocorticoids have been shown to modulate renal sulfate transport (211). Renal BBMV Na\(^+\) -sulfate cotransport was shown to decrease in response to dexamethasone (202). Three-week-old chickens treated with dexamethasone (60 \(\mu\)g/100 g body wt) showed a significantly reduced BBMV Na\(^+\) -sulfate cotransport, without having any effect on BLM sulfate/bicarbonate exchange (202). Kinetic analysis showed an almost twofold decrease in \(V_{\text{max}}\) (but no change in \(K_{\text{m}}\)) for BBMV Na\(^+\) -sulfate cotransport in the dexamethasone-treated animals compared with controls (202), suggesting glucocorticoids may have regulatory role in renal sulfate reabsorption. Recently, this laboratory found treatment of
young rats with dexamethasone for 1 wk led to a reduced BBMV Na\(^+\)-sulfate cotransport, which correlated with a downregulation of the NaSi-1 protein and mRNA levels in the renal cortical BBM (unpublished data). In agreement with this, a recent study looked at the effect of another glucocorticoid, methylprednisolone (MPL), on renal sulfate transport. Male adrenalectomized rats treated with MPL showed a 2.2-fold increase in the urinary sulfate excretion rate and a 2.7-fold increase in renal sulfate clearance compared with adrenalectomized controls (211). MPL treatment significantly reduced the $V_{\text{max}}$ by 23% (without causing any changes in $K_m$) in renal BBMV Na\(^+\)-sulfate cotransport, but produced no changes in the $V_{\text{max}}$ or $K_m$ in renal BLM sulfate/bicarbonate exchange (211). The reduction in renal BBMV Na\(^+\)-sulfate cotransport was correlated closely with similar decreases in NaSi-1 protein and mRNA levels in MPL-treated rats compared with controls (211). These data suggest that glucocorticoids increase the renal clearance of sulfate by downregulating (in part) renal NaSi-1 mRNA and protein expression. Furthermore, the Nas1 promoter possesses several GREs that may act in regulating the transcription of the Nas1 gene. Recent data from this lab suggest that dexamethasone does in fact inhibit (by up to 60%) the basal transcription of the Nas1 gene (unpublished data). Further work is needed to fully characterize the mechanisms of glucocorticoid regulation of the Nas1 gene and its effect(s) on sulfate homeostasis.

G. Growth Hormone

Early studies have shown growth hormone (GH) to regulate radiosulfate uptake into mouse cartilage and skeletal cells (163). Recently, the effects of human GH (hGH) treatment on renal BBM Na\(^+\)-sulfate cotransport were investigated in mature adult (9–10 mo) and aged (22–23 mo) rats (213). Both NaSi-1 mRNA and protein levels were lower in the aged rats compared with mature adult rats; however, upon hGH treatment, NaSi-1 mRNA levels increased after 2–3 days in both mature adult and aged rats, and thereafter gradually decreased to control levels. NaSi-1 protein levels increased significantly after 4 days of hGH treatment in mature adult rats but were unaltered in the aged rats (213). Insulin-like growth factor 1 (IGF-I) levels increased significantly and remained stable after 2 days of hGH treatment in both age groups; however, there was no significant difference in plasma IGF-I levels between the two age groups (213). These data demonstrate that hGH can increase NaSi-1 mRNA and protein levels in vivo (by yet unknown mechanisms), suggesting that NaSi-1 expression in the kidney may (in part) be regulated by circulating GH concentrations.

H. NSAIDs and Prostaglandins

NSAIDs are known to increase sulfate renal clearance and decrease renal fractional reabsorption of sulfate. NSAIDs such as indomethacin, salicylic acid, tiaprofenic acid, and ibuprofen have been shown to inhibit renal sulfate reabsorption (13, 173). Infusion of indomethacin, tiaprofenic acid, or ibuprofen led to a significant increase in the urinary excretion rate and renal clearance of sulfate, without altering GFR in rats (13, 173). This effect was accompanied by a significant decrease in the fractional reabsorption of sulfate by the kidneys and a decrease in serum sulfate concentration (13, 173). Indomethacin and ibuprofen (at therapeutic concentrations) produced a >80% decrease in urinary excretion of PGE$_2$ (173). PGE$_2$ administration abolished the effects of ibuprofen on renal sulfate clearance and increased sulfate reabsorption in indomethacin-treated rats, without significantly altering the renal clearance of sulfate or creatinine (173). These data suggest that prostaglandins may be able to control the effects of NSAIDs on the renal clearance and reabsorption of sulfate. Tiaprofenic acid (1 mM) led to a 36% inhibition in BLMV sulfate uptake but to no effect in BBMV sulfate uptake (12). Ibuprofen and indomethacin (1 mM each) decreased sulfate uptake in rat kidney cortex BBMVs by 22 and 40% and in BLMVs by 38 and 60%, respectively (12). A more recent investigation showed a lower ibuprofen concentration (30 μM) led to a significant decrease in $V_{\text{max}}$ (with no change in $K_m$) for BBMV Na\(^+\)-sulfate cotransport, without affecting the $V_{\text{max}}$ or $K_m$ in BLMV sulfate/bicarbonate exchange, compared with control rats (210), suggesting that ibuprofen administration at a reduced concentration (30 μM) was unable to inhibit BLMV sulfate uptake. No differences were observed in the membrane motional order of BBMV and BLMV in ibuprofen-treated and control rats (210), suggesting that altered membrane fluidity was not responsible for the changes observed in sulfate transport. The decrease in renal BBMV Na\(^+\)-sulfate cotransport was correlated well with parallel decreases in NaSi-1 mRNA and protein levels in kidney cortex of ibuprofen-treated animals when compared with control animals (210). These data indicate that NSAIDs alter Na\(^+\)-sulfate cotransport by downregulating NaSi-1 mRNA and protein in renal BBMs and may play an important role in controlling sulfate homeostasis.

I. Cell Membrane Fluidity

The membrane motional order or lipid packing order (i.e., membrane fluidity) is known to affect the activity and kinetics of membrane transporters. Salicylic acid has been shown to decrease the lipid order parameter of renal BBM and BLM in a concentration-dependent manner,
making the membrane more fluid (5). The change in membrane fluidity could possibly interfere with conformational transitions of proteins, thereby resulting in altered protein function. This theory was tested in a recent study aimed at determining whether membrane fluidity can affect the functional activity of the renal \( \text{Na}^+ \)-sulfate cotransporter rNaSi-1 (138). Exposure of rNaSi-1 stably transfected MDCK cells to cholesterol (200 \( \mu \text{M} \)) and benzyl alcohol (20 \( \text{mM} \)) resulted in a 35\% decrease and a 20\% increase, respectively, in the \( V_{\text{max}} \) (with no change in \( K_{\text{m}} \)) for \( \text{Na}^+ \)-sulfate cotransport (138). Similar effects were observed by these compounds on membrane fluidity: fluorescence polarization of 1,6-diphenyl 1,3,5-hexatriene (an indicator of membrane motional order) was significantly decreased by cholesterol (200 \( \mu \text{M} \)) and increased by benzyl alcohol (20 \( \text{mM} \)) in NaSi-1-expressing MDCK cells (138). These data suggest that alterations in membrane fluidity, which could arise during diseased states, aging, or pregnancy, may play an important role in the modulation of renal \( \text{Na}^+ \)-sulfate cotransport and sulfate homeostasis.

J. Chronic Renal Failure

Early clinical studies showed that children suffering from chronic renal failure (CRF) had elevated serum sulfate levels, with hypersulfatemia occurring even in early stages of renal failure (168). This is of particular importance in children suffering from CRF, due to their raised protein turnover during growth compared with adults. In nondialyzed children with CRF, plasma sulfate levels rose to seven times the upper normal limit, similar to the increase observed in nondialyzed adult CRF patients (168). It has been reported that sulfate seems to be the only ion that accumulates in such high amounts in uremic plasma, where it represents a large fraction of the anion gap in CRF (77). With decreasing GFR, plasma sulfate levels in children with CRF were shown to rise exponentially to a maximum of 2.54 \( \text{mM} \), which is 10 times the mean normal level in healthy children \( (240 \pm 60 \ \mu \text{M}; \text{Ref. 169}) \). A similar hyperbolic relationship was observed between plasma sulfate and PAH clearance \( (C_{\text{PAH}}) \) (168). From these data, it was suggested that the degree of hypersulfatemia in CRF mainly depends on the reduction in GFR. In patients with decreasing renal function, absolute urine sulfate excretion was well maintained; however, a marked fall in fractional tubular reabsorption was observed (169). Some children with CRF even showed tubular sulfate secretion, which is not generally observed in normal children or adults. Thus the kidney does seem to have a high adaptive capacity to compensate for sulfate retention in the presence of reduced GFR. Further studies are needed to determine if altered intestinal sulfate absorption or intermediary sulfate metabolism may contribute to hypersulfatemia in CRF.

A recent CRF study in rats showed that 6 wk after 80\% nephrectomy, these rats had significant increases in NaSi-1 mRNA and protein levels, but significant decreases in sat-1 mRNA and protein levels compared with control (sham-operated) rats (74). Renal clearance measurements showed a 50\% reduction in total sulfate reabsorption in CRF rats; however, the total amount of sulfate excreted was not altered. Despite this, serum sulfate levels were significantly higher in rats both 3 and 6 wk after nephrectomy. Further work is needed to determine the modulation of NaSi-1 and sat-1 transporters in CRF.

Sulfate accumulation in CRF could lead to the pathogenesis of renal osteodystrophy, possibly due to complex formation of sulfate with calcium (\( \text{Ca}^{2+} \)) in the plasma (139). CaSO\(_4\) complex formation is relatively insoluble (with a dissociation constant of \( K = 0.006 \ \text{M} \)) and has been previously used therapeutically for the treatment of hypercalcemia, by reducing the absorption of calcium from the gut (131). Raised plasma sulfate could also contribute to uremic bone disease by ion association with calcium in plasma. The resultant decrease of ionized serum \( \text{Ca}^{2+} \) could stimulate the parathyroid glands to synthesize parathyroid hormone. This hypothesis is compatible with the observation that hyperparathyroidism develops early in children with CRF, even in the presence of normal serum \( \text{Ca}^{2+} \) levels (164). In addition, infusion of sodium sulfate into dogs with normal renal function has been shown to lead to an increase in urinary \( \text{Ca}^{2+} \) excretion, with a dramatic fall in serum \( \text{Ca}^{2+} \) leading to tetanic symptoms (263). Sulfate addition (at concentrations 7–48 \( \text{mM} \)) to serum was shown to be followed by a dose-dependent increase of complexed \( \text{Ca}^{2+} \) levels in serum, with a corresponding decrease of free \( \text{Ca}^{2+} \) concentration (262). These studies suggest that the increases in serum sulfate levels in CRF may aggravate complex formation with \( \text{Ca}^{2+} \), resulting in hyperparathyroidism.

K. Pregnancy and Postnatal Growth

Early studies have proposed that steady-state serum sulfate levels vary during pregnancy and postnatal development. When comparing pregnant versus nonpregnant animals, the \( V_{\text{max}} \) and \( K_{\text{m}} \) values for BBM \( \text{Na}^+ \)-sulfate cotransport were significantly higher in pregnant guinea pigs, whereas the \( K_{\text{m}} \) value was significantly greater (but not the \( V_{\text{max}} \)) for the BLM bicarbonate/sulfate exchanger in pregnant animals, compared with the nonpregnant group (137). The higher \( V_{\text{max}} \) in BBMV \( \text{Na}^+ \)-sulfate cotransport could be due to a greater membrane fluidity observed in pregnant and younger animals (137). Newborn infants have a mean serum sulfate concentration of 470 \( \mu \text{M} \) compared with adult levels of 330 \( \mu \text{M} \) (42). The higher serum sulfate levels in the newborn may be attributed in part to a difference in amino acid and protein

\( \text{Physiol Rev} \), VOL 81, OCTOBER 2001, www.prv.org
intake and the fact that GFR is lower in young infants compared with adults (63). Young guinea pigs have been shown to have an increased renal sulfate reabsorption compared with adult animals (186, 194). The increased renal sulfate reabsorption was correlated with an increase in $V_{\text{max}}$ (without affecting $K_m$) for BBM Na$^+$-sulfate cotransport (194). Neonatal guinea pigs (<10 days) had a threefold higher $V_{\text{max}}$ (with no change in $K_m$) in BBMV Na$^+$-sulfate cotransport, whereas $V_{\text{max}}$ and $K_m$ were unaltered in BLM sulfate/bicarbonate transport compared with adult animals (137). Furthermore, an increased density of NaSi-1 proteins or an increased turnover rate of NaSi-1 protein could be responsible for the raised $V_{\text{max}}$ in young and pregnant guinea pigs that may be required for an increased demand for sulfated substrates, such as sulfated glycosaminoglycans, during growth and development. However, since the total renal tubular mass and the BBM and BLM surface areas of the proximal tubule are much smaller at birth than in adulthood (75, 134) and the fact that the proximal tubular segment (S1) where the majority of sulfate reabsorption occurs is poorly developed in the newborn compared with the adult (95, 108, 119, 240), we could expect to see a reduced number of sulfate transporters in the young compared with adults. To resolve this issue, a recent study in this laboratory looked at the expression levels (mRNA and protein) of NaSi-1 and sat-1 transporters in rats of various ages (156). It was demonstrated that renal sulfate transport changed with postnatal age: both NaSi-1 and sat-1 mRNA levels increased by 10-fold (per $\mu$g RNA) from day 1 to day 77 (11 wk) rats (156). A similar age-dependent increase was observed for sodium-dependent and sodium-independent sulfate uptakes in Xenopus oocytes injected with kidney mRNAs from rats of increasing age. Interestingly, no significant changes in NaSi-1 and sat-1 protein levels (per mg BBM or BLM protein) were observed in renal membranes from day 1 to day 77 rats (156), suggesting translational or posttranslational mechanisms may be maintaining equal numbers of sulfate proteins on proximal tubular membranes during early maturation. This study demonstrated that the mRNA expression of the renal sulfate transporters NaSi-1 and sat-1 are upregulated during postnatal development, whereas their respective protein contents in the kidneys remain constant from birth to early adulthood. At the other end of the life cycle, to determine whether renal sulfate transporter expression varied during aging, NaSi-1 mRNA and protein levels were measured in mature adult (9–10 mo) and aged (22–23 mo) rats (213). The aged rats had a significantly reduced NaSi-1 mRNA and protein levels compared with mature adult rats, suggesting NaSi-1 expression decreased during aging (213). These data show that the expression of renal sulfate transporters is highly regulated postnaturally and that they play an important role in sulfate homeostasis during growth and development.

## L. Posttranslational Regulation

### 1. Glycosylation

The effect of glycosylation on the expression of mammalian sulfate transporters has not been studied in great detail. In vitro translation of NaSi-1 cRNA in rabbit reticulocyte lysate was shown to produce a major band of 66-kDa protein, which was shifted upward in the presence of microsomes (157), suggesting core glycosylation of NaSi-1 was occurring. On immunoblots with rat renal BBMV proteins, polyclonal NaSi-1 antisera detected a broad band at 65–70 kDa (148, 156, 160, 161), which upon treatment with the endoglycosidase enzyme PNGaseF for 1 h resulted in a thinner band at ~66 kDa (unpublished data). Tunicamycin (an N-glycosylation inhibitor) was shown to lead to a significant reduction in the NaSi-1-induced and sat-1-induced sulfate uptakes in Xenopus oocytes (unpublished data), further suggesting N-glycosylation may be important for NaSi-1 and sat-1 function. Site-directed mutagenesis of the putative N-glycosylation sites of rNaSi-1 (asparagines 140, 174, and 591 to serines) and rsat-1 (asparagines 158, 163, and 587 to serines) led to a significant reduction (when compared with wild-type proteins) in the induced sulfate transport activities in Xenopus oocytes, for the N140S and N591S mutants and for the N158S and N587S mutants (but not N163S) rsat-1 mutants, respectively (unpublished data). These data suggest asparagines N140 and N591 (on rNaSi-1) and N158S and N587S (on rsat-1) may be essential for correct protein delivery to the plasma membranes of Xenopus oocytes, as previously demonstrated for N-glycosylation mutants of the NaPi2 protein (96). The functional significance of these N-glycosylation sites on NaSi-1 and sat-1 expression in mammalian cells still needs to be determined.

### 2. Phosphorylation by cAMP-dependent protein kinase (protein kinase A)

To date, very little information is known regarding the role of intracellular signaling-second messenger pathways and their involvement in the regulation of mammalian sulfate transporters. Recently, it was demonstrated that pharmacological activation of the adenylate cyclase/protein kinase A pathway, using 8-bromo-cAMP, led to a significantly decreased transport activity of NaSi-1 and sat-1 proteins in Xenopus oocytes (154). The inhibitory effect of 8-bromo-cAMP on transport activity was dose dependent, with 200 $\mu$M 8-bromo-cAMP leading to 45 and 32% inhibition of the rNaSi-1- and rsat-1-induced sulfate transport activities, respectively (unpublished data).
Since the rsat-1 protein does not contain any putative protein kinase A sites, the inhibitory effect of 8-bromo-cAMP on rsat-1 transport would argue against a direct effect of phosphorylation on the rsat-1 protein, but rather may be due to an indirect effect on protein retrieval from the plasma membrane, as previously postulated to occur with the Na+-glucose cotransporter SGLT-1 (100). Presently, it is not known whether the single putative protein kinase A sites on the NaSi-1 proteins (Thr240 on hNaSi-1, Thr404 on mNaSi-1, and Thr405 on rNaSi-1) are involved in phosphorylation and whether protein kinase A phosphorylation of NaSi-1 is essential for membrane sorting or protein expression.

3. Phosphorylation by phospholipid-dependent protein kinase (protein kinase C)

Recently, it was shown that pharmacological activation of the phospholipase C/protein kinase C pathway by DOG led to a significantly decreased transport activity of NaSi-1 and sat-1 proteins in Xenopus oocytes (154). The degree of inhibition on transport activity by DOG was dose dependent and using 10 μM DOG led to a 33 and 41% inhibition of the rNaSi-1- and rsat-1-induced sulfate transport activities, respectively (unpublished data). As suggested for the inhibitory effect of 8-bromo-cAMP on NaSi-1 and sat-1 function, it could be postulated that DOG may be having an indirect effect on protein retrieval from the plasma membrane, as proposed to occur with SGLT-1 (100). However, the presence of the numerous putative PKC sites found on both NaSi-1 and sat-1 proteins does not rule out that PKC phosphorylation may be taking place and that it may be important for protein function. The significance of the putative tyrosine kinase and casein kinase sites on NaSi-1 and sat-1 proteins is yet unknown but may suggest that other forms of phosphorylation could also be important for their expression and sorting to the plasma membranes. Clearly further work is needed to elucidate the precise roles of phosphorylation on the expression of NaSi-1, sat-1, and other mammalian sulfate transport proteins.

M. Heavy Metals

Heavy metal intoxication is known to lead to defects in cellular uptake mechanisms in the mammalian liver and kidney. In particular, a number of reabsorptive and secretory defects in renal transport systems can arise. Recently, the effects of several heavy metals (mercury, lead, cadmium, and chromium) were tested on the function of the NaSi-1 and sat-1 transporters in Xenopus oocytes (158, 159). rNaSi-1 and rsat-1 proteins were expressed in Xenopus oocytes, and sulfate uptakes were performed (under initial rates) in the presence or absence of the metals mercury, lead, cadmium, or chromium. Mercury strongly and irreversibly inhibited NaSi-1 transport ($K_i = 7 \mu M$) by reducing both $V_{max}$ and $K_m$ for sulfate, whereas mercury strongly inhibited sat-1 transport ($K_i = 2.5 \mu M$) by reducing its $V_{max}$ by eightfold but not its $K_m$ for sulfate (158, 159). Lead inhibited NaSi-1 transport ($K_i = 21 \mu M$) reversibly by decreasing $V_{max}$ but not $K_m$ for sulfate but was unable to significantly inhibit sat-1 transporter activity (up to 1 lead) (158, 159). Cadmium (500 μM) showed weak reversible inhibition of NaSi-1 (no $K_i$ calculated) and sat-1 transport ($K_i = 762 \mu M$) by decreasing only $V_{max}$ (but not $K_m$) for sulfate uptake (158, 159). Chromium strongly inhibited NaSi-1 ($K_i = 520 \mu M$) and sat-1 transport ($K_i = 49 \mu M$) reversibly by reducing the affinity for sulfate by sevenfold without affecting the $V_{max}$ for sulfate uptake (158, 159). The mechanism of inhibition by chromium is most probably by competitive binding for the substrate site on the proteins. In fact, due to the strong structural similarity between CrO$_4^{2-}$ and SO$_4^{2-}$, chromium (VI) oxide has been previously suggested to mimic the sulfate anion and was suggested to cross plasma membranes using sulfate transport systems (33, 151, 267). These studies demonstrated that heavy metals can inhibit the activity of renal Na$^+$-sulfate cotransporter NaSi-1 and the hepatic/renal sulfate/bicarbonate transporter sat-1 by various mechanisms that may be responsible (in part) for sulfaturia after heavy metal intoxication (158, 159).

VI. PATHOPHYSIOLOGY OF SULFATE TRANSPORTERS

There is extensive evidence to suggest that sulfate transport mechanisms are highly regulated in the body. Slight imbalances in sulfate homeostasis, perhaps due to defects in renal reabsorptive or secretory processes, can lead to clinical manifestations. There are several known human syndromes/diseases in which the formation of the sulfate ion or the metabolism of oxidized sulfur is disturbed: Hunter’s syndrome, Morquio’s syndrome, Maroteaux-Lamy syndrome, metachromatic leukodystrophy, and multiple-sulfophydrase deficiency (244). Furthermore, several genetic diseases have been linked to defects in specific membrane sulfate transporters, including DTDSST (see sect. νB3) and CLD (see sect. νB2) (93, 226). The autosomal dominant form of familial distal renal tubular acidosis has been linked to mutations in AE1 (25; see sect. νB4). In this disease, tubular secretion of hydrogen ion in the distal nephron is impaired, leading to the development of metabolic acidosis, which is frequently accompanied by hypokalemia, nephrocalcinosis, and metabolic bone disease (177). Hereditary spherocytosis and Southeast Asian ovalocytosis have also been linked to mutations in AE1 (246). The common feature of these latter diseases is a mutation in the sulfate transporter.
protein AE1, whose function is disrupted or lost due to either an early termination, incorrect protein formation, or aberrant processing to the plasma membrane. For more details on diseases associated with membrane sulfate transporters, see References 71 and 247.

VII. CONCLUSION AND FUTURE DIRECTIONS

This review has focused on the physiology of mammalian sulfate transporters and the processes that govern their expression. The cloning of mammalian sulfate transporters in the last 10 years has prompted extensive research into the functional and developmental relevance of these proteins. It has become obvious that sulfate transporters exist in a variety of cell types and contribute to essential functions in the body. However, we are only scratching the surface of the mechanisms by which these proteins and their respective genes are regulated. The presence of other regulatory proteins that may bind to and affect the expression of sulfate transporters is yet unknown but may be an important process for the regulation of sulfate transporters in cells. Naturally, the existence of other yet uncloned sulfate transporters is not ruled out, with modern technologies permitting further cloning of proteins encoding novel sulfate transporters. This direction is clearly aided by rising interest in physiological genomics and completion of the genome sequencing projects that are being undertaken for several mammalian species.

More than 30% of the gene products in the mammalian genome are encoded by polytopic transmembrane proteins such as membrane transporters. Despite extensive advances over the past 20 years in the characterization, purification, and modification of these proteins using molecular biology and biochemistry, only a handful of these membrane-bound proteins have been crystallized to date. Due to the present lack of structural information of membrane transporters at high-level resolution, it can be foreseen that in the coming years extensive efforts will be directed at elucidating the structure/function relationships of membrane proteins at the molecular level. Such research will not only determine the tertiary structures of membrane transporters at a high resolution, but will also increase our knowledge of structural components essential for proper function and how changes in protein structure (due to genetic mutations) can lead to clinical diseases.

Recently, several laboratories (including my own) have initiated gene targeting studies in mice to gain a greater understanding of the overall importance of sulfate transport genes in normal states. This approach, pioneered by the AE1 knock-out mouse in the field of membrane sulfate transporters, has shed extensive light on the physiological significance of the AE1 gene in vivo. The targeted disruption technology will be extremely useful for determining the precise role(s) of NaSi-1 and sat-1 in the body (being presently endeavoured in the author’s laboratory), particularly since no clinical condition(s) has yet been linked to defect(s) in these genes (unlike with CLD, DTDST, and AE1 genes).

Furthermore, it has become quite evident that numerous diseases, such as diabetes, cystic fibrosis, nephrogenic diabetes insipidus, Alzheimer’s disease, and Parkinson’s disease, result from trafficking defects in membrane transporters. Among the transporters in which defective trafficking has been linked to human diseases are the GLUT4 glucose transporter, the CFTR Cl− channel, and the aquaporin-2 water channel (225). The number of reported membrane transporters undergoing regulated trafficking is increased tremendously, such that it may be a widespread modality for the in vivo regulation of proteins including sulfate transporters. To date, very little is known about the posttranslational (trafficking) mechanisms that regulate sulfate transporter expression, and it is foreseen that many laboratories will aim to elucidate these sorting processes. Such information will be crucial in understanding the pathophysiological states (such as hyposulfatemia, hypersulfaturia, and altered sulfate metabolism) that arise due to aberrant protein sorting or synthesis. In the next few years, the precise physiological and developmental relevance of these important sulfate transporter genes will be elucidated and will form the basis of future strategies toward the treatment of patients with disturbances in sulfate transport, metabolism, and homeostasis.

Much appreciation and gratitude is given to Heini Murer, who paved the path toward the discovery of mammalian sulfate transporters.

Work in the author’s laboratory is funded by the Australian National Health and Medical Research Council, the Australian Research Council, and the University of Queensland.

Address for reprint requests and other correspondence: D. Markovich, Dept. of Physiology and Pharmacology, Univ. of Queensland, St. Lucia, Queensland 4072, Brisbane, Australia (E-mail: danielm@plpk.uq.edu.au).

REFERENCES


Physiol Rev • VOL 81 • OCTOBER 2001 • www.prv.org


68. HASTBACKA J, SUPERT-FURGA A, WILCOX WR, EIMDL DL, COHN DH, AND LANDER ES. Atelectogenesis type II is caused by mutations in the diastrophic dysplasia sulfate-transporter gene (DTDST): evi-


115. Karlstedt E, Kaitila I, and Pirinen S. Phenotypic features of den-
L’hypersulfaufémie n’est-elle pas la cause des ostéodystrophies de l’insuffisance rénale chronique? Presse Med 70: 211, 1902.


MURER H, MANGANEL M, AND ROCH-RAMEL F. Tubular transport of monocarboxylates, Krebs cycle intermediates and inorganic sul-


210. Salgak H, Benincosa LJ, Murer H, and Morris ME. Phospho- 

