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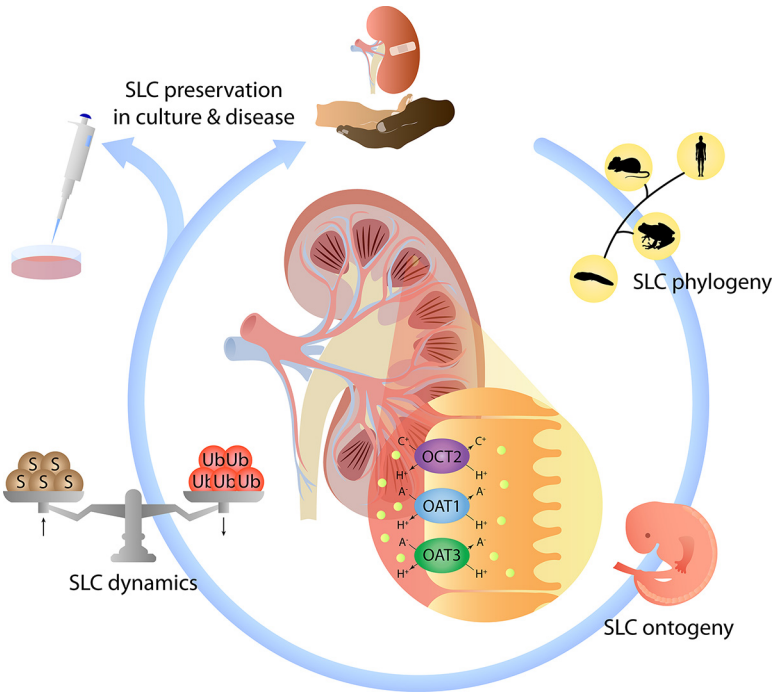

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KEY WORDS

active secretion; evolution; kidney disease; nephrogenesis; nephrology; organic anion transporter; organic cation transporter; proximal tubule; regenerative medicine

CLINICAL HIGHLIGHTS

- The solute carriers (SLCs) organic anion transporters 1 and 3 (OAT1/3) and organic cation transporter 2 (OCT2), expressed in kidney proximal tubule cells, are crucial mediators of metabolic waste removal and active drug secretion.
- SLC downregulation in kidney disease leads to the retention of >100 uremic solutes, which progressively worsens clinical outcome.
- Loss of intrinsic SLC expression in vitro hampers the translational value of current kidney cell models for drug-transporter interaction studies, which are demanded for all new molecular entities before entering the market.
- Given their major role in kidney physiology and drug development, a good understanding of SLC regulation is needed to develop both transporter-targeted therapies and improved cell models.

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REGULATION OF SOLUTE CARRIERS OCT2 AND OAT1/3 IN THE KIDNEY: A PHYLOGENETIC, ONTOGENETIC, AND CELL DYNAMIC PERSPECTIVE

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Abstract

Over the course of more than 500 million years, the kidneys have undergone a remarkable evolution from primitive nephric tubes to intricate filtration-reabsorption systems that maintain homeostasis and remove metabolic end products from the body. The evolutionarily conserved solute carriers organic cation transporter 2 (OCT2) and organic anion transporters 1 and 3 (OAT1/3) coordinate the active secretion of a broad range of endogenous and exogenous substances, many of which accumulate in the blood of patients with kidney failure despite dialysis. Harnessing OCT2 and OAT1/3 through functional preservation or regeneration could alleviate the progression of kidney disease. Additionally, it would improve current in vitro test models that lose their expression in culture. With this review, we explore OCT2 and OAT1/3 regulation from different perspectives: phylogenetic, ontogenetic, and cell dynamic. Our aim is to identify possible molecular targets both to help prevent or compensate for the loss of transport activity in patients with kidney disease and to enable endogenous OCT2 and OAT1/3 induction in vitro in order to develop better models for drug development.

active secretion; evolution; kidney disease; nephrogenesis; nephrology; organic anion transporter; organic cation transporter; proximal tubule; regenerative medicine

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1. FROM BENCH TO BEDSIDE: THE IMPORTANCE OF KIDNEY SOLUTE CARRIERS

As intricate filtration-reabsorption systems, the kidneys maintain the fine internal balance of water, salts, and nutrients in blood plasma and interstitial fluids. At the same time, unwanted endogenous metabolites and foreign substances, such as toxins and drugs, are efficiently removed from the body. However, a large part of solutes do not readily pass the glomerular filtration barrier because of size, charge, or protein binding (1). Here, removal relies on active secretion by the proximal tubule epithelial cells (PTECs), primarily through members of the solute carrier (SLC) family 22: organic cation transporter 2 (OCT2), encoded by the gene *SLC22A2*, and organic anion transporters 1 and 3 (OAT1/3), encoded by the genes *SLC22A6* and *SLC22A8* (**FIGURE 1**). Because of their shared ancestry, these membrane transport proteins have in common 12 predicted membrane-spanning domains with a large extracellular loop between transmembrane domains 1 and 2 and a large intracellular

*C. Pou Casellas and K. Jansen contributed equally to this work.

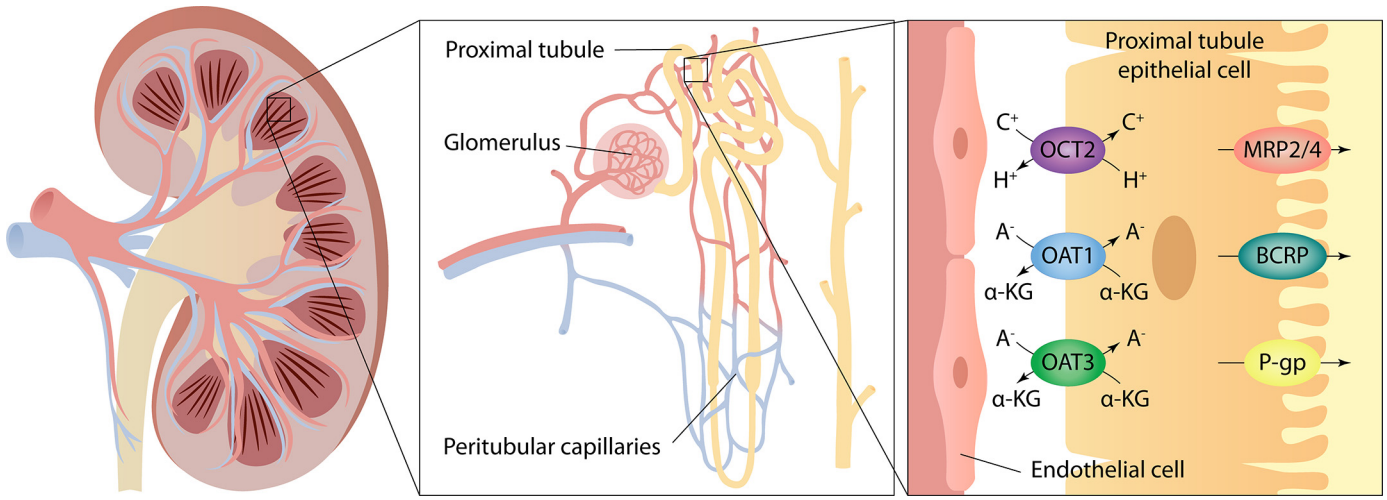

FIGURE 1. Schematic illustration of the kidney, the nephron as its functional unit, and the proximal tubule epithelial cell as its site for active secretion. Organic anion transporters 1 and 3 (OAT1/3) transport a broad range of organic anions (A^-) into the cell in exchange for α-ketoglutarate (α-KG), whereas the organic cation transporter 2 (OCT2) functions as an organic cation (C^+)/H^+ antiporter. : BCRP, breast cancer resistance protein; MRP2/4, multidrug resistance proteins 2 and 4; P-gp, P-glycoprotein.

loop between transmembrane domains 6 and 7 and intracellular amino and carboxy termini (**FIGURE 2**). With their multispecific binding pockets, OAT1/3 and OCT2 together are responsible for the basolateral uptake of a wide variety of anionic and cationic metabolites and drugs in the kidney (**TABLE 1**) (2, 3). For efficient solute excretion, OAT1/3 and OCT2 work in concerted action with kidney efflux transporters on the luminal cell side, such as breast cancer resistance protein (BCRP), P-glycoprotein (P-gp), and multidrug resistance proteins 2 and 4 (MRP2/4), but the excretion rate is mainly determined by functional activity of the uptake transporters (**FIGURE 1**).

When kidney function falls below 10–15%, uremic retention solutes accumulate in blood and quickly reach life-threatening concentrations. Dialysis treatment can replace the kidney's filtration-reabsorption function to remove many metabolic end products, including urea and creatinine, yet ~25% of the retention solutes are poorly dialyzable, mostly because of protein binding (4). These retention solutes, also known as uremic toxins because of their various biological adverse effects, accelerate chronic kidney disease (CKD), increase the risk of cardiovascular disease and bone dysfunction, and contribute to the high morbidity and mortality rates in CKD patients (4–7). Notably, residual kidney function (RKF) remains an important prognostic factor for patient survival, while increasing exposure to dialysis fails to decrease mortality rates among kidney patients (8). This confirms that active renal secretion via PTECs is an indispensable process. In 2016, Lowenstein and Grantham (8) argued for a paradigm shift from the "glomerulocentric"

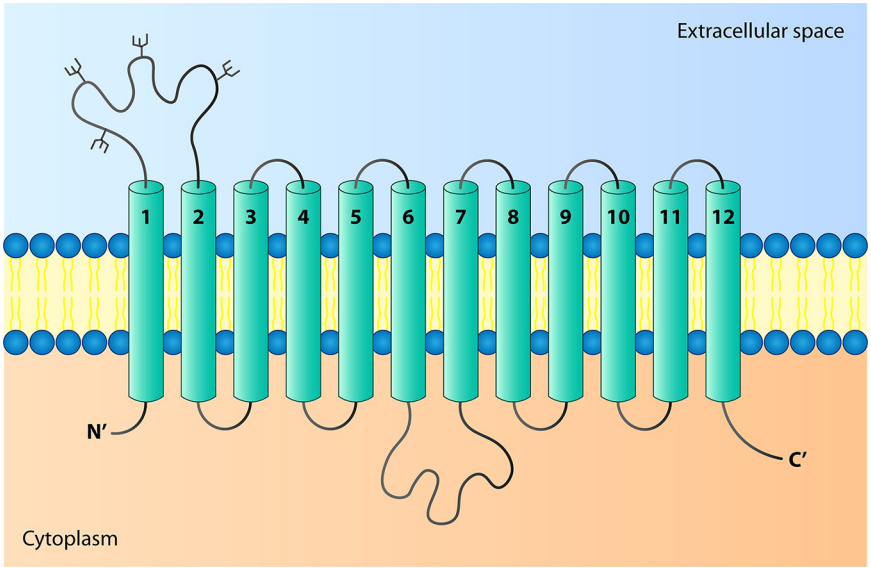

FIGURE 2. Molecular structure of organic cation transporter 2 and organic anion transporters 1/3. All 3 solute carriers contain twelve putative transmembrane domains (TMDs) with the N′ and C′ termini located at the cytosolic side of the cell membrane. A large hydrophilic loop between TMDs 1 and 2 contains several glycosylation sites, whereas an intracellular loop between TMDs 6 and 7 contains multiple potential phosphorylation sites.

Table 1. ***Examples of endogenous and xenobiotic substrates for organic cation transporter 2 and organic anion transporters 1/3***

Transporter	Endogenous Substrates	Drug Substrates
OCT2 (*SLC22A2*)	Monoamine neurotransmitters (e.g., dopamine, serotonin) Histamine Polyamines (e.g., putrescine, spermine) Guanidino compounds (e.g., guanidine, methylguanidine)	Metformin Pindolol Cisplatin Famotidine Lamivudine Cimetidine
OAT1 (*SLC22A6*)	Cyclic nucleotides Prostaglandin E_2 and F_{2a} Uric acid Indoxyl sulfate	Adefovir Ciprofloxacin Methotrexate Pravastatin
OAT3 (*SLC22A8*)	Conjugated hormones Prostaglandins Uric acid *Para*-cresyl sulfate	Adefovir Ciprofloxacin Methotrexate Pravastatin NSAIDs

For an exhaustive list, visit DrugBank (https://go.drugbank.com). OAT1/3, organic anion transporters 1/3; OCT2, organic cation transporter 2.

view of RKF to tubular solute excretion, and evidence for the significance of residual tubular secretion in patient survival is accumulating (8–12).

Besides the critical role of OCT2 and OAT1/3 in patient survival, expression levels can influence the pharmacokinetics and hence the therapeutic (or toxicologic) effects of drugs in healthy subjects. Likewise, drug-drug interactions (DDIs) or interactions with endogenous solutes at the transporter binding sites can result in altered pharmacokinetics and adverse drug effects, especially in patients with comorbidities and polypharmacy. As a consequence, the International Transporter Consortium and regulatory bodies including the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) urge new molecular entities to undergo interaction studies on OAT1/3 and OCT2 before entering the clinic and the market (13). With ethical, societal, and political pressure toward animal-free drug testing, adequate in vitro test models for interaction studies are highly warranted, but kidney cell models are hampered by their rapid loss of transporter expression in culture (**TABLE 2**). For drug screening, this problem is currently circumvented by overexpression of SLCs in different cell lines, but a cell model reflecting the physiological situation would be given preference (14, 15). Moreover, genetic modifications limit the applicability of in vitro models for fundamental research and for regenerative and clinical purposes (e.g., implantation of kidney organoids or bioartificial kidneys). For all kidney models available, including primary cells and stem cell-derived organoids, it is yet unknown how intrinsic uptake transporter expression can be preserved or induced (**FIGURE 3**).

Altogether, harnessing OCT2 and OAT1/3 through functional preservation or regeneration could alleviate the progression of CKD, improve kidney replacement therapies, and refine in vitro test models. On the one hand, if we could restore or enhance transport functionality short term, we might be able to boost active secretion of uremic toxins. On the other hand, short-term reversible inhibition could eliminate dose-limiting drug toxicities, e.g., for the chemotherapeutic and OCT2 substrate cisplatin. Moreover, the expression-modulating activities of drugs should be taken into account in case of kidney disease or polypharmacy. Besides the known detrimental potential of drug-drug interactions (DDIs), drug-induced transporter regulation might just as well cause accumulation of uremic toxins or drug-induced side effects, e.g., by diminishing the RKF.

With this review, we explore the regulation of OCT2 and OAT1/3 using different perspectives: phylogenetic, ontogenetic and cell dynamic. In sect. 2, the study of evolutionary nephrology and comparative physiology provides the reader with an understanding of how and why the kidney has developed and retained active secretion mechanisms. In sect. 3, we provide an overview of the transcription factors and signaling pathways involved in proximal tubule (PT) development and, potentially, SLC ontogeny. Pathways involved could serve as molecular targets for regenerative therapies. In sect. 4, an exploration of cell dynamic, short-term regulation mechanisms in the adult kidney might uncover opportunities for functional boosts or pharmacotherapeutic interventions. Finally, in sect. 5 we translate the findings from evolution and comparative physiology, ontogeny, and regulation into new perspectives to reverse or compensate for the loss of SLCs in the kidney. This review serves as an information toolbox by providing perspectives of the transporters' past and present, which can give directions

Table 2. *Organic cation transporter 2 (SLC22A2) and organic anion transporter 1/3 (SLC22A6/8) expression levels in commercial proximal tubule epithelial cell lines*

Cell Line	Origin	SLC Expression *SLC22A2*	*SLC22A6*	*SLC22A8*	Reference
RPTEC-TERT1	Human	Low	ND	ND	(16)
ciPTEC	Human	Low	0	0	(15, 17)
HK2	Human	0	0	0	(18)
HKC11	Human	Medium	0	0	(18)
HKC8	Human	Medium	0	0	(18)
HPTC-05-CLA	Human	0	0	0	(18)
HPTC-05-LTR	Human	0	0	0	(18)
C57BL6/J	Mouse	0	0	0	(18)
NRK-52E	Rat	0	0	0	(18)
SHR	Rat	0	0	0	(18)
WKY	Rat	0	0	0	(18)
OK-ATCC	Opossum	0	0	NR	(18)
OKH	Opossum	Low	0	NR	(18)
OKWT	Opossum	Medium	Low	NR	(18)
LLC-PK1	Pig	Medium	0	0	(18)
MDCK	Dog	Low	0	0	(18)

ND, not detected; NR, not reported.

to a better therapeutic future. The identification of possible targets can help to prevent or compensate for the loss of transport activity in patients with kidney disease and induce or restore transporter expression and function in vitro (**FIGURE 4**).

2. FROM FISH TO PHILOSOPHER: A PHYLOGENETIC PERSPECTIVE ON KIDNEY SOLUTE CARRIERS

In 1959, H. W. Smith published *From Fish to Philosopher* (19), a celebrated book about the evolutionary development of the kidney. By peeking into the theater of evolution with ever-changing environmental pressures, he explained why the kidney has developed into an intricate and energy-consuming filtration-reabsorption system. At the time of publication, active tubular secretion in the PT had been demonstrated experimentally and suggested to be comprised of two separate systems (20). Later, these systems would be known as OATs and OCTs.

2.1. The Proximal Tubule: A Conserved Secretion System

Active tubular secretion has been conserved in the course of evolution, suggesting an indispensable role for SLCs (19, 21). Kidney PTs have probably been equipped with SLC homologs since the beginning of the Paleozoic era, in the early Cambrian period more than 500 million years ago (**FIGURE 5**). Once simple diffusion became insufficient in primitive marine organisms to discharge waste solutes, the mesoderm developed primordial nephric tubules: meager funnellike outlets that connected the body cavity (the coelom) to the outside world. These nephric tubules epithelialized to retain useful substances while secreting excess salts and organic molecules with the aid of osmotic water flow (10).

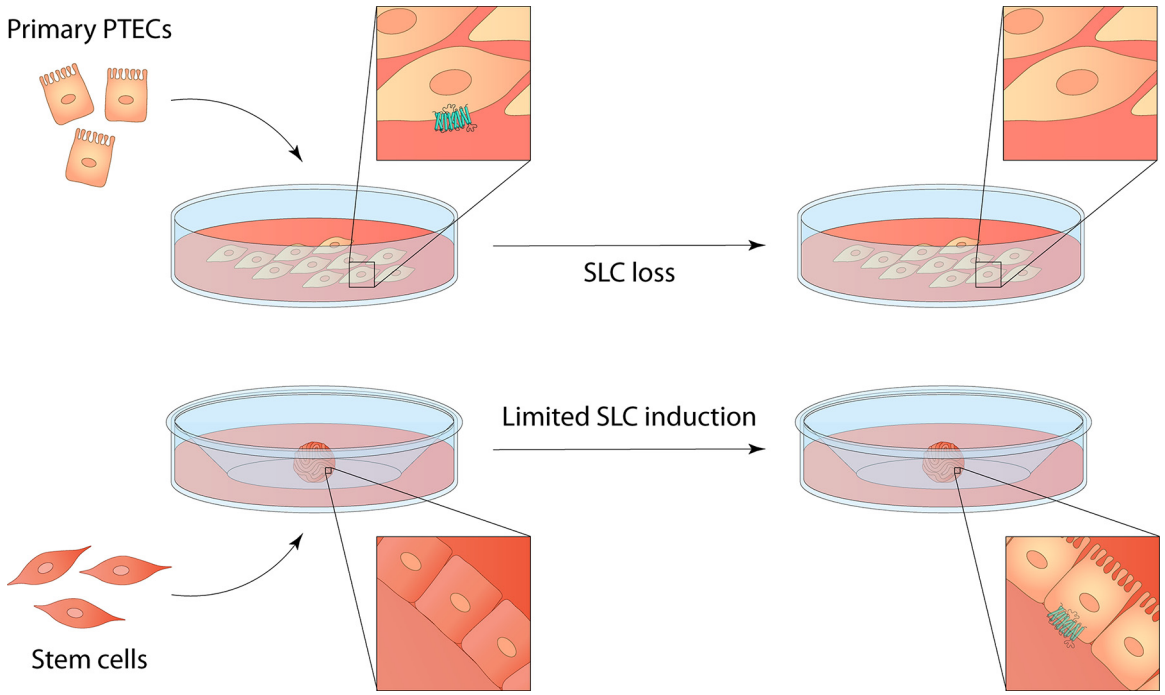

FIGURE 3. Solute carrier expression changes in cell culture. Upon culture, primary proximal tubule epithelial cells (PTECs) rapidly lose expression of *SLC22A2/6/8*. Meanwhile, differentiation of stem cells in vitro yields limited expression of *SLC22A2/6/8*. SLC, solute carrier.

Slc22 homologs can be found in invertebrates, including *Drosophila*, *Caenorhabditis elegans*, and sea urchins, which supports the existence of SLCs during the early Cambrian (22–24). However, even though these distant homologs transport organic anions and cations, intron phasing analysis indicated that OATs and OCTs are likely phylogenetically distinct from their invertebrate homologs. Eraly et al. (25, 26) and Zhu et al. (24)

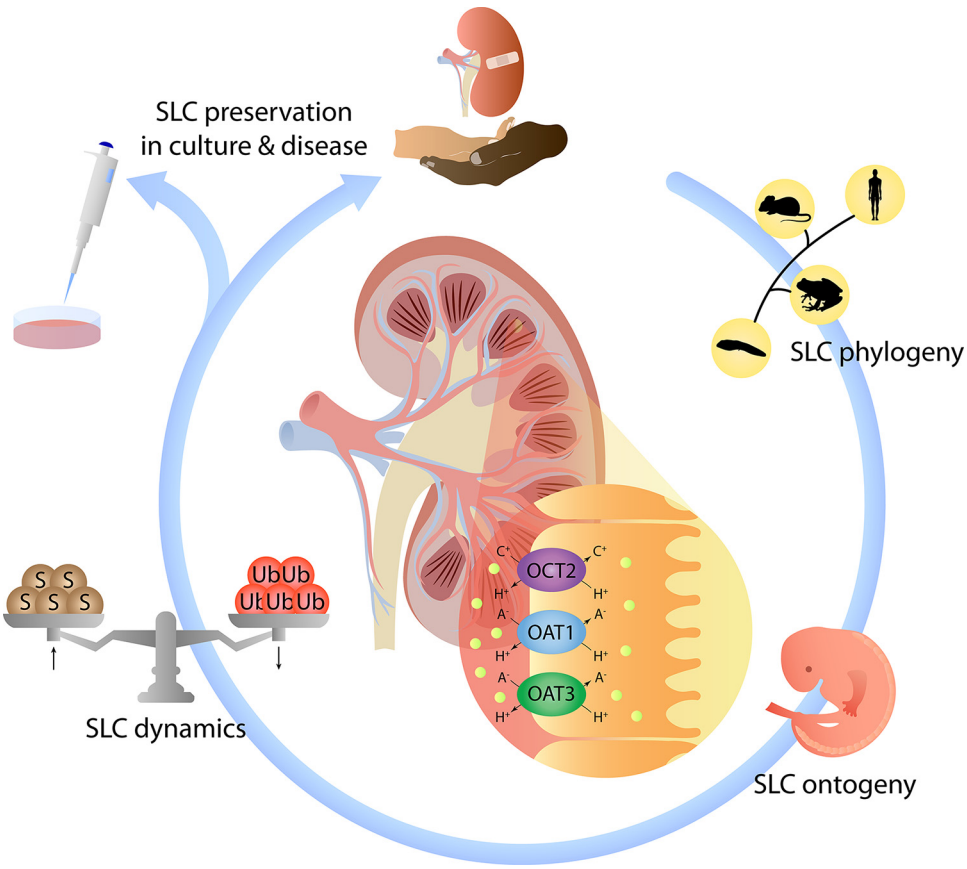

FIGURE 4. Considering solute carrier (SLC) phylogeny, ontogeny, and dynamics can lead to new insights on their preservation in proximal tubule epithelial cells in vitro and in kidney disease.

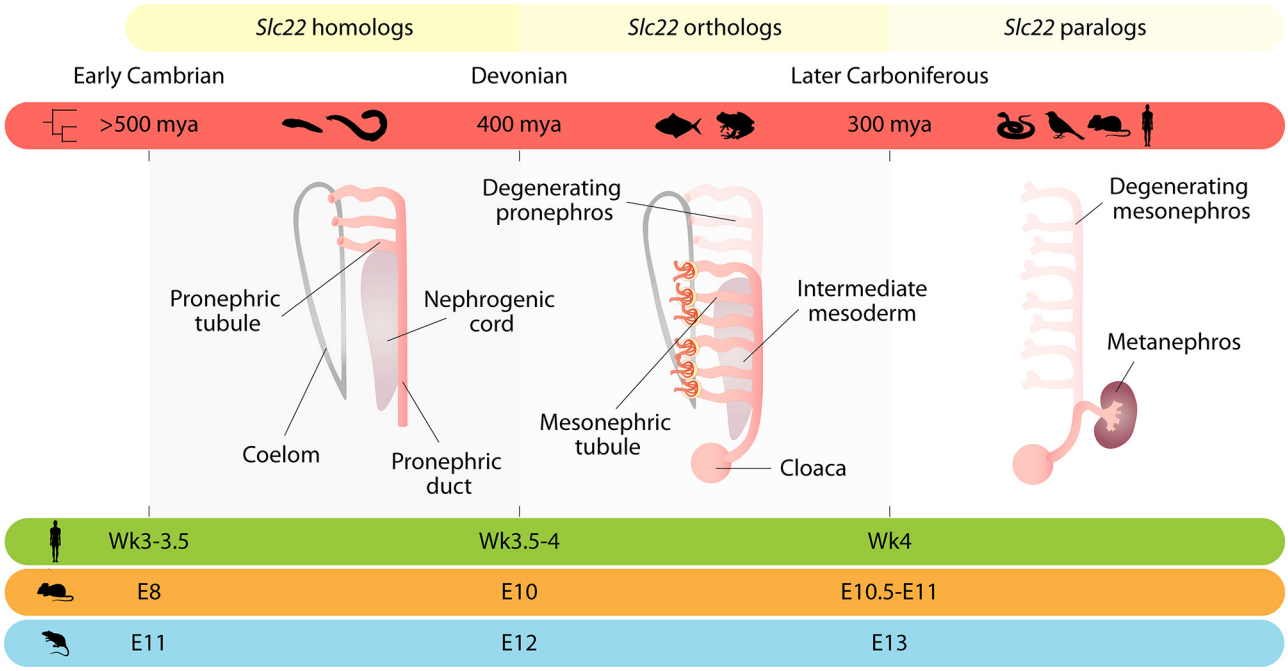

FIGURE 5. Evolutionary and ontogenetic timeline of kidney development and solute carrier (SLC) appearance. During the early Cambrian period, organisms developed pronephros as excretory systems, which contained *Slc22* homologs. Later, during the Devonian period, pronephros evolved into mesonephros, which presented with *Slc22* orthologs. Finally, with the appearance of more complex organisms during the later Carboniferous period, the metanephric kidneys developed, which contain *Slc22* paralogs in different species. Pronephroi and mesonephroi still transiently exist in the present during nephrogenesis. E, embryonic day; Wk, week of life.

concluded from phylogenetic analyses that the OAT and OCT lineages might have formed somewhere after the divergence of vertebrates and invertebrates and before the separation of bony fish and terrestrial vertebrates.

Smith believed that when the marine ancestors of vertebrates invaded low-salt fresh waters, they had to compensate for osmotic water influx. By making use of the blood hydrostatic pressure, water was easily filtered out through a tuft of leaky capillaries, which would be engulfed by the nephric tubules: the glomerulus was born. Species presenting with glomeruli date back to at least the Devonian era around 400 million years ago, maybe even to the Silurian (27–29). Recently, however, Meyer and Hostetter (30) argued that glomerular filtration did not primarily develop for water excretion, because some invertebrates also seem to possess an "ultrafilter" system; accordingly, the generated fluid stream might be necessary to support the clearance of secreted solutes. Regardless of the teleological explanation, extensive blood filtration forced reabsorption mechanisms to speed up to prevent the loss of salts, glucose, amino acids, vitamins, hormones, and more.

Although glomerular filtration led to extensive removal of metabolic end products, there seemed to be the need for SLC conservation in kidneys. Fish *slc22* and mammalian *slc22* genes evolved from a common ancestral gene by speciation. Nonetheless, fish *slc22* genes are considerably divergent from their mammalian orthologs (e.g., 49% similarity between zebrafish and murine *Slc22a6*), which is logical considering their different ecological niches and physiological requirements (24). Whereas in fish and amphibians the gills and skin still contributed to salt and solute excretion, the kidneys became the master regulator of body fluid composition for terrestrial life forms that emerged in the Carboniferous period (29). In the absence of a wet environment, filtered water and solutes had to be retained in the body. Instead of taking the easy road of reducing glomerular filtration, the mammalian kidney retained its filtration capacity. Smith contemplated that "the filtration-reabsorption system is now so firmly established that there is no easy way to overhaul it and to convert it to a purely tubular kidney, as the marine fishes have done." According to him, glomerular filtration and tubular reabsorption had to increase further when mammals became warm-blooded, which resulted in increased blood circulation to keep up with the higher oxygen and nutrient demand (19). Moreover, a carnivorous lifestyle provided an additional selection pressure to maintain a high glomerular filtration rate (GFR) for the excretion of urea produced during protein breakdown (31, 32). Interestingly, meat consumption increases the production of gut-derived metabolites, such as indoxyl sulfate and *p*-cresyl sulfate, which are almost exclusively removed through proximal tubular secretion (33). Therefore, it is conceivable that meat consumption

promoted the evolutionary conservation of SLC expression in kidney tubules (34).

Phylogenetic analysis by Eraly et al. (26) uncovered a phenomenon of transporter expansion and gene pairing in the evolution of mammals as a result of tandem duplications: the majority of the OAT subclade is located on chromosome 11, where paralogs *SLC22A6* and *SLC22A8* are only 8 kb apart from each other (24). The research group led by S. K. Nigam hypothesized that, founded on increasing physiological complexity and exposure to a broader spectrum of endogenous and exogenous substrates, transporter redundancy and broader substrate specificity could have offered a survival benefit. In need of a more robust secretion system, mammals have developed an interorgan network of *SLC22* transporters with mono- and multispecific substrate specificities that interact through remote sensing and signaling (35, 36). This system includes the kidney-abundant paralogs *SCL22A6* and *SLC22A8*, as well as *SLC22A2* and *SLC22A1*, with the respective former being more ancestral as evidenced by their presence in nonmammals (24, 37).

2.2. Energy Investment: Evolutionary Trade-Off

Environmental stress factors throughout evolutionary history pushed the development of the kidney into an energy-consuming filtration-reabsorption system that, despite extensive filtration, conserved active tubular secretion in most species. In his reprint of Smith's classic, Vize comments on the kidney's energetic householding: "There is enough waste motion here to bankrupt any economic system—other than a natural one, for Nature is the only artificer who does not need to count the cost by which she achieves her ends" (38). However, as R. L. Chevalier argued, this becomes the kidney's pitfall once energy investments shift. Intensive tubular solute reclamation in mammals comes at the price of high energy consumption: although the kidneys account for only 1% of our weight, they consume 10% of oxygen in the body. During the reproductive phase, the energetic costs of the kidney are borne because of the pressure of natural selection (i.e., survival of the fittest). However, selection is determined by reproductive fitness, not by long-term health: with age, energy investment strategies shift, and investment into an energy-draining organ seems inefficient. CKD becomes, in combination with an evolutionary mismatching meat- and salt-rich diet, a common eventuality (32). Could it perhaps be that the downregulation of SLCs in disease and primary kidney cells in culture, just like in aging, is the result of shifting priorities in energy investment? We seize upon this in sect. 5.

3. FROM EMBRYOS TO ADULTS: AN ONTOGENETIC PERSPECTIVE ON KIDNEY SOLUTE CARRIERS

Ernst Haeckel's famous phrase "ontogeny recapitulates phylogeny" describes embryonic development as a flashback into history as the embryo goes through stages that resemble the course of evolution. Although this quotation has gained a bad repute, the embryonic development of the kidney undeniably represents some phylogenetic stages (**FIGURE 5**). In human gestational weeks 3–3.5 (wk3–wk3.5), which corresponds with murine embryonic day 8 (mE8), and rat embryonic day 11 (rE11), the segmented intermediate mesoderm gives rise to the pronephros: pairs of nephric tubules that compare with the primitive kidney of our early marine ancestors (39). The pronephros is essential for embryonic survival, but it is very rudimentary and quickly degenerates. Subsequently, the intermediate mesoderm forms a new tubular system at wk3.5–wk4 (mE10 and rE12), the mesonephros, which in some species is connected to glomeruli (40). The mesonephros persists as a functional kidney in fish and amphibians but is replaced in reptiles, birds, and mammals by the definitive kidney, the metanephros (41). The metanephros develops in the human embryo at wk4–wk5 (mE10.5–mE11 and rE13) (40).

Currently, relatively little is known about metanephric transporter ontogeny. For a better understanding of when and how SLCs develop in the PT, this section presents the nephrogenesis (**FIGURE 6**) and SLC expression timeline in mice, rats, and humans (**FIGURE 7**) as well as the biochemical factors that contribute to PT maturation. Metanephric expression of SLCs follows a general pattern with three stages: a prenatal induction and gradual increase in expression (sect. 3.1), followed by a sudden perinatal rise (sect. 3.2), and a postnatal, sex-biased upregulation during sexual maturity (sect. 3.3).

3.1. Prenatal Expression of Kidney Solute Carriers

3.1.1. The process of nephrogenesis.

In all species, the commencement of nephrogenesis is defined by the interaction between the Wolffian duct (WD) and the metanephric mesenchyme (MM) (**FIGURE 6**). At embryonic wk4 (mE10.5 and rE13), the MM secretes factors that cause the WD to sprout a single bud: the ureteric bud (UB) (RET^+, $HOXB7^+$) (42–47). MM and UB meet at approximately wk5 (mE11–mE11.5 and rE13–rE13.5) (42, 43, 46). Upon physical interaction, MM progenitors closest to the UB condense around its tips, thereby generating the cap mesenchyme (CM). The CM constitutes the so-called nephron progenitor cells, characterized by the expression of the transcription factors

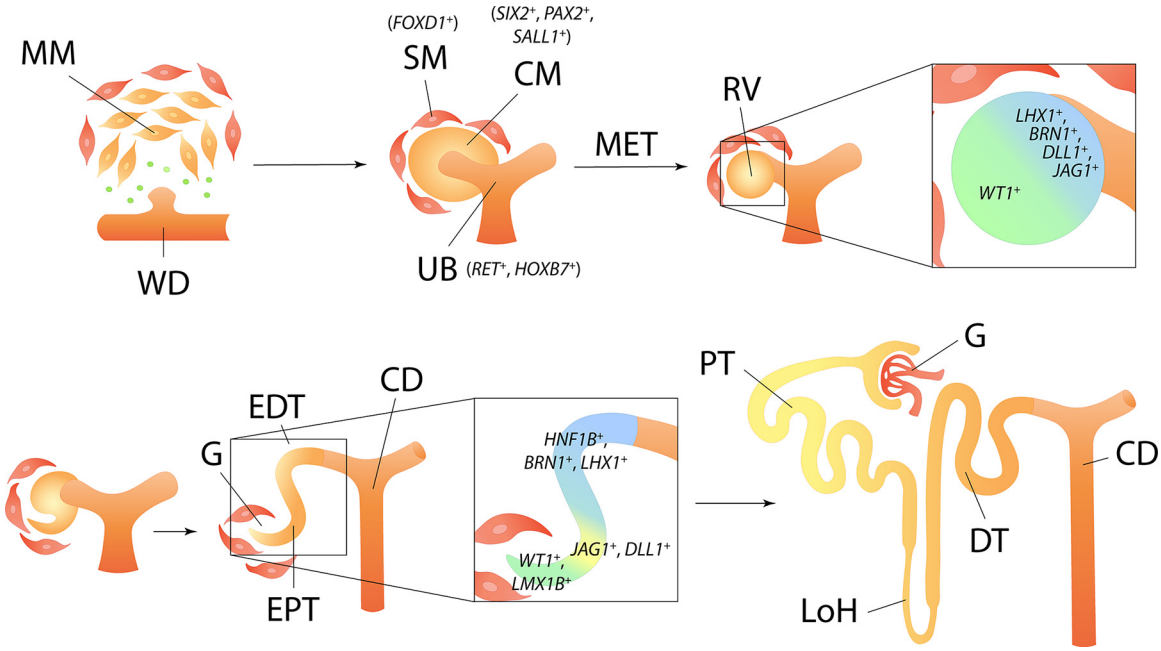

FIGURE 6. Schematics of the process of nephrogenesis. CD, collecting duct; CM, cap mesenchyme; DT, distal tubule; EDT, early distal tubule; EPT, early proximal tubule; G, glomerulus; LoH, loop of Henle; MET, mesenchymal-to-epithelial transition; MM, metanephric mesenchyme; PT, proximal tubule; RV, renal vesicle; SM, stromal mesenchyme; UB, ureteric bud; WD, Wolffian duct.

SIX2, *PAX2*, and *SALL1*, which are essential for both progenitor maintenance and differentiation. Through elongation and segmentation, nephron progenitor cells eventually give rise to the glomerulus, the PT, the loop of Henle, and the distal tubule. MM that does not form the CM develops into stromal mesenchyme (*FOXD1*$^+$), which will mature into mesangial, pericytic, and interstitial cells. Finally, the UB will form the collecting duct system and ureter (43, 48).

When CM and UB interact, the UB signals the CM to promote differentiation of some nephron progenitor cells and to maintain the self-renewing stemness potential of others. (48–50). CM cells fated for differentiation undergo mesenchymal-to-epithelial transition (MET), which is mostly driven by Wnt and Notch signaling (51, 52), and form the renal vesicle at wk6 (mE12.5 and rE13.5–rE15) (42, 43). MET causes the downregulation of CM marker genes and the upregulation of epithelial markers in renal vesicles, including *WNT4*, and cadherins 4 and 6 (*CDH4* and *CDH6,* respectively) (50, 52–54). The renal vesicle shows regionalized expression of a diverse set of markers: the distal part highly expresses *LHX1* and *BRN1*, as well as Notch ligands *DLL1* and *JAG1*, whereas the proximal part presents with high expression of *WT1* (53, 55–57). The most distal part of the renal vesicle fuses to the UB tip, which leads to the maintenance of an undifferentiated CM population that keeps promoting UB branching (43, 58, 59).

By wk7 (mE13–mE14 and rE16), the renal vesicle forms a lumen and "unwinds," leading to the formation of comma-shaped bodies, which develop into s-shaped bodies at wk8–wk9 (mE14–mE14.5 and rE18) (42, 60–62). When s-shaped bodies start forming, the neighboring stromal and endothelial progenitors incorporate into them and differentiate into the mesangial and endothelial cells of the glomerulus (43). Comma- and s-shaped bodies present with a more evident polarization than renal vesicles, and segmentation of these structures into the different tubular segments is primarily directed by Notch signaling (53, 56, 63). The most proximal domain of the s-shaped body expresses *WT1* and *LMX1B* and possesses a podocytic fate; the less proximal domain is the prospective PT region, and it is characterized by low levels of *PAX2* and exclusively high levels of *JAG1* and *DLL1*. Finally, the mid- and distal regions of s-shaped bodies express hepatocyte nuclear factor 1B (*HNF1B)*, *BRN1*, and *LHX1*, which have a crucial role in the formation of both mature loop of Henle and distal tubule (55, 64–67).

By wk32 and mE16.5, mature nephrons can already be observed (68–70). In rats, nephrons mature only after birth, but fully formed nephrons containing immature structures can be found around rE20 (71). Nephrogenesis is considered complete by wk34–wk36 (mP4–mP6, and rP4–rP10) (42, 68, 72–74) (**FIGURE 7*A***).

For a more complete and detailed explanation of nephrogenesis and the signaling molecules involved, we recommend the reviews by Little and McMahon (75) and Costantini and Kopan (56) and the book chapters by Kopan et al. (43) and Bush et al. (46).

3.1.2. The emergence of SLCs during nephrogenesis.

Surprisingly little is known about the exact time point of SLC induction in human kidneys. The only evidence of OAT1 appearance was generated through in situ hybridization studies by A. P. McMahon's research group. With data provided in the GUDMAP database, they showed that the first transcripts of *SLC22A6* can be detected by wk11 (76). The ontogeny of OAT3 and OCT2 in humans remains to be elucidated. Fortunately, studies on rodents have provided more information: *Slc22a2* appears around mE15.5 and rE13, *Slc22a6* has been detected earliest at mE14 and rE14, and *Slc22a8* arises around mE14.5 and rE14 (**FIGURE 7**). In general, however, protein expression of these three transporters is only observed later with the formation of s-shaped bodies (77, 78). All studies reporting (first) appearance of these transporters in humans, mice, and rats and the applied techniques are listed in **TABLE 3**.

3.1.3. Molecules/pathways involved in PT formation and prenatal SLC induction.

It remains unknown how transporter expression is first induced during nephrogenesis, but it is most likely tightly linked to the molecular dynamics during PT formation and maturation. This subsection gives an overview of the signaling pathways and molecules known to affect PT formation or maturation during nephrogenesis.

3.1.3.1. NOTCH SIGNALING. For a long time, Notch was considered the main pathway to induce PT fate. In a study conducted by Cheng et al. (82), homozygous Notch2 knockout in *Pax3*+ progenitor cells of murine embryos allowed epithelialization but no clear formation of s-shaped bodies, tubules, or glomeruli. In heterozygous Notch2 knockouts, only distal nephrons appeared, whereas no proximal or podocytic structures could form, indicating that Notch2 is needed for PT segmentation (82). Comparable data was obtained when treating metanephroi with DAPT, an inhibitor of Notch signaling (83). However, when either Notch1 or Notch2 was knocked out in *Pax2*+/*Six2*+ progenitor cells, all nephron segments were formed, including PTs (82, 84). Only when both Notch1 and Notch2 were depleted, was nephrogenesis impaired at the renal vesicle stage (53). The difference in outcome between these studies was pointed out by Chung et al. (53): *Pax3*+ cells are not only mesenchymal progenitor cells but also stromal cells, and DAPT targets all cell types equally, including stromal cells. Therefore, the effect of Notch2 knockout specifically on PTs might have been due to the targeting of the stromal population, since stromal cells have been shown to have a profound influence on nephron formation and maturation (53, 85, 86).

More recently, the role of Notch in nephron segmentation was investigated by deleting both Notch1 and Notch2 in *Wnt4*+ cells in murine embryos, which appear during the differentiation of renal vesicles. In double-mutant kidneys, all mature nephron segments failed to form (57). With these findings it could be concluded that, contrary to previous assumptions, Notch signaling is required for the segmentation of the nephron into all nephron segments, not specifically the PT. Yet, Notch signaling has a PT-specific function, as it has been found to regulate the expression of two key transcription factors, *Lhx1* and *Hnf1b* (57), whose effects on PT formation and SLC regulation are described below.

3.1.3.2. HEPATOCYTE NUCLEAR FACTORS. Among all HNFs, HNF1β was first shown to be crucial for the formation of PTs during nephrogenesis. In murine embryos, knockdown of *Hnf1b* both in the MM and at the renal vesicle stage causes the formation of aberrant nephrons with a critically low number of PTs, loops of Henle, and distal tubules. *Hnf1b*-deficient kidneys show a major reduction in Notch signaling from the comma-shaped body onward and increased expression of *Pax2* in the prospective PT region. Moreover, these kidneys display no *Lotus tetragonolobus* lectin (LTL) staining characteristic of PTs and have significantly reduced expression levels of differentiation markers such as *Hnf1a*, *Hnf4a*, *Brn1*, and *Cdh6* (55, 87). Confirming that the observed genotype in these kidneys is due to the lack of *Hnf1b*, its overexpression in *Xenopus* animal caps induced a variety of genes, including *lhx1*, *hnf1a*, *hnf4a*, and, most importantly, *slc22a6*. *Hnf1b* has been shown to regulate *lhx1* transcription by direct binding to its promoter (88), and *Lhx1,* in turn, is thought to promote Notch signaling and the subsequent formation of proximal segments (82).

Two other HNFs reputed to have a major role both in PT differentiation and *SLC* regulation are HNF1α and HNF4α. Gallegos et al. (89) and Martovetsky et al. (90) investigated the process leading to PT differentiation using rodent microarray data and found that both *Hnf1a* and *Hnf4a* had the highest connectivity to the emergence of the PT.

These three HNFs, HNF1α, HNF4α, and HNF1β, have been shown to affect *SLC* expression in vitro, which is discussed below. Therefore, they might have a role not only in the formation of the PT but also in its subsequent maturation.

3.1.3.3. OTHER NUCLEAR RECEPTORS AND TRANSCRIPTION FACTORS. Retinoic acid (RA), acting through

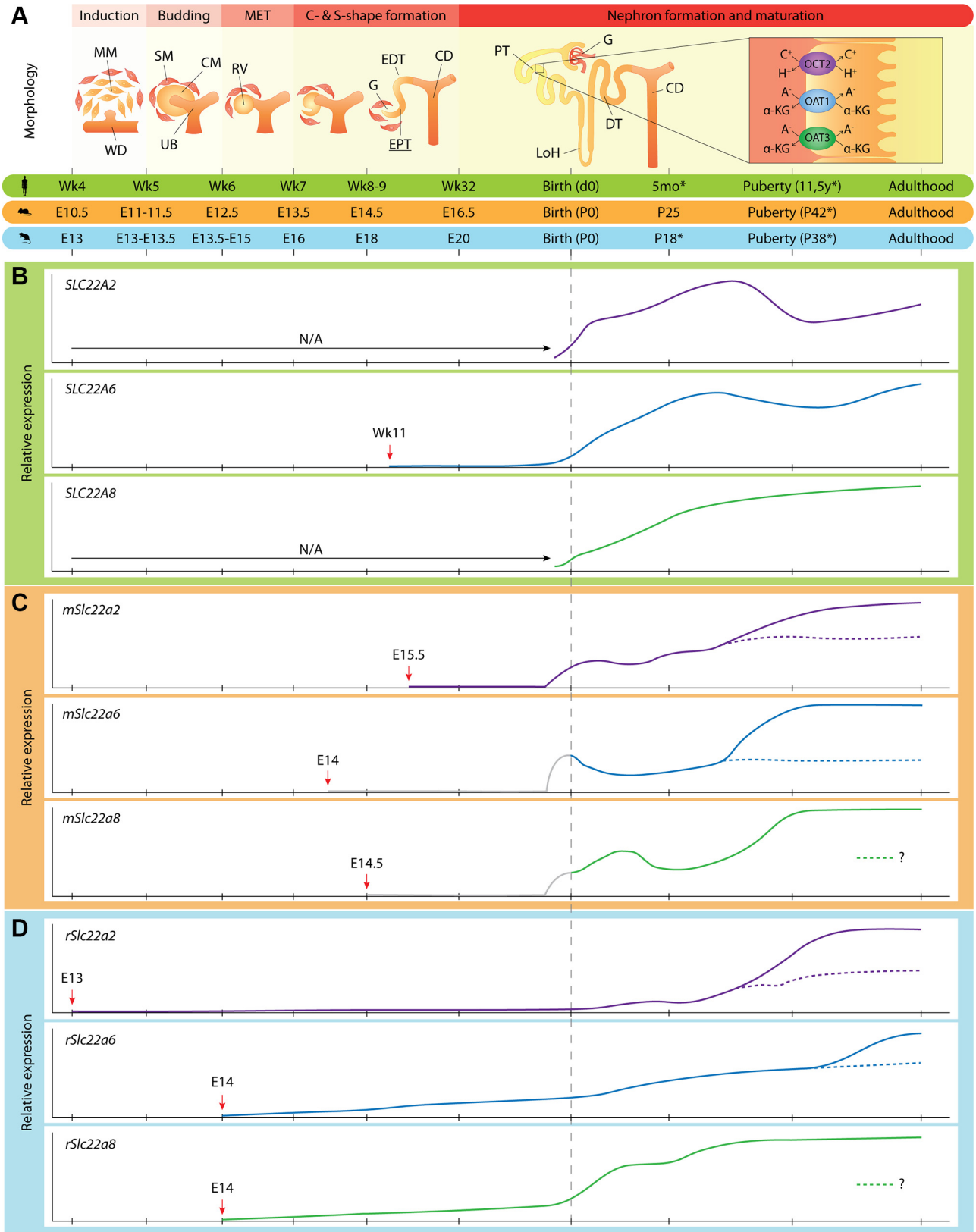

Table 3. ***Ontogeny of organic cation transporter 2 and organic anion transporters 1/3 in humans, mice, and rats***

Species	Time Point of Appearance	Technique	Reference
OCT2 (SLC22A2)			
Humans	?		
Mice	E15.5	RT-qPCR	(79)
Rats	E13	RT-qPCR	(77)
OAT1 (SLC22A6)			
Humans	Wk11	In situ hybridization	(76)
Mice	E14	In situ hybridization	(80)
	E14.5	RT-qPCR	(79)
	E15.5	Immunohistochemistry	(78)
Rats	E14	RT-qPCR	(77)
	E18	Northern blotting	(81)
	E19	Immunohistochemistry	(77)
OAT3 (SLC22A8)			
Humans	?		
Mice	E14.5	RT-qPCR	(79)
	E16	In situ hybridization	(80)
	E16	Immunohistochemistry	(78)
Rats	E14	RT-qPCR	(77)

E, embryonic day; Wk, week of life.

the nuclear receptors retinoic acid receptor (RAR) and retinoic X receptor (RXR), has been shown to coordinate pronephros segmentation and PT formation in zebrafish. Zebrafish with impaired RA signaling exhibit more distal than proximal segments, and this has been related to the activity of some major RA target genes, including *irx3b*, *hnf1b*, and *mecom* (65, 91–93). Both *irx3b* and *mecom* are negatively regulated by RA and are necessary for the differentiation of zebrafish kidney progenitors toward distal segments (91, 93). Conversely, *hnf1b* promotes PT formation in zebrafish embryos (65), which supports the findings in murine embryos. Induction of pronephros segmentation by RA has also been confirmed in *Xenopus* animal caps, which

FIGURE 7. Timeline of prenatal, perinatal, and postnatal kidney development (*A*) with the associated changes in expression of organic cation transporter 2 (OCT2), organic anion transporter 1 (OAT1), and organic anion transporter 3 (OAT3) in humans (*B*), mice (*C*), and rats (*D*). Dotted lines represent expression in females, and solid lines represent expression in males. Expression curves represent the average of the data obtained from GUDMAP (gudmap.org; Refs. 164, 165) and the studies by Cheung et al. (105), Oswald et al. (106), Breljak et al. (107), Basit et al. (109), Pavlova et al. (80), Lawrence et al. (79), Alnouti et al. (102), Buist et al. (103), Breljak et al. (108), Sweet et al. (77), Ahmadimoghaddam et al. (234), Nakajima et al. (81), Xu et al. (96), de Zwart et al. (99), Slitt et al. (101), Urakami et al. (114), Sweeney et al. (72), Tsigelny et al. (100), Buist et al. (97), Nomura et al. (98), Ljubojevic et al. (110), Cerrutti et al. (111), and Wegner et al. (112). Red arrows indicate the appearance of the first corresponding SLC transcripts. *Ages were converted for the different species using the approaches dictated by Dutta et al. (116) and Sengupta (117). α-KG, α-ketoglutarate; CD, collecting duct; CM, cap mesenchyme; DT, distal tubule; E, embryonic day; EDT, early distal tubule; EPT, early proximal tubule; G, glomerulus; LoH, loop of Henle; MM, metanephric mesenchyme; P, postnatal day; PT, proximal tubule; RV, renal vesicle; SM, stromal mesenchyme; UB, ureteric bud; WD, Wolffian duct; Wk, week of life; y, year of life.

start expressing markers such as *hnf1b*, *lhx1*, and *pax8* after treatment (88).

Martovetsky et al. (90) also identified other nuclear receptors and transcription factors presumably important for the differentiation of PTs: nuclear factor erythroid 2-related factor 2 (*Nrf2*), glucocorticoid receptor (*Nr3c1*), peroxisome proliferator-activated receptor α (*Ppara*), and tumor protein p53 (*Trp53*). Some of these have a role in the regulation of SLCs during adulthood and are discussed in sect. 3.3.1.

3.1.3.4. EPIGENETIC MODULATORS. Epigenetic modulators have shown to regulate *SLC* transcription in vitro, which is described in sect. 3.3.1.4, but to our knowledge only one study has reported the importance of epigenetics in PT formation and *SLC* induction during nephrogenesis. Liu et al. (94) found that in murine embryonic kidneys lacking both histone deacetylases 1 and 2 (encoded by *Hdac1* and *Hdac2*), there was a dramatic decrease in nephron progenitor cells, and even though they could observe renal vesicle formation, only sporadic comma- and s-shaped bodies developed. These mutant kidneys also had reduced expression of *Lhx1*, *Hnf1a*, *Hnf4a*, and members of the Notch signaling pathway such as *Dll1*, *Hes5*, *Hey1*, and *Jag1*. Notably, the double *Hdac1/2* knockout also led to reduced levels of *Slc22a6* (94). Nevertheless, this particular effect of acetylation on *SLC* expression could have been merely due to the fact that PT formation itself was already impaired by histone acetylation. Only conditional knockouts at a later stage of tubulogenesis would confirm whether *Hdac1/2* indeed specifically regulate *Slc22a6*.

In relation to the factors mentioned in sects. 3.1.3.1 and 3.1.3.2, this study also provided more insight on the relationship between HNFs and Notch during nephrogenesis. They found that *Lhx1* acts upstream of *Hnf1a* and *Hnf4a*, as well as many components of the Notch pathway, such as *Hes5* and *Dll1*, and proposed that *Lhx1* controls *Hnf1a/4a* expression via Notch signaling (94).

3.2. *Perinatal Expression of Kidney Solute Carriers*

In utero, the major role of the metanephros is the production of large volumes of urine for adequate fetal fluids, whereas waste products are mainly exchanged via the placenta for disposal through the maternal blood circulation (40). However, this drastically changes at birth when the neonate encounters radical changes in its environment. Suddenly, it depends on its own metabolism and excretion system to survive. It is thus unsurprising that within the first 2 wk of life the GFR doubles (95). During that time, the expression of *SLC*s is also subjected to a substantial change (**FIGURE 8**). Given that both GFR and *SLC* expression are negatively affected in CKD, one might question whether they are interrelated. It is not certain whether GFR affects *SLC* expression, but it is conceivable that the concomitant increase in tubular flow might signal PTECs, which are mechanosensitive,

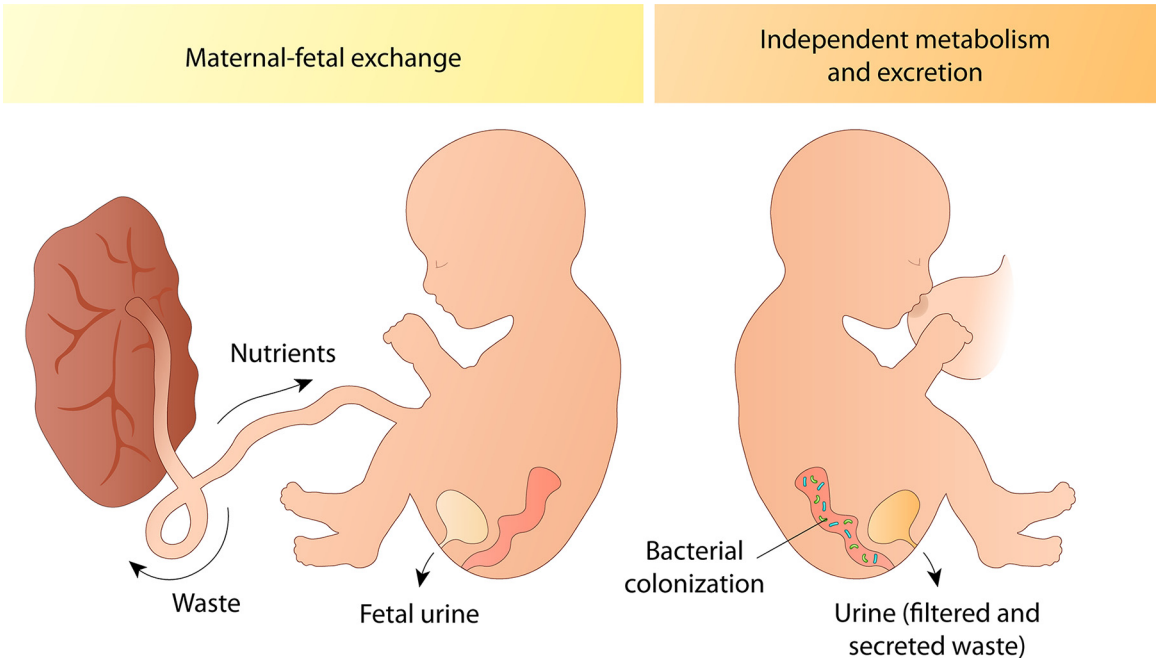

FIGURE 8. Differences in waste exposure and elimination in utero versus postpartum. Unborn children remove waste products mostly via the placenta, whereas upon birth and establishment of the gut microbiome, the child relies on independent metabolism and urinary waste excretion. This necessitates drastic changes, among others in the expression of kidney solute carriers (*SLC*s).

to upregulate *SLC* expression to compensate for the increased abundance of toxins and metabolites.

Although perinatal expression of OAT1/3 and OCT2 in humans remains to be investigated, in rodents (particularly in rats) perinatal expression has been well documented. With the exception of one study showing that *Slc22a6* levels in rats boosted at rat postnatal day 0 (rP0, birth) with even higher expression at birth than in adult rats (81), all studies agree that both *Slc22a6* and *Slc22a8* expression at rP0 is very similar to that at rE20–rE21. However, this expression significantly increases from rP7 to rP15 (72, 80, 96–100), indicating that it might take some days of adaptation for the kidneys to induce *SLC* transcription. The same pattern applies for *Slc22a2*, although this transporter might be upregulated less dramatically from rP7 to rP15 compared to *Slc22a6/8* (96, 99, 101) (**FIGURE 7**). In mice, perinatal (or early postnatal) *Slc* expression follows a different pattern. In case of *Slc22a2*, from murine postnatal day −2 (mP−2) to mP0, expression levels rise up to 25% of the adult levels in male mice and up to 93% of the adult levels found in females. From mP0 until early postnatal days, *Slc22a2* expression levels remain relatively constant (102). Regarding *Slc22a6/8*, it has been reported that mice at mP0 already express 30–40% the levels of these transporters found in adults and that these levels remain constant through early postnatal days (103) (**FIGURE 7**). This indicates that, similar to Oct2, Oat1/3 in mice are upregulated right at birth and thus earlier than in rats. The fact that nephrogenesis lasts a few days longer in rats than in mice might explain this phenomenon.

Although the exact timeline and magnitude of the observed increases in *SLC* expression depend on the species and the transporter, all studies support the hypothesis that SLCs are crucial for the adaptation of the body to the extrauterine environment. There is accumulating evidence that transporters and enzymes involved in drug and metabolite disposition communicate on an interorgan level (i.e., the gut-liver-kidney axis), or even on an interorganismal level (i.e., between gut microbiome and host), to sustain homeostasis. The so-called remote sensing and signaling hypothesis argues that small molecules (i.e., the to-be-excreted metabolites themselves, antioxidants and other signaling molecules) regulate the expression of relevant transporters and enzymes based on solute concentrations in the blood (35, 36). It is conceivable that intestinal bacterial colonization and increased metabolite concentrations in the blood induce, at least partly, the perinatal increase in SLC expression (104).

3.3. Postnatal Expression of Kidney SLCs

Postnatal expression of OCT2 and OAT1/3 in humans has only recently been investigated by Cheung et al. (105), who measured the expression in different age groups. According to this study, OAT1 levels increase from birth to childhood, followed by a slight decrease in adolescence and a prominent increase in adulthood (105). No sex differences have been detected in adult expression of human *SLC22A6* (106, 107). In mice, between birth (mP0) and mP30, *Slc22a6* expression remains constant, but at the latter time point expression levels start increasing more dramatically in males than in females. This results in adult male mice expressing from two- to fourfold higher *Slc22a6* expression levels than females (78, 103, 108, 109). A similar pattern can be observed in rats: *Slc22a6* expression keeps increasing from rP0 to the same extent for both sexes. However, after rP40, *Slc22a6* levels generally increase more in male rats than in females, resulting in adult females expressing 20–80% of the levels found in adult males, depending on the study (97, 99, 109–112). Of note, one study reported that adult female rats express around twofold higher levels than males (113), and two other studies reported no difference in expression between the sexes (114, 115). The variability in these results does not appear to be strain related (**FIGURE 7**).

OAT3 levels in humans increase in an escalated manner with age, reaching a peak in adulthood, where no sex differences in expression are observed (105–107). In mice, postnatal expression of *Slc22a8* remains unclear. Buist et al. (103) observed, just as for *Slc22a6*, that *Slc22a8* expression showed a sex-dependent difference at mP30 but that at mP40 no difference could be observed. In contrast, another study showed that adult female mice express around twofold higher levels of *Slc22a8* than male mice (108). The same discrepancies can be seen in the literature with *Slc22a8* expression in rats: one study showed no difference in Oat3 adult levels between male and female rats (97), other studies have noted that adult female rats express levels of Oat3 that are ~1.5-fold higher than the levels found in males (99, 113, 115), and others showed that female rats express ~66% of the levels found in males (110, 112). Based on these data, more research is needed to confirm sex-specific expression levels of *Slc22a8* in rodents (**FIGURE 7**).

Finally, human OCT2 levels have been shown to reach a peak in childhood and drop significantly in adolescence, after which they slightly recover in adulthood (105). Once again, human *SLC22A2* expression has not been reported to be sex dependent (106). In mice, however, after mP30 males start expressing around twice as much *Slc22a2* levels as adult female mice (102, 109). Similarly, after rP30 male rats express from 1.3- to 5-fold higher *Slc22a2* levels than females (99, 101, 109, 114). Only one study found no differences in rat *Slc22a2* expression between the sexes in adulthood (113) (**FIGURE 7**).

In conclusion, puberty has a great impact on *SLC* expression during postnatal development but, as far as we know, only in rodents: mice and rats show a sex-dependent difference in *Slc* expression on average around mP42 and rP38, which corresponds to puberty (116, 117). Sex dependence differences between rodents and humans is further discussed in sects. 3.3.1.2 and 3.3.1.3.

3.3.1. Molecules involved in postnatal SLC expression regulation.

Postnatal regulation of *SLC*s might not necessarily be driven by the same factors that direct PT differentiation and SLC induction. One example of such divergence is Notch signaling. Once nephrogenesis is completed, the Notch pathway remains mostly silenced during adulthood and only gets reactivated in kidney disorders, which might result from cellular dedifferentiation (118). In contrast, HNFs and other nuclear receptors continue playing an important role in modulating adult kidney SLC expression.

3.3.1.1. HEPATOCYTE NUCLEAR FACTORS. Besides a role in promoting *SLC* induction during nephrogenesis, HNFs modulate SLC expression in adult animals and in vitro. Numerous studies have noted that HNF1α, HNF4α, and HNF1β bind to the promoter regions of *SLC22A6* and *SLC22A8* and modulate their activity and expression (89, 90, 119–123). As can be seen in **TABLE 4**, the effect of HNFs on SLC expression is very much dependent on the cell model employed, which could be explained by the fact that certain cell models (e.g., OK cells) might have some endogenous SLC expression (124, 125) and others (e.g., HEK293) do not. Nonetheless, the majority of studies indicate that HNF1α, HNF4α, and HNF1β positively regulate *SLC22A6* and *SLC22A8* expression postnatally, either alone or in combination. Interestingly, one study found that the induction of *SLC22A6* by overexpression of *Hnf1a* and *Hnf4a* can be inhibited by coexpressing *Gata4* or *Foxa2*/3, which leads to the upregulation of hepatocyte markers instead (123). This shows that for HNFs to induce a desired phenotype (e.g., a mature PT), one must avoid the presence of repressor molecules. In addition, given the variety of effects in different cell systems, there might also be a need to find crucial coactivators for HNFs to effectively increase *SLC* expression.

Table 4. ***Hepatocyte nuclear factor overexpression/knockout approaches performed to modulate adult renal uptake transporter expression in vivo and in vitro***

Gene	Approach	(Animal) Model	Effect on Transporter	Reference
HNF1A/Hnf1a	Knockout	Mice	↓ *Slc22a6* expression	(119, 120)
			↓ *Slc22a8* expression	(119)
			↑ *Slc22a2* expression	
	Overexpression	MEFs	Unchanged *Slc22a6* expression	(90, 123)
	Overexpression	HEK293 cells	↑ *SLC22A6* promoter activity	(120)
	Overexpression	HEK293 cells	↑ *SLC22A8* promoter activity	(122)
	Overexpression	OK cells	Unchanged *SLC22A6* promoter activity	(121)
HNF1B	Overexpression	HEK293 cells	↑ *SLC22A6* promoter activity	(120)
	Overexpression	HEK293 cells	↑ *SLC22A8* promoter activity	(122)
	Overexpression	OK cells	Unchanged *SLC22A6* promoter activity	(121)
HNF4A/Hnf4a	Pharmacological antagonism	MEFs	↓ *Slc22a2, Slc22a6, and Slc22a8* expression	(90)
	Overexpression	MEFs	Unchanged *Slc22a6* expression	(90, 123)
	Overexpression	OK cells	↑ *Slc22a6* promoter activity	(121)
Hnf1/4a	Concomitant overexpression	MEFs	↑ *Slc22a6* expression	(90, 123)

MEFs, mouse embryonic fibroblasts; OK, opossum kidney.

As for *SLC22A2*, its adult regulation by HNFs has been the least studied. One study reported that the pharmacological antagonism of Hnf4α led to reduced levels of *Slc22a2* in vitro (90), and another study showed increased mRNA levels upon Hnf1α knockout in mice (119). This implies that *SLC22A2* is, just like *SLC22A6/8*, positively regulated by HNF4α but, contrary to the other two *SLCs*, negatively regulated by HNF1α. On one hand, this seems unlikely given that HNF1α and HNF4α are very tightly related and in many cases promote each other's expression (126–129). On the other hand, a study using conditionally immortalized PTECs (ciPTECs) overexpressing OAT1 or OAT3 showed an associated downregulation of endogenous OCT2 (130). Given these discrepancies, more research should be carried out to unravel the effect of HNFs on *SLC22A2* transcription and the relation to *SLC22A6/8* expression.

3.3.1.2. SEX HORMONES. Given that puberty noticeably affects *Slc* expression in rodents, sex hormones are bound to be a major player driving regulation. This has indeed been confirmed both in vivo and in vitro. Gonadectomy in male mice and rats results in a decrease in mRNA expression and protein levels of Oct, and Oat1 compared with control rats, whereas in females it has no effect (101, 102, 108, 110, 131, 132). Treatment of gonadectomized male rodents with testosterone rescues *Slc22a2* and *Slc22a6* expression, whereas treatment with estradiol has shown no effect or even a further decrease in expression (102, 108, 110). Supporting these findings, testosterone increases the levels of *Slc22a2* in healthy male mice and both in male and female rats, and estradiol significantly decreases them in male rodents (133, 134). Regarding *Slc22a8*, only one study reported that gonadectomy increased Oat3 protein levels in male mice and that treatment with testosterone downregulated *Slc22a8* expression (108). This would imply that *Slc22a8* is regulated in the opposite manner as *Slc22a2* or *Slc22a6*, which seems rather unlikely. Being paralogs, *Slc22a6* and *Slc22a8* are expected to be concomitantly expressed in different tissues and sexes (25).

In conclusion, androgens have a positive effect on Slc expression, but this effect is most likely rodent specific. In humans, expression of *SLC22A2/6/8* is independent of sex (106, 107) and hence unlikely to be regulated by androgens. To our knowledge, only one study has been performed investigating the effect of estradiol on the human *SLC22A6* promoter activity in vitro. Here, estradiol increased *SLC22A6* transcription through activation of the estrogen receptor and the cofactors core binding factor (*CBF*) and heterogeneous nuclear ribonucleoprotein K (*HNRNPK*) (135). However, more studies are needed to confirm this.

As a side note, in contrast to SLCs, sexual dimorphism in transporter abundancy has been shown for human BCRP (*ABCG2*), which is actively involved in steroid metabolism (136). For other ABC transporters, in both humans and rodents, sex differences are less pronounced, contradictory, or absent (113, 137–139).

3.3.1.3. OTHER NUCLEAR RECEPTORS AND TRANSCRIPTION FACTORS. Apart from the regulatory role of sex hormones, the transcription factor B cell lymphoma 6 (Bcl6) has also been proposed to have a role in the sex-dependent expression of *Slc22a6/8* in rats. In conjunction with Hnf1α, Bcl6 is able to bind to the promoter regions of *Slc22a6/8* and activate their expression (112, 140). In contrast to *Hnf1a*, *Bcl6* is more highly expressed in male rats than in females, which might explain sex-dependent differences in *Slc* expression, in addition to the effect exerted by sex hormones (112, 113). However, whether Bcl6 and sex hormones act through a shared pathway has not yet been investigated.

There are also several nuclear receptors that contribute to the regulation of kidney SLCs postnatally, including the liver X receptor (LXR), the vitamin D receptor (VDR), PPAR, the estrogen-related receptor α (ESRRA), and the glucocorticoid receptor. Of note, activation of LXR has only been shown to reduce protein levels of both OCT2 and OAT1 in vitro, whereas mRNA levels of *SLC22A2* remain unchanged (*SLC22A6* levels were not measured). Thus, LXR might have an effect on SLCs posttranslationally (141, 142). VDR also appears to be a negative regulator of *SLC* expression, as activation by vitamin D3 leads to reduced expression levels of both *Slc22a6* and *Slc22a8* in rats. Furthermore, vitamin D3 can downregulate the expression of several transcription factors including *Hnf1a* and *Hnf4a*, which might mediate the subsequent reduction in *Slc* expression (143, 144).

Contrary to LXR and VDR, PPAR, ESRRA, and the glucocorticoid receptor have shown positive effects on Slc expression and function. In a study performed in rats with hyperuricemic nephropathy, *Slc22a6/8* levels were decreased and could be recovered by treatment with the Pparγ agonist rosiglitazone (145). Similarly, knockout of *Ppara* in mice downregulated *Slc22a2* expression, and treatment of control mice with the Pparα agonist gemfibrozil increased expression (146). Recently, a study demonstrated that the knockdown of *Esrra* in mice caused the downregulation of *Slc22a6* expression and the overexpression of *Esrra* increased *Slc22a6* levels (147). Regarding the effect of the glucocorticoid receptor, treatment of Madin-Darby canine kidney (MDCK) cells with the agonists dexamethasone and hydrocortisone

increased endogenous *SLC22A2* expression (148), and dexamethasone dose-dependently increased *para*-aminohippurate excretion in young rats, which is predominantly mediated by Oat1 and, to a lesser extent, Oat3 (149–151).

3.3.1.4. EPIGENETIC MODULATORS. Modulation of either DNA methylation or acetylation has notable effects on the transcription of many transporters in different tissues, including *SLC*s (152). For kidney *SLC22A2*, and *SLC22A6/8*, methylation has a negative effect on expression, while acetylation could be beneficial.

In a variety of in vitro studies, expression inhibition by DNA methylation has been described for *SLC22A2* and *SLC22A8* by impairing the binding of transcription factors to their promoter regions (122, 153, 154). DNA demethylating agents, such as decitabine, have therefore been used to induce or increase transporter expression in vitro (122, 154, 155). For *SLC22A2*, promoter methylation has shown to dramatically reduce transcription by inhibiting promoter transactivation by the upstream transcription factor 1 (USF1) or MYC (153, 154). Chen et al. (155) contributed to these findings by unraveling the role of hypoxia in the methylation capacity of *SLC22A2*: decitabine was found unable to demethylate DNA under hypoxic conditions and thus could not increase *SLC22A2* expression. This was attributed to the fact that hypoxia blocks the uptake of decitabine and reduces expression levels of methylating enzymes (155).

In the case of *SLC22A8*, depending on the cell line, DNA demethylation only increases expression in combination with *HNF1a* transfection or with *HNF1a* and *HNF1b* cotransfection, supporting the importance of HNFs (122). For *SLC22A6,* the effect of demethylating agents has not been explored, but there is indirect evidence pointing toward a negative effect of methylation on *SLC22A6* expression: when *Hnf4a* was knocked out in murine livers, the authors could detect higher levels of methylating enzymes such as *Dnmt1* and *Tet3*, together with higher levels of methylation at the locus in *Slc22a6* exon 1, compared with wild-type mice (156). As discussed above, *HNF4A* is a positive regulator of *SLC22A6*, which implies that hypermethylation resulting from the absence of Hnf4α impairs *SLC22A6* expression. Furthermore, another study demonstrated that the CpGs found downstream of the transcriptional starting site of *SLC22A6/8* are more methylated in the liver than in the kidneys (157). This coincides with the fact that both *SLC22A6/8* are highly expressed in the kidneys and absent in the liver (16).

Contrarily to methylation, acetylation has shown to increase transcription of *SLC22A2*. By blocking histone deacetylation with HDAC inhibitors, *SLC22A2* expression has been successfully induced in vitro, and this process has been found to be MYC dependent (155, 158). In a comparable manner as for methylation, hypoxia appears to negatively regulate *SLC22A2* expression by upregulating HDACs (155). Concerning *SLC22A6/8*, the effect of acetylation on their expression remains to be elucidated.

3.3.1.5. MIRNAS. Several studies have shown downregulation of *SLC*s by a variety of microRNAs (miRNAs). miR-21 has been found to negatively regulate transcription of *Slc22a2* and *Slc22a6/8* in mice and *SLC22A6* in primary PTECs. Moreover, miR-21 significantly affected the expression levels of *Ppara*, suggesting that the observed effects on transporter expression are given through *Ppara* regulation (159, 160). Two other miRNAs, miR-489-3p and miR-630, have been shown to downregulate OCT2 mRNA and protein levels in vitro (161). Finally, miR-223 was found not to affect *SLC22A6* expression by itself but to inhibit the indoxyl sulfate-dependent upregulation of this transporter (34).

3.3.1.6. GUT-DERIVED METABOLITES. Finally, our group recently proposed an upregulating effect on SLC expression for gut-derived metabolites within the framework of the remote sensing and signaling hypothesis (34, 35). Essentially, metabolite-induced SLC upregulation (as shown for indoxyl sulfate and *SLC22A6*) could be a regulatory mechanism in the adult kidney to maintain plasma levels upon increased metabolite production. This sensing and signaling pathway involves, among others, activation of epidermal growth factor receptor (EGFR) and the aryl hydrocarbon receptor (AhR) (34, 162). It is conceivable that this remote sensing system is for the first time activated upon a sudden increase in functional load after birth, and it remains active in adulthood to counteract daily food-induced fluctuations in plasma concentrations (104, 163).

3.4. Considerations on Transcriptional Targeting Approaches

A variety of molecules are putatively involved in the transcriptional regulation of *SLC22A2* and *SLC22A6/8* during nephrogenesis, postnatally and in vitro, as schematically summarized in **FIGURE 9, *A–C***, for each transporter, respectively. To assert which molecules might contribute to the induction of *Slc22a2* and *Slc22a6*/8, we report their appearance in time during nephrogenesis and expression in adult PTs, which is depicted in **FIGURE 9*D***. Here, we considered appearance during nephrogenesis only in the cell lineage developing into PT (i.e., MM, renal vesicle, s-shaped body, and PT), using murine data derived from the GUDMAP database (164, 165). Expression in the adult PT

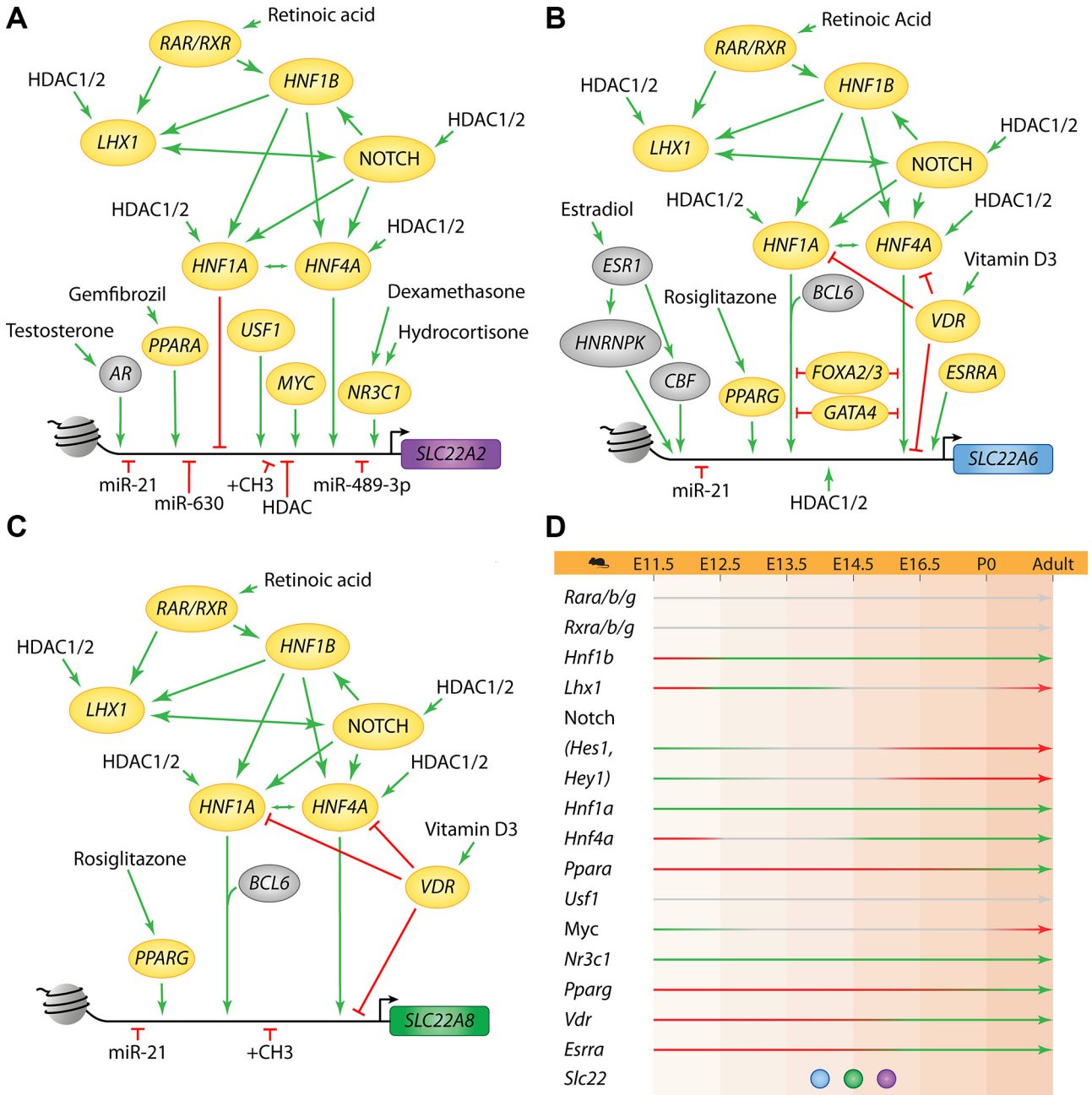

FIGURE 9. Pathways and molecules found to modulate kidney *SLC22A2* (*A*), *SLC22A6* (*B*), and *SLC22A8* (*C*) transcription and their expression timelines (*D*). *A–C*: green arrows indicate a positive induction; red lines indicate inhibitory actions. Gray molecules indicate a (possible) species-dependent involvement. *D*: expression levels of the different genes from embryonic day (E)11.5 to postnatal day (P)0 were based on murine data, whereas expression levels in adults were taken from human data. Green arrow, expression; red arrow, no expression; gray arrow, expression unclear. Dots at *bottom* indicate the time point of induction of *SLC22A2* (purple), *SLC22A6* (blue), and *SLC22A8* (green). AR, androgen receptor; BCL6, B-cell lymphoma 6; CBF, core binding factor; ESR1, estrogen receptor 1; ESRRA, estrogen-related receptor α; FOXA2/3, Forkhead Box A2/3; GATA4, GATA binding protein 4; HDAC(1/2), histone deacetylases (1/2); HNF1A/B: hepatocyte nuclear factor 1α/β, HNF4A: hepatocyte nuclear factor 4α; HNRNPK, heterogeneous nuclear ribonucleoprotein K; LHX1, LIM homeobox 1; MYC, Myc protooncogene; NR3C1, nuclear receptor subfamily 3 group C member 1; PPARA/G, peroxisome proliferator-activated receptor α/γ; RAR, retinoic acid receptor; RXR, retinoic X receptor; SLC22A2/6/8, solute carrier 22A2/6/8; USF1, upstream stimulatory factor 1; VDR, vitamin D receptor; +CH3: methylation.

was specified by means of the Human Protein Atlas (16) and the single-cell RNA sequencing data sets by Wu et al. (166) and Humphreys (167), which were accessed through the transcriptomic database generated by the group of B. Humphreys (168).

Being involved in tubulogenesis, Notch (as shown with target genes *Hes1* and *Hey1*) is active as early as mE11.5, but becomes silenced in PTs after mE15.5, the point at which all SLCs have already appeared. Similarly, *Lhx1* and *Myc* are expressed during early nephrogenesis but

eventually disappear in the adult PT. It is conceivable that these determinants of PT fate acquisition predispose the cells for the expression of SLCs but they are insufficient to induce transcription. Interestingly, Slc induction occurs together with the appearance of *Hnf4a* and *Esrra*. This makes the latter prime candidates for Slc induction and maintenance, but conditional knockout studies are needed to confirm their actual necessity. *Hnf1a/b* and *Nr3c1* are expressed throughout development and adulthood, possibly acting as the basal transcription factors required for Slc expression. The nuclear receptors *Ppara* and *Pparg* appear in PTs only after birth, which implies a potential regulatory role postpartum rather than an inducing one. Finally, for *Rara/b/g*, *Rxra/b/g*, and *Usf1*, expression patterns over time are unclear; thus we cannot confirm their importance in Slc induction (**FIGURE 9*D***).

We would like to highlight that time relations in expression levels alone cannot prove any causal relations. First, transcription factors needed for PT maturation might not necessarily be linked to *SLC* induction. Second, transcription factors that appear after SLC induction might not be essential for induction but might have a major role in regulating their expression. Third, some of these transcription factors might be individually insufficient and require either the presence of coactivating factors or the absence of repressing factors. This overview might not be complete and merely serves as an information toolbox to summarize current knowledge and direct future research. Scientific evidence has been partly controversial, as discussed in the respective sections above (sects. 3.1-3.3). Furthermore, different techniques (e.g., knockout and overexpression experiments, in situ hybridization) each bring their own advantages and disadvantages, which should be carefully considered during interpretation. For instance, knockout experiments can affect all cell types, including putative supporting cell types if a nonconditional knockout approach is used, and these methods are often accompanied by the induction of compensatory mechanisms. In parallel, overexpression systems might not properly reflect endogenous expression regulation mechanisms. Finally, interspecies differences, as observed with the effect of sex hormones on SLC expression, should always be considered.

4. FROM PHYSIOLOGY TO PHARMACOLOGY: A CELL DYNAMIC PERSPECTIVE ON KIDNEY SOLUTE CARRIERS

The lack of SLC expression in vitro is mostly due to impaired transcription, and successfully inducing mRNA expression would be a paramount step. In vivo, mRNA levels decrease in disease, but cell dynamic processes form another regulatory layer, which might be easier to target for short-term beneficial effects. Transport functionality is constantly altered by environmental stimuli: endogenous and exogenous substances cause shifts in posttranslational modifications that determine transporter protein levels at the cell membrane. By pharmacologically interfering with these physiological processes, changes in transport functionality can be reached much faster than with gene-based regulation.

4.1. Physiological Cell Dynamics: Transporter Phosphorylation

To facilitate rapid expression changes, OAT1/3 undergo heavy trafficking between the cell surface and intracellular compartments; it is estimated that 10% of OAT1 protein is internalized and recycled every 5 min (169). As a consequence, steady-state distribution of OAT1/3 can shift almost immediately, e.g., upon meal intake. Duan and You (170) reviewed mechanisms involved in OAT short-term regulation. Also for organic cation transport, several short-term regulation mechanisms have been reported as the reaction to increased amounts of catalytic end products, which were summarized by Ciarimboli and Schlatter (171).

For all kidney solute carriers, the extracellular loop between transmembrane 1 and 2 is important for membrane targeting and stability, whereas the intracellular loop between transmembrane domains 6 and 7 and the carboxy terminus contains putative phosphorylation sites for a possible modification of the transport protein. The attachment of a negatively charged phosphate group is a key mechanism by which quick changes in protein activity can be induced.

4.1.1. Phosphorylation of OCT2.

Human OCT2 possesses several potential protein kinase C (PKC), protein kinase A (PKA), and tyrosine kinase phosphorylation sites in the intracellular loop and carboxy terminus (171, 172). Notably, studies by Ciarimboli and Schlatter's research group showed that regulation of organic cation uptake highly depends on transporter isoform, tissue, sex, and species/expression system (171, 173, 174). Thus, the effect of phosphorylation on OCT2 can be opposing in different systems. According to the authors, this might be caused by divergent activity levels of various involved pathways. For example, PKC has no role in OCT2 regulation in transfected HEK293 cells, whereas isolated human and mouse PTs suggest PKC-mediated uptake inhibition (173–175). In contrast, in isolated rabbit PTs, PKC increases OCT2 activity (176). With regard to cAMP-

dependent PKA, there is an induced downregulation of OCT2 activity in isolated human PTs and HEK293 cells transfected with human OCT2 but an induced activation in isolated mouse PTs and HEK293 cells transfected with rat OCT2 (173–175).

The only truly conserved regulatory pathway is the Ca^{2+}-calmodulin (CaM) signaling pathway: Ca^{2+}-activated CaM keeps endogenous OCT2 functionality up, probably through direct phosphorylation as well as indirect phosphorylation through CaM kinase II and myosin light chain kinase (MLCK). Inhibition of CaM reduces the affinity of OCT2 substrates, suggesting a phosphorylation-induced conformational change that affects the OCT2 binding site. Interestingly, CaM is more highly expressed in isolated PTs from male rats compared with female rats, and CaM inhibition only affected OCT2 activity in male PTs (177). This sex-dependent difference in OCT2 regulation might provide an additional explanation for the sex-dependent differences in *Slc22a2* gene expression discussed above, which were specific to rodents.

Independently of CaM signaling, activation of G protein-coupled receptor signaling inhibits OCT2. This pathway is likely driven through phosphatidylinositol 3-kinase (PI3K), although the subsequent mitogen-activated protein kinase (MAPK) pathway is only involved in OCT2 regulation in rats (174, 177). Also, for cGMP an inhibitory effect has been observed (178). Most recently, tyrosine protein kinase Yes1, tethered to the cell membrane, has been shown to be strongly involved in OCT2 regulation; also, kinase insert domain receptor (KDR) and Lck/Yes novel tyrosine kinase (LYN) were proposed candidates based on a >75% OCT2 inhibition when the respective kinase-targeting siRNA was applied (179). Based on this and Ciarimboli and Schlatter's review (171), **FIGURE 10*A*** provides an overview of regulatory pathways involved in the posttranslational regulation of human OCT2.

4.1.2. Phosphorylation of organic anion transporters 1 and 3.

For OATs, okadaic acid has been shown to decrease transport activity by inhibiting the removal of a phosphate group from a serine residue by phosphatases (PP1/PP2A) (180). In other words, in contrast to OCT2, an increased phosphorylation state decreases OAT activity. Duan and You (170) hypothesized that a negatively charged phosphate group could dampen the affinity of the negatively charged counterion α-ketoglutarate or induce a conformational change that reduces OAT affinity. This finding implies a regulatory role for OAT phosphorylation, which is supported by two papers that studied the possible involvement of CaM kinase II and PKA, respectively (181, 182). However, despite the presence of phosphorylation sites, evidence for physiological relevance is still limited. For instance, mutagenic changes in four putative PKC consensus sites did not affect transport activity of OAT1 (183). On the other hand, phosphorylation can also occur outside conventional motifs; in short, the extent to which protein kinases directly control OAT activity remains to be elucidated (182). In contrast, the effect of PKC and PKA on OAT membrane trafficking is better understood.

4.2. *Physiological Cell Dynamics: Membrane Trafficking*

Transporter phosphorylation is undoubtedly a key regulatory mechanism, but oftentimes intermediate targets are involved. Especially for organic anion uptake, PKC and PKA are key players with opposite indirect effects on transporter membrane trafficking through a dynamic cross talk between ubiquitination and SUMOylation.

4.2.1. Decreased organic anion uptake through ubiquitination.

Since 1994 it has been known that PKC activation inhibits the uptake of organic anions. Among others, this could be evoked by angiotensin II, bradykinin, diacylglycerol, parathyroid hormone (PTH), phenylephrine, phorbol esters, and progesterone (149, 176, 184–186). PKC activation results in transporter retrieval from the cell membrane through a dynamin- and clathrin-dependent pathway and ubiquitination (169, 187, 188). Ubiquitination is an enzymatic modification of a target protein, in this case OAT1/3, through covalent attachment of a ubiquitin protein by substrate recognizing E3 ubiquitin ligases. Ubiquitination leads to endosomal protein internalization, but different ubiquitin linkages can result in different outcomes: the target protein can be either deubiquitinated and recycled back to the plasma membrane or sorted for lysosomal degradation. The research group led by G. You has significantly contributed to our current knowledge on OAT1/3 trafficking and has summarized their findings in a comprehensive review (189). A central role is given to E3 ubiquitin ligase Nedd4-2: PKC directly phosphorylates Nedd4-2, which subsequently interacts with both OAT1 and OAT3 to induce their ubiquitination (187–191) (**FIGURE 10*B***). It should be noted that PKC subtypes can affect OAT expression differentially: the above-described mechanisms are predominantly ascribed to conventional PKC-α, whereas PKC-γ, -ε, and -ζ showed opposite effects by increasing OAT trafficking toward the cell membrane (192, 193). A possible explanation follows below.

Interestingly, serum- and glucocorticoid-inducible kinase 2 (sgk2) also regulates OAT activity via Nedd4-2,

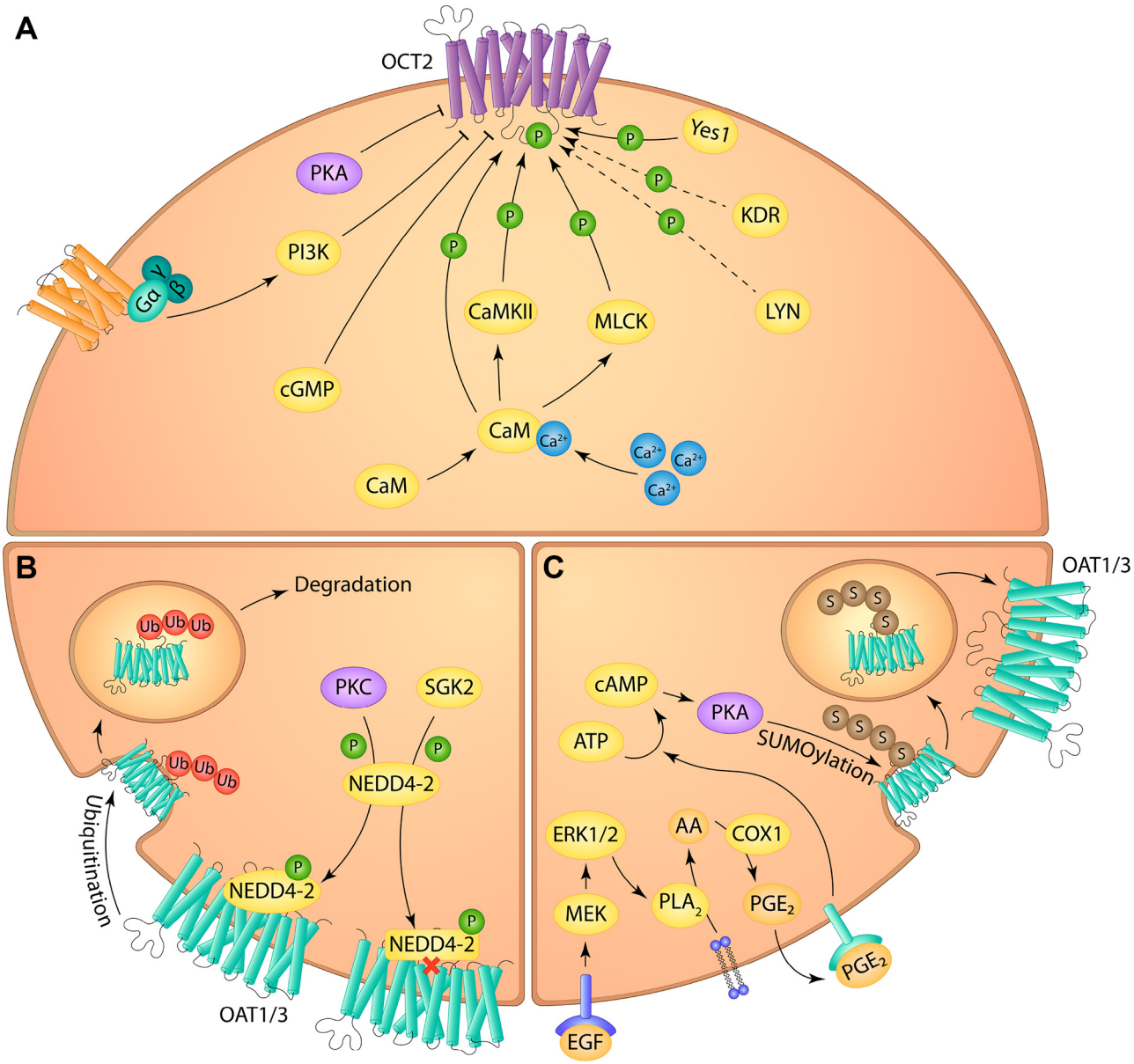

FIGURE 10. Posttranslational regulation pathways of kidney organic cation transporter 2 (OCT2) (*A*) and organic anion transporters 1/3 (OAT1/3) (*B* and *C*). Rapid changes in transporter membrane expression are regulated through phosphorylation. OCT2 is directly phosphorylated by multiple kinases, whereas OAT1/3 are predominantly regulated indirectly through protein kinase C (PKC) and A (PKA), which influence membrane trafficking through ubiquitination and SUMOylation, respectively. Dotted lines represent putative interactions. AA, arachidonic acid; ATP, adenosine triphosphate; CaM, calmodulin; cAMP, cyclic adenosine monophosphate; CaMKII, calmodulin kinase II; cGMP, cyclic guanosine monophosphate; COX1, cyclooxygenase 1; EGF, epidermal growth factor; ERK1/2, extracellular signal-regulated kinase 1/2; KDR, kinase insert domain receptor; LYN, Lck/Yes novel tyrosine kinase; MEK, mitogen-activated protein kinase kinase; MLCK, myosin light chain kinase; NEDD4-2, Ubiquitin Ligase Neural Precursor Cell Expressed Developmentally Down-Regulated 4-like; P, phosphoryl group; PGE_2: prostaglandin E_2; PI3K, phosphatidylinositol 3-kinase; PLA_2, phospholipase A_2; S, small ubiquitin-like modifier (SUMO); SGK2, serum- and glucocorticoid-inducible kinase 2; Ub, ubiquitin; Yes1, tyrosine kinase Yes1.

but with an opposite effect: Sgk2 inhibits Nedd4-2 and hence suppresses transporter internalization (189, 194).

4.2.2. Increased organic anion uptake through SUMOylation.

In contrast to (conventional) PKC, cAMP-dependent PKA has been shown to short-term stimulate renal organic anion uptake (195). In a series of successive publications using different kidney in vitro systems, Sauvant et al. (125, 196–198) linked epidermal growth factor (EGF)-induced OAT stimulation to PKA and described the following events: EGF stimulates the mitogen-activated protein kinases MEK and ERK1/2; tyrosine kinase, phosphatidylinositol-3-kinase (PI3K), and AKT have also been shown to be involved in this step (162, 199). ERK1/2 then activates downstream phospholipase A_2 (PLA_2), leading to an increased release of arachidonic acid (AA), which

is metabolized by cyclooxygenase 1 (COX1) into prostaglandin E_2 (PGE_2). PGE_2 is thought to bind to its receptor EP4 for a G protein-mediated activation of adenylate cyclase (cAMP), which activates PKA and results in OAT upregulation (**FIGURE 10C**). Continuous and excessive PGE_2 production, however, leads to a transcriptional downregulation (200). Interestingly, inhibition of the aforementioned PKC-ζ blocked the EGF-induced increase in organic anion uptake, suggesting that atypical PKCs act downstream of the PKA signaling pathway (192).

Although the OAT-inducing effect of cAMP and PKA was known for almost two decades, the underlying mechanism was, until recently, a mystery. Interestingly, cAMP-response element (CRE) is a key player in the transcriptional regulation of OAT3: CRE-binding protein (CREB)-1 and activating transcription factor (ATF)-1 constitutively regulate *SLC22A8* expression via binding to CRE. PKA has been shown to phosphorylate CREB-1 and ATF-1, which could enhance *SLC22A8* expression (201). However, cAMP and PKA are effective within minutes, suggesting that there is also a posttranslational mechanism at play (197).

In 2019, it was found that PKA specifically increases OAT protein levels at the cell membrane by promoting OAT3 SUMOylation: the covalent attachment of small ubiquitin-like modifiers (SUMOs) to lysine residues via an enzymatic cascade similar but not identical to ubiquitination (202, 203). Whereas PKC-dependent ubiquitination accelerates OAT3 internalization, SUMOylation accelerates OAT3 recycling to the cell membrane. Accordingly, in vivo knockdown of the SUMO-specific protease SENP2 leads to more OAT3 SUMOylation and thereby to higher protein levels and transport activity, whereas SENP2 overexpression in vitro decreases SUMOylation and transport activity (204).

Interestingly, increased OAT3 SUMOylation correlates with reduced OAT3 ubiquitination, suggesting a competitive targeting of the same OAT lysine residues (202). Thus, PKC and PKA enforce opposite fates on OATs at the cell membrane through opposing posttranslational modification mechanisms.

5. FROM KNOWLEDGE TO APPLICATION: GETTING HOLD OF KIDNEY SOLUTE CARRIERS IN VITRO AND IN DISEASE

Using phylogenetic, ontogenetic, and cell dynamic perspectives, we have presented various opportunities to get hold of the expression of SLCs in kidney PTs. However, to improve current in vitro models and to advance therapies, knowledge must be translated into new paradigms, better in vitro culture conditions, and therapeutic applications.

5.1. Stress: A New Paradigm?

Intriguingly, SLC expression seems to be closely linked to environmental stress factors, which have been a recurrent topic in all sections. First, continuous mismatches between evolutionary solutions and newly arising problems forced the kidney into establishing remarkable but energetically inefficient mechanisms, which become detrimental during acute stress situations and age-dependent degeneration. Second, although induced prenatally, SLC expression is truly unfolded perinatally, during a phase of extreme metabolic changes, including the exposure to gut-derived metabolites. Third, in the adult kidney SLC expression is highly dynamic to allow quick adaptations to metabolite fluctuations and other cell dynamic changes. Moreover, epigenetic mechanisms enable environmental stimuli to switch genes on or off, and it has been shown that oxidative stress can induce epigenetic reprogramming (205). Could stress control be the key to kidney SLC induction and preservation?

Short-term low-level stress can promote transport activity, e.g., through increased plasma levels of gut-derived metabolites or activation of the COX1/PGE_2 cascade (34, 198). In line with this, cellular stress has been shown to cause a massive increase in SUMOylation, which promotes OAT trafficking to the cell membrane (203). On the other hand, high or chronic stress downregulates transporters and should hence be avoided. Seizing on Chevalier's hypothesis of energy investment strategies (discussed in sect. 2), it might be possible that high and prolonged stress shift energetic priority to the maintenance of salt and nutrient reabsorption for homeostasis and cellular survival. In such conditions, active secretion might be of less importance because of the presence of glomerular filtration and can even turn out to be detrimental because of the influx of harmful substances. The glucose and peptide reabsorption transporters SGLT2 and PEPT2 have been shown to be downregulated only after 16 wk of 5/6 nephrectomy in rats. In contrast, functional downregulation of OCT2 and OAT1/3 has been observed within 2 wk (206–208). Moreover, although kidney injury causes lower expression of OCT2 and OAT1/3, expression of apical ATP-binding cassette (ABC) transporters remains unchanged or even gets upregulated (209–211). The apparent difference in basolateral SLC and apical ABC transporter regulation might hint toward a protective response against nephrotoxicity: cellular uptake of harmful substances is depressed, whereas cellular excretion is promoted (**FIGURE 11**).

5.2. Cell Culture: New Molecular Targets?

Also in vitro, stress drives cell behavior: when brought into culture, kidney cells not only lose SLC expression

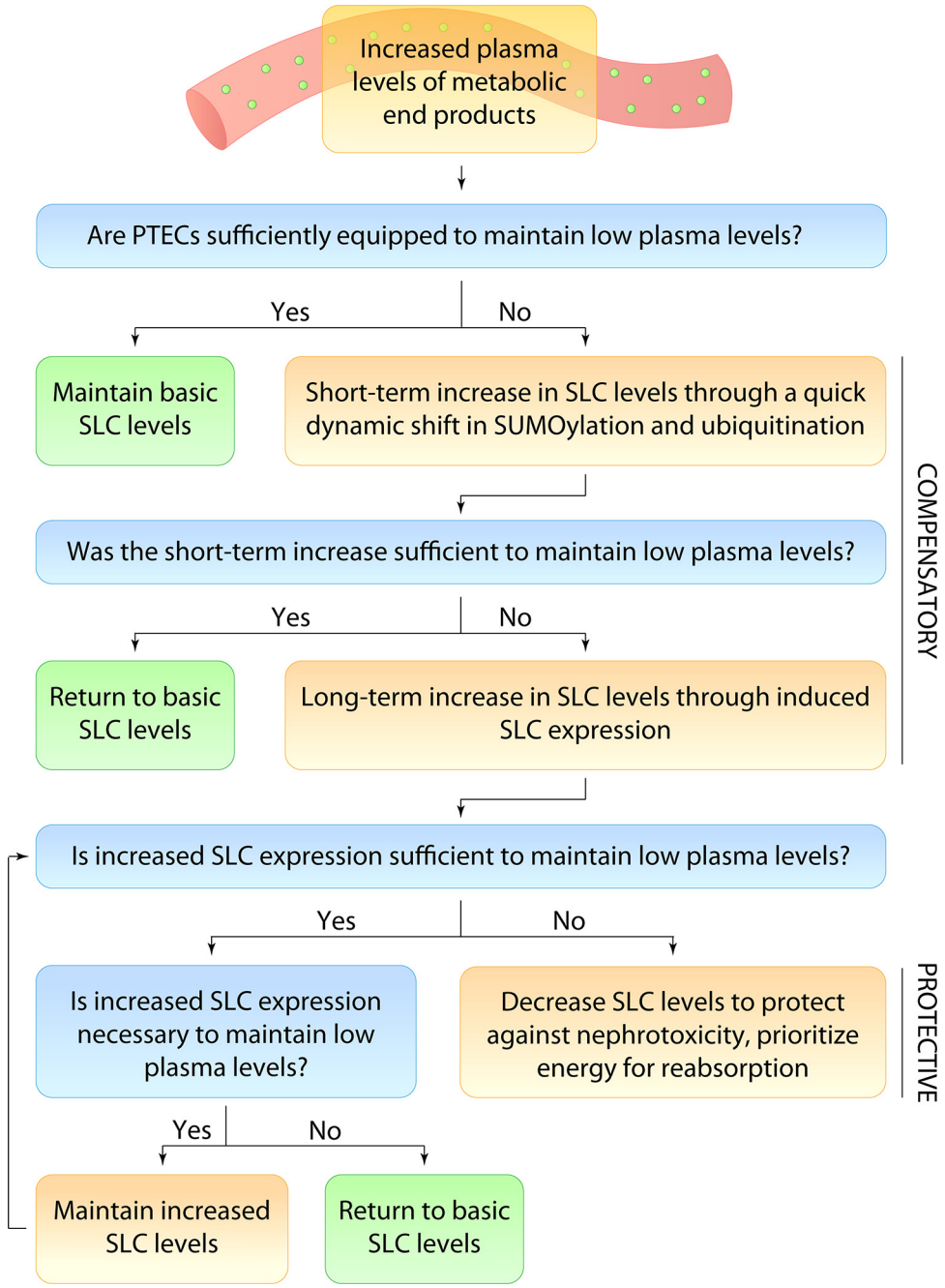

FIGURE 11. Proposed decision tree for *SLC22A2/6/8* expression in kidney proximal tubule epithelial cells. Changes in plasma levels of metabolic end products might induce a compensatory solute carrier (SLC) upregulation to counteract the increased workload, whereas a continued high level of cellular stress might induce a protective SLC downregulation to prevent nephrotoxicity through limiting xenobiotic influx. PTECs, proximal tubule epithelial cells.

but also experience a "culture shock" with strong effects on cellular metabolism (212), imposed among others by oxidative stress. Oxidative stress mediates epigenetic dysregulation and activates reactive oxygen species (ROS)-dependent signal transduction pathways that usually do not operate in vivo (213). Interestingly, immortalization of freshly isolated cells by overexpression of *TERT* and the SV40 large T antigen makes cells more resistant to oxidative stress and has been shown to preserve SLC expression (214–216). Accordingly, p53-deficient cells have been shown to retain *SLC22A2* expression (217). More physiological culture systems, e.g., organoids and three-dimensional (3-D) hollow fiber cultures, demonstrate biomimetic superiority to two-dimensional (2-D) cultures and go along with higher transporter expression (218, 219). Therefore, stress reduction, e.g., through physiological culture conditions with adequate oxygen tension, might be a key point to focus on for culture optimization.

Another culture condition that deserves serious consideration is the glucose content of culture mediums, which is with 10–20 mM often in diabetic ranges. It is known that high glucose levels promote oxygen radicals that induce cell lipid peroxidation and activation of PKC, which, as discussed above, is directly linked to OAT

ubiquitination (220). Interestingly, PKC-α and -β expression is enhanced in diabetic nephropathy (221). In patients with type 2 diabetes and nephropathy, treatment with ruboxistaurin, a selective PKC-β inhibitor, reduced albuminuria and maintained the glomerular filtration rate (222). It would be interesting to investigate whether high glucose levels negatively affect SLC expression and whether ruboxistaurin can prevent the loss of OATs in the PT.

Apart from stress reduction strategies, molecular targeting of pathways and molecules involved in PT formation and SLC expression (discussed in sect. 3) holds the potential to improve cellular maturation and SLC expression in culture. Organoid culture makes use of cellular self-organization and cell-cell communication, which leads to complex structures and various cell populations. However, cellular immaturity remains a challenge, and targeted approaches might be able to promote maturation and unlock the expression of SLCs. We have reviewed numerous molecules that upregulate SLC expression both in vitro and in vivo, but perhaps the ones with a most important role are nuclear receptors such as HNFs, ESRRA, PPAR, and RAR/RXR and epigenetic modulators inhibiting DNA methylation. Future studies should confirm that these transcriptional factors indeed have a crucial role in the regulation of *SLC22A2/6/8* expression alone or in combination. For instance, the ambiguous relationship between *HNF1A* and *SLC22A2* should be clarified (e.g., by overexpressing *HNF1A* in human cells/organoids and subsequently assessing *SLC22A2* levels). Similarly, the influence of epigenetic modulators on SLC expression should be further investigated with human material (e.g., the effect of acetylation on *SLC22A6/8* expression could be explored by treating cells in vitro with HDAC inhibitors).

Most information is currently based on rodent data and cell lines of divergent origins, whereas throughout all sections we have uncovered various cell systems and species differences in SLC expression and regulation. For more predictive and translational data, as well as for the development of clinically appropriate therapeutics, clinical data and the experiments on organoids from human-derived adult cells or induced pluripotent stem cells deserve more attention (223, 224). Nonetheless, we might also learn from species differences through comparative physiology and "kidney biomimicry" as suggested by Stenvinkel and Johnson (225): various animals, including diving seals, hibernating bears, and constantly azotemic vampire bats, can provide clues on how to protect the kidney from hypoxia or oxidative stress.

5.3. Therapeutic Applications: Intervention or Regeneration?

Considering the fact that most posttranslational regulation pathways involve protein kinase-induced phosphorylation of either the SLCs directly or intermediate targets, kinase-targeted drug therapy is the first pharmacotherapeutic approach that comes to mind. Accordingly, an in vivo proof of concept showed that the clinically approved tyrosine kinase inhibitor dasatinib mediates an OCT2 repression through Yes1 inhibition (179). However, kinases such as PKC and PKA are difficult candidates because of their multifunctionality; to exploit the potential of kinase-targeted drug therapies, it is crucial to take into account which isoforms are involved in the regulation of which proteins (226). An interesting example is the abovementioned PKC-β inhibition through ruboxistaurin.

In some cases, pharmacotherapeutic interventions might be more successful upstream or downstream. An example for upstream intervention in OAT regulation could be the use of prostaglandin analogs (e.g., misoprostol) or inhibitors (e.g., indomethacin). As described above, PGE_2 short-term activates but long-term inhibits OAT functionality. Therefore, prostaglandin analogs might enhance short-term functionality, whereas inhibitors have been shown to have the potential to restore OAT expression and function after kidney injury (200, 226, 227).

Downstream targets could be factors involved in the dynamics between ubiquitination and SUMOylation. For instance, proteasome inhibitors like bortezomib and carfilzomib, used against hematologic malignancies, target the ubiquitin-proteasome system, thereby preventing protein degradation (228). Although adverse reactions common to antineoplastic agents might be expected in vivo, their extracorporeal applicability might still be interesting, e.g., to boost transport activity of cells in kidney assist devices. Molecules targeting the SUMOylation pathway are also worth an investigation. To enhance membrane trafficking toward the cell membrane, SUMOylation should be promoted or, conversely, deSUMOylation should be prevented. For the latter, protease SENP2 has been proposed as a novel target because of its experimentally confirmed role in OAT deSUMOylation. Pharmacological SENP2 inhibition could cause a therapeutic shift of OAT trafficking toward the cell membrane. For this purpose, 1,2,5-oxadiazoles have been proposed as a new class for the development of novel therapeutic agents (204, 229).

The number of possible therapeutic targets to preserve or increase SLC expression in the clinics seems encouraging, but the reality is rather sobering: a large proportion of clinical trials testing treatments to improve morbidity and mortality in CKD ended in negative or neutral results (230). To date, the most efficient intervention to prevent disease progression is a change of lifestyle. Perhaps the next therapeutic breakthroughs, including the ones targeting active tubular secretion, are to be sought in regenerative medicine. The

development of a bioartificial kidney is still in its infancy, but it would complement dialysis with secretory capacities (219, 231). Furthermore, Chevalier described the phase of late nephrogenesis as a critical window of opportunity for regenerative therapies because the fetus can generate additional nephrons to compensate for any functional constraints (32, 232, 233). Both in vivo and in vitro, progenitor cell fate manipulation could enhance nephron number and thereby create a secretory reserve for later life stages.

6. CONCLUSION

Over the course of evolution, the kidney's secretory function has been developed and conserved in the majority of living species, despite the availability of extensive glomerular filtration. In mammals, OAT3 arose as a paralog to OAT1 with overlapping and even extended substrate specificities, which often result from selective pressure. From a phylogenetic perspective, we can confirm the indispensability of OCT2 and OAT1/3 for efficient blood clearance. Their essential contribution becomes especially evident in patients with kidney failure: kidney function cannot be entirely compensated for with dialysis, partly because current modalities lack secretory capabilities. In kidney patients, RKF remains the strongest predictor of survival. Yet, surprisingly little is known about the transcriptional induction of OCT2 and OAT1/3 during development, or their maintenance in the healthy adult kidney. Over the last two decades, a better understanding of nephrogenesis and the development of complex kidney organoids in vitro has broadened our knowledge of SLC regulation, which has been extensively reviewed in the work at hand. In this review, we have presented information from different perspectives to provide new insights and promising therapeutic directions, not only to control in vitro SLC expression for drug research but also for potential clinical application to improve current kidney replacement therapies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.P.C., K.J., M.B.R., H.C., M.C.V., and R.M. conceived and designed research; C.P.C. and K.J. prepared figures; C.P.C., K.J., M.B.R., M.C.V., and R.M. drafted manuscript; C.P.C., K.J., M.B.R., H.C., M.C.V., and R.M. edited and revised manuscript; R.M. approved final version of manuscript.

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