Saccharomyces cerevisiae as a model system for kidney disease: what can yeast tell us about renal function?

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Kolb AR, Buck TM, Brodsky JL. Saccharomyces cerevisiae as a model system for kidney disease: what can yeast tell us about renal function? Am J Physiol Renal Physiol 301: F1–F11, 2011. First published April 14, 2011; doi:10.1152/ajprenal.00141.2011.—Ion channels, solute transporters, aquaporins, and factors required for signal transduction are vital for kidney function. Because mutations in these proteins or in associated regulatory factors can lead to disease, an investigation into their biogenesis, activities, and interplay with other proteins is essential. To this end, the yeast, Saccharomyces cerevisiae, represents a powerful experimental system. Proteins expressed in yeast include the following: 1) ion channels, including the epithelial sodium channel, members of the inward rectifying potassium channel family, and cystic fibrosis transmembrane conductance regulator; 2) plasma membrane transporters, such as the Na\(^+\)-K\(^+\)-ATPase, the Na\(^+\)-phosphate cotransporter, and the Na\(^+\)-H\(^+\) ATPase; 3) aquaporins 1–4; and 4) proteins such as serum/glucocorticoid-induced kinase 1, phosphoinositide-dependent kinase 1, Rh glycoprotein kidney, and trehalase. The variety of proteins expressed and studied emphasizes the versatility of yeast, and, because of the many available tools in this organism, results can be obtained rapidly and economically. In most cases, data gathered using yeast have been substantiated in higher cell types. These attributes validate yeast as a model system to explore renal physiology and suggest that research initiated using this system may lead to novel therapeutics.

A number of systems have been employed to study cellular aspects of kidney disease, including mouse models, primary and immortalized tissue culture cell lines, and Xenopus oocytes. Mouse or other rodent models for disease are most desirable, but these systems are costly, and the generation of mutations is an uncertain and long-term undertaking. Tissue culture systems have been used to successfully model renal epithelia, but genetic manipulation of mammalian cells may not be trivial, and there is often debate on which cell type represents the best model. To study ion and water channel physiology and gating, functional assays may be performed in Xenopus oocyte expression systems, but biochemical techniques using this system are laborious and limited by the amount of material that can be obtained. While critical discoveries have certainly been made using these systems, there is always a need for faster, easier, cheaper, and more genetically amenable systems. One such system, and the focus of this review, is the baker’s yeast, Saccharomyces cerevisiae.

Nearly all cellular processes in eukaryotes are conserved between yeast and humans, and, in many cases, the molecular underpinnings of basic cellular events were first described in yeast. As a model organism, yeast possess critical advantages. First, the growth of large quantities of cells is rapid and inexpensive, thus aiding the development of biochemical assays. Second, glycosylation and processing, which are important for protein function, occur similarly in yeast and mammalian cells. Third, yeast are easy to transform and can be engineered to express heterologous proteins. Fourth, yeast exist in a haploid form or diploid state and recombine genes through homologous recombination. This makes genetic ablation a simple undertaking and allows for genomewide screens for genetic modifiers of essential processes. Fifth, compared with other model organisms, abundant tools are available, either commercially or through the yeast community. These tools include the yeast gene knockout collection, which encompasses deletions in every nonessential gene (~85% of all genes) (139); a hypomorphic allele collection, in which essential yeast genes are placed under the control of a repressible promoter or contain destabilizing sequences in their messages.
Ion Channels

Ion channel function in the kidney is vital to maintain osmotic and salt homeostasis, and, as discussed above, a number of diseases are the direct result of mutations and/or misregulation of kidney-localized ion channels. Ion channels, including ENaC (epithelial sodium channel), several members of the Kir (inward rectifying potassium channel) family, and CFTR (the cystic fibrosis transmembrane conductance regulator) have been successfully characterized in yeast, an organism that normally lacks these proteins (Fig. 1; Table 1).

ENaC. ENaC, a heterotrimeric sodium channel composed of α-, β-, and γ-subunits, is expressed in the kidney, colon, and airway, where it functions to maintain osmotic homeostasis (114, 116). ENaC is responsible for final sodium absorption in the distal nephron. Thus ENaC function and levels are tightly regulated, and either gain- or loss-of-function mutations in ENaC alter sodium homeostasis in the kidney, resulting in hypertension (Liddle’s Syndrome), or hypotension (Pseudohypoaldosteronism Type I), respectively. In addition, common polymorphisms in the genes encoding ENaC subunits may affect blood pressure variation in the population as a whole (55).

To examine how ENaC is regulated during biosynthesis, the three ENaC subunits were expressed individually in yeast. Immunofluorescence microscopy was used to determine that ENaC localized primarily to the ER (endoplasmic reticulum), although other immunostaining regions were observed that may represent secretory vesicles and/or the plasma membrane (18, 41). The primary ER residence of ENaC is consistent with the fact that <20% of the channel resides at the plasma membrane in epithelial cells (44, 103, 117, 130, 137). This makes yeast an ideal system to monitor early events during ENaC biogenesis, such as ER-associated degradation (ERAD) (137).

ERAD is a quality control pathway exemplified by the chaperone-mediated recognition of misfolded proteins within the ER and the subsequent targeting of these aberrant proteins for ubiquitination and degradation by the cytosolic 26S proteasome. Notably, ERAD was first discovered in yeast, and the components and basic elements of this pathway are completely conserved (39, 131). Consistent with studies in vertebrate systems (77, 78, 117, 130), ENaC degradation was slowed in yeast strains with defects in the proteasome pathway when analyzed by a cycloheximide chase assay (18, 61). Using recently developed biochemical techniques, it was then determined that the ER resident E3 ubiquitin ligases, Hrd1 and Doa10, append ubiquitin onto the ENaC subunits and facilitate their degradation (18). Interestingly, the extent of ubiquitination and stabilization in the E3 mutant strains varied among the subunits. This result supports data from other systems, suggesting that the three subunits are differentially regulated (19, 50, 85, 117).

Because molecular chaperones aid in secretory protein folding and select misfolded proteins for ERAD, the chaperone requirements for ENaC degradation were also assessed in yeast. Cycloheximide chase assays revealed that the small heat shock proteins, Hsp26 and Hsp42, facilitate the degradation of α-ENaC (61), and the ER luminal Hsp40 chaperones, Sec1 and Jem1, promote the degradation of each of the three ENaC subunits. By employing reagents obtained from wild-type and mutant strains in defined cellular and in vitro assays, the Hsp40s were shown to function before substrate ubiquitination (18). The results obtained from the yeast studies were then validated in vertebrate cells. Specifically, overexpression of the mammalian homologs of the small HSPs or the Hsp40 in Xenopus oocytes accelerated ENaC degradation, and their expression decreased the amiloride-sensitive ENaC current and ENaC residence at the plasma membrane (18, 61). These studies indicate that yeast can provide a means to identify...
factors involved in ion channel biogenesis, and that results in this system can be translated into higher cell types. Earlier, another yeast expression system for ENaC, consisting of an inducible /H9251/H9252 -ENaC concatamer, was established (41). Immunoblotting of secretory vesicles and plasma membrane preparations confirmed that ENaC traffics to the plasma membrane, as it does in epithelial cells (see above). Interestingly, ENaC expression led to defective cell growth when yeast were incubated on high (1 M) sodium-containing media (41). These data indicate that ENaC is active in yeast, which sets the stage for genomewide studies to identify and characterize additional regulators of ENaC function.

**Kir channels.** A group of unrelated ion channels has also been expressed in yeast. The Kir family is composed of seven subfamilies (Kir1–7) that share ~60% sequence homology and ~40% sequence identity within subfamilies (27). Several members of the Kir potassium channel family are expressed in the kidney, including Kir1.1, Kir4.1, Kir5.1, and Kir6.1 (27, 46). Kir1.1 (also known as ROMK) functions at the apical membrane, and Kir4.1 and 5.1 function at the basolateral membrane of polarized epithelial cells. These channels maintain potassium homeostasis and provide potassium to the Na+/K⁺-2Cl⁻ cotransporter and to the Na⁺-K⁺-ATPase, thus contributing to the cellular flux of sodium and chloride. Mutations

### Table 1. Yeast studies of renal proteins

<table>
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ENaC, epithelial sodium channel; Kir, inward rectifying potassium channel; CFTR, cystic fibrosis transmembrane conductance regulator; SGK1, serum/glucocorticoid-induced kinase 1; RhGK, Rh glycoprotein kidney; TREH, trehalase; ERAD, endoplasmic reticulum-associated degradation; EM, electron microscopy.
in Kir1.1 result in Type 2 Bartter syndrome, which is characterized by potassium and sodium wasting (46), and mutations of Kir4.1 were recently shown to cause EAST/SeSAME syndrome, which also gives rise to a Bartter-like phenotype (16, 110). Although the role of Kir6.1 in the pancreas is better characterized, Kir6.1 is an ATP-sensitive potassium channel and resides with Kir1.1 and CFTR on the basolateral membrane of renal epithelial cells (46, 104). Kir6.1 is also localized to the mitochondria in the kidney, where it may act as a mitochondrial potassium channel (89). While there are insignificant levels of other Kir family members in the kidney, all family members are closely related. Therefore, studies that involve Kir homologs that reside in other tissues may provide insights into the function of renal Kir family members.

Multiple Kir proteins have been expressed in yeast. In fact, 10 of 11 Kir proteins, representing every Kir subfamily, have been expressed in this organism and traffic to the plasma membrane, as they do in mammalian cells. Some of these channels were purified from yeast, reconstituted into proteoliposomes, and shown to have channel activity using a Rb+ flux assay (26).

Another strategy to study channel function in yeast is to express Kir channels in a strain deleted for the gene encoding two endogenous plasma membrane-resident potassium transporters, Trk1 and Trk2. This strain is inviable when grown on low-potassium media, but cell growth is rescued upon the expression of a foreign, functional potassium channel that resides at the plasma membrane. Kir1.1 was the first Kir channel expressed in this strain background and restored cell growth on low-potassium-containing media (123). This system was then optimized for genetic screens (87).

For example, a genomewide synthetic gene array screen was used to isolate factors that impact the trafficking of Kir3.2. Seven genes that affect the protein’s residence at the plasma membrane were identified, including the following: COPII cargo receptors, which mediate the transport of specific proteins from the ER to the Golgi (ERV25, EMP24, and TED1), a COPII vesicle packaging chaperone (ERV14), a fatty acid elongase (SUR4), a GPI inositol deacylase (BST1), and a regulatory subunit of mannosyl-transferases (CGS2) (42). Each of these factors plays a designated role in the secretory pathway. Screens in Kir2.1-expressing yeast were also employed to uncover small-molecule inhibitors of Kir and to define how the protein’s transmembrane domains are organized and contribute to channel function (83, 145, 146).

In addition, an expression system for Kir6.1 was established, and, like other Kir family members, the channel rescued the growth of trk1Δtrk2Δ mutant yeast on low-potassium-containing media. Mutated residues in Kir6.1 that ablate ATP sensitivity and trafficking in Xenopus oocytes were then scored as gain or loss-of-function mutations in this system (40). In principle, monitoring the growth of trk1Δtrk2Δ cells on low-potassium-containing media should facilitate continued studies on the function, trafficking, and regulation of any potassium channel that can be expressed in yeast.

CFTR. The structure, function, biogenesis, and regulation of a third ion channel, CFTR, have also been extensively examined in the yeast system. Although its specific activity in the kidney has not been well characterized, CFTR function in airway cells and in tissue culture has been extensively studied because mutations in CFTR destabilize the protein and result in cystic fibrosis. The most common disease-causing mutation is a deletion of phenylalanine at position 508 (11, 22, 102). Although kidney pathology is not associated with cystic fibrosis, it has been proposed that, as patient survival increases due to improved treatment of the airway pathology, a renal pathology may become apparent (115).

CFTR is a chloride channel of the ATP-binding cassette transporter family, consisting of 12 transmembrane domains and containing 2 prominent cytoplasmic loops. The first loop harbors a nucleotide binding domain (NBD1) and a regulatory domain, and the second loop contains a second NBD (NBD2). In the active form, CFTR’s regulatory domain is phosphorylated, and the NBDs are in the ATP-bound state (25, 37). The most common cystic fibrosis-causing mutation, ΔF508, is located near the end of NBD1 and is thought to affect the docking of this domain onto the transmembrane domains (111). Consequently, the mutation severely affects CFTR folding in the ER, such that the protein is targeted for ERAD (54, 136). In some cell types, a significant fraction of the wild-type protein is trapped in the ER and degraded by the ERAD pathway. However, improvements in the folding environment, by altering chaperone levels or by reduced temperature (29), rescue the folding defect, such that the protein can escape ERAD and function at the plasma membrane. Thus there is a profound need to better define the factors that play a role in the folding and degradation of CFTR, which might then become therapeutic targets.

One approach that was successfully used to explore the protein-folding pathway of CFTR was to use a chimera in which the NBD1 of the yeast ATP-binding cassette transporter, Ste6, was replaced with the NBD1 from CFTR. Ste6 is required for the successful mating between haploid strains in yeast, and the expression of the Ste6-CFTR-NBD1 chimera supported this event. However, mating efficiency was reduced when the cystic fibrosis causing mutation, ΔF508, was introduced into NBD1. The system was then used to identify suppressor mutations that restored function. Strikingly, the mutations also corrected the folding and trafficking defect of full-length ΔF508 CFTR when expressed in mammalian cells (28, 124, 125). Even though the nature of the defect in the Ste6-CFTR-NBD1(ΔF508) chimera was subsequently reinterpreted (93), these studies exemplified how yeast could be used to uncover determinants within a domain that permit the proper folding and trafficking of a disease-causing mutant protein.

A yeast expression system for full-length CFTR has also been used to define how the protein is targeted for ERAD. A variety of biochemical and cellular assays showed that CFTR resides primarily in the ER (62, 121, 148). Cycloheximide and pulse-chase experiments in yeast strains with mutations or deletions in ER-associated factors demonstrated that CFTR degradation is dependent on the proteasome; the ER localized E2 ubiquitin-conjugating enzymes, Ubc6 and Ubc7, and E3 ubiquitin ligases, Hrd1 and Doa10 (see above); and the Cdc48 complex, which is required to extract ubiquitinated proteins from the ER and present them to the proteasome (38, 62, 68, 88, 148). The mammalian homolog of Cdc48, p97 or valosin-containing protein, is also important for degrading CFTR in higher cells (132). Yeast chaperones that contribute to the degradation of immature forms of CFTR include the cytosolic Hsp40s (Ydj1 and Hlj1) and an associated Hsp70 (Ssa1), which together help present misfolded ER membrane proteins.
to the ubiquitination machinery. Interestingly, yeast Hsp90 prevented the aggregation of purified NBD1 and aided the folding of CFTR (143). The ability of Hsp90 to fold CFTR has been confirmed in higher cell types (75, 135).

To more globally identify factors involved in CFTR biogenesis, a microarray screen analyzed the yeast transcriptome in CFTR-expressing cells, with the underlying assumption that factors required for CFTR degradation would be upregulated. From this effort, the small HSPs, Hsp26 and Hsp42, were found to be upregulated, and their deletion had a profound effect on CFTR degradation in yeast. In human embryonic kidney-293 cells, a mammalian Hsp26/Hsp42 homolog preferentially selected ΔF508 over wild-type CFTR for degradation (1). This result indicated that the yeast system could be used to uncover a component that can be specifically modulated to rescue the biogenesis of the disease-causing protein in higher cells.

Of note, CFTR degradation in yeast may occur in discrete ER microdomains, termed ER-associated compartments (51), which are maintained by the COPII/Sar1 vesicle transport machinery (36). Real-time fluorescent microscopy of an inducible EGFP-CFTR construct indicated a diffuse ER localization for the protein that was either degraded immediately or accumulated in distinct foci. Using fluorescence recovery after photobleaching technology, the authors determined that the CFTR population in the foci was not as mobile as the CFTR in the diffuse ER pool, and the former pool appeared to be degraded via autophagy (35). Links between autophagy and defects in CFTR function, at least under some conditions, were recently reported (76).

Transporters

Na\(^+\)-K\(^+\)-ATPase. Osmotic homeostasis in kidney epithelia depends on the coordinated efforts of ion channels and a diverse group of transporters. For example, the ubiquitously expressed Na\(^+\)-K\(^+\)-ATPase imports two K\(^+\) ions for every three Na\(^+\) ions exported and is composed of an α-, β-, and γ-subunit (60). The α- and β-subunits are sufficient to form an active channel, while the γ-subunit regulates activity. There are three α-subunit isoforms and four β-subunit isoforms, but the α1- and β1-subunit isoforms are the major isoforms expressed in the kidney. In kidney epithelia, the Na\(^+\)-K\(^+\)-ATPase functions at the basolateral membrane and provides the electrochemical gradient required for Na\(^+\) transport at the apical membrane, which is mediated by other ion channels, such as ENaC. Na\(^+\)-K\(^+\)-ATPase function in the kidney has been linked to some forms of hypertension and has thus been targeted for therapeutics (30, 72). The Na\(^+\)-K\(^+\)-ATPase also functions in the heart and has been targeted for the treatment of congestive heart failure through the use of cardiac glycosides, such as digoxin, which inhibit the transporter (34, 82). Although not used clinically, another cardiac glycoside, ouabain, has been utilized extensively to characterize the function of the Na\(^+\)-K\(^+\)-ATPase, because each ouabain molecule binds one holoenzyme and inhibits pump activity (71).

Yeast, unlike animal cells, lack the Na\(^+\)-K\(^+\)-ATPase and can, therefore, be used to characterize its activity. Yeast compensate for the expression of an exogenous transporter by derepressing Gcn4, a transcription factor that mediates a response to nutrient starvation (118, 119). To establish a yeast expression system for the Na\(^+\)-K\(^+\)-ATPase, a sheep α-subunit and a canine β-subunit were individually expressed (48). The resulting transporter exhibited potassium-sensitive ouabain binding and ouabain-sensitive ATPase and γ-nitrophenolphosphatase activities, as in mammalian cells (47). This system was then adapted to study the activities of the many possible α- and β-isoformal combinations. This was accomplished by placing each subunit isoformal on plasmids with different selectable markers (94). For example, each of the human α-isoformal with the β1-isoformal were expressed in yeast, and each assembled enzyme exhibited similar potassium-sensitive ouabain binding affinities and activities, as observed in mammalian cells (86).

The yeast system has also contributed to structure-function analyses of the Na\(^+\)-K\(^+\)-ATPase. In one study, truncated ATPase subunits were fused to a β-galactosidase reporter, which is only functional in the cytoplasm and, therefore, allows one to determine transmembrane segment orientation. It was found that the α-subunit contains 10 transmembrane segments, while the β-subunit contains one transmembrane segment, consistent with results from other studies (31, 60). The yeast system also helped to establish that the α- and β-subunits constitute minimal transporter activity (106). Furthermore, the yeast system and yeast two-hybrid assays were used to identify regions within the α- and β-subunit that contribute to subunit-subunit association: a 63-amino acid tract (E63-D125) in the extracellular loop of the β-subunit and the amino acid tract SYGQ, along with V904, T890, and C908 in the extracellular loop between the seventh and eighth transmembrane segment of the α-subunit, were shown to mediate intermolecular associations (23, 134). Yeast two-hybrid assays were also employed to provide evidence that the α-subunits may self-associate via the first cytoplasmic loop (23, 142). The Na\(^+\)-K\(^+\)-ATPase cycles through ATP-bound, ADP-bound, and nucleotide-free conformations, but the residues that mediate this cycle and the contributions of bound ions during the cycle were at first poorly understood. To this end, mutated enzymes of the form, such as one containing the phosphorylation site mutant, D369N, and cation binding site mutants in the α-subunit were expressed, assembled, and assayed using ouabain binding and ATPase activity assays in yeast (97). As a result of these efforts, phosphorylation at D369 was shown to be critical for a major conformational change of the K\(^+\)-bound enzyme (98, 99), and the D804, D808, E327, and E779 residues were found to coordinate sodium and potassium ions as the transporter changes conformation (58, 90, 100). Multiple other residues were isolated that affect the transporter’s ATPase activity (52, 109, 141). For example, mutations in residues 691 and 708–714 are important for Mg\(^2+\) binding and D369 phosphorylation (57, 120). In addition, mutations in this region (amino acids 708–720) induce the unfolded protein response when expressed in yeast at higher temperatures, which indicates that the mutations likely affect protein stability (56). In fact, misassembly of the Na\(^+\)-K\(^+\)-ATPase is known to trigger its degradation through the ERAD pathway in mammalian cells (9), which is consistent with the observed unfolded protein response induction upon the expression of the misassembled protein in yeast.

As described above for the Kir proteins, drugs that target the Na\(^+\)-K\(^+\)-ATPase can be identified and characterized in yeast. The yeast expression system was employed to show that the Na\(^+\)-K\(^+\)-ATPase is the target of palytoxin and sanguinarine.
(105, 107), and that the activity of palytoxin does not depend on a catalytically active enzyme (108). In addition, cardiac glycosides, such as ouabain, were found to have different isoform specificities (45). Finally, the purified, detergent-solubilized enzyme from yeast membranes was used to validate the use of electrochemical dyes that can report on Na⁺-K⁺-ATPase activity in vitro (43).

Sodium, hydrogen antiporter/sodium, hydrogen exchanger.

A yeast expression system for another transporter, the Na⁺-H⁺ antiporter (NHA), has been developed. NHA are widely expressed and help maintain the intracellular pH, which is important for a variety of cellular functions, including cell division (79). There are two NHA families, and alterations in the activities of these enzymes may also affect blood pressure (79, 140). Na⁺/H⁺ exchanger (NHE) is the first family, and there are nine paralogs in humans (79). The second group is the NHE family, which is characterized by the presence of a shorter COOH-terminal tail and is encoded by two genes in mammals, NHA1 and NHA2, and one homolog in yeast (140). In the proximal tubule of the kidney, NHA contribute to sodium reabsorption (15).

When NHE1 was expressed in yeast, it localized primarily to the ER, as opposed to the plasma membrane; however, the protein was active after its reconstitution into proteoliposomes (84). When NHE2 and NHE3 were expressed from a strong promoter, the salt tolerance of yeast lacking endogenous sodium transporters increased slightly, despite the fact that most of the transporters remained in the ER (32, 33). However, a decrease in the amount of the E3 ubiquitin ligase, Rsp5, increased the number of channels at the plasma membrane (32). Rsp5 is the yeast homolog of the mammalian ubiquitin ligase, Nedd4–2, which is required for the endocytosis and lysosomal degradation of ENaC (59, 138, 149) and possibly CFTR (20) after retrieval of the plasma membrane; therefore, these data suggest that later steps during the secretion, recycling, and quality control of renal proteins can be characterized in yeast. Moreover, and consistent with these data, a related E3, Nedd4–1, was recently shown to play a role in mediating NHE1 endocytosis in mammalian cells (113).

Two human NHA genes were also characterized in yeast. NHA1 and 2 rescued growth on high-sodium media when expressed in a strain deleted for endogenous sodium transporters, including yeast NHA1 (140). Rescue required plasma membrane residence. These data further emphasize the possibility that yeast may prove to be a new model to identify factors that impact NHA stability and function.

Sodium, phosphate cotransporter.

Another manner in which yeast can be leveraged to explore renal protein biogenesis and structure-function relationships is to express the protein in a yeast strain in which the homolog has been deleted, leaving only the mammalian protein to function in its place. This is analogous to the ability of the human Kir proteins to support the growth of trk1Δtrk2Δ yeast on low-potassium-containing media (see above). The Na⁺-phosphate cotransporter is expressed in many epithelial cells, where it functions to maintain phosphate homeostasis, and defects in transporter function lead to diseases such as X-linked hypophosphatemia and autosomal-dominant hypophosphatemic rickets (13, 133). As anticipated, the wild-type cotransporter, but not an inactive mutant form of the protein, trafficked to the plasma membrane and rescued the growth of yeast lacking the high-affinity phosphate transport system (12). These data further support the ability of yeast to properly fold and secrete renal proteins.

Water Channels

The kidney plays a critical role in maintaining water homeostasis, and this role depends on the function of aquaporin (AQP) water channels. AQPs are six transmembrane, ~30-kDa proteins that tetramerize (91). Humans express 13 AQPs that are divided into three families based on their channel selectivity for water and/or other solutes. Seven of the AQPs are expressed in the kidney: AQP1, AQP2, AQP3, AQP4, AQP6, AQP7, and AQP11. Mutations in two of these channels have been identified that lead to urine concentration defects (AQP1) and nephrogenic diabetes insipidus (AQP2). Four of the renal AQPs (AQP1–4) have been successfully expressed in yeast.

AQP1 was the first human AQP to be expressed in yeast (65). The AQP1 expression system was used to assess channel function by isolating AQP1-containing vesicles and subjecting them to stop-flow experiments, which spectrophotometrically measure changes in vesicle size in response to hypertonic conditions. To increase the signal to noise, these experiments were carried out in a mutant yeast strain, sec6–4, which has an exocytosis defect that results in the accumulation of intracellular vesicles. To further improve the sensitivity of this assay, some experiments were performed by first detergent solubilizing and purifying the AQPs from yeast and then reconstituting the channels into synthetic proteoliposomes, in which the stop-flow experiments could be performed. This approach permitted tighter control of the AQP-to-lipid ratio (24, 64). The effects of channel inhibitors, such as CuSO₄, AgNO₃, tetraethylammonium, and p-chloromercuribenzenesulfonate, could then be assayed (65, 101). In fact, it was found that HgCl₂ inhibits AQP4, contrary to previous studies in oocytes (144).

Yeast expression systems for AQPs have also been used to study channel selectivity, as well as the structure-function relationship of channels that were subjected to site-directed mutagenesis. To compare the properties between AQP family members, a protoplast-bursting assay was carried out. This study determined that AQP1 has a larger water conductance than AQP3, 5, or 9 (101). Similar to what was shown for AQP1 isolated from red blood cells, AQP2 appears to be water selective, as urea, glycerol, or formamide were not transported by the channel (24, 147). Additional studies of AQP pore selectivity focused on different AQP classes, which are defined by their selectivity for solutes and the Asn-Pro-Ala (NPA) amino acid motif. Class I AQPs are primarily water selective, class II AQPs are both water permeable and permeable to small neutral solutes, such as glycerol and urea, and class III AQPs are the least selective. It was found that class I AQPs were impermeable to H₂O₂, whereas the class II AQP, AQP3, was H₂O₂ permeable (14). Studies investigating the properties of class III AQPs found that AQP7 and AQP9 transport arsenite, providing a hint as to the route through which arsenic may enter cells (73). The residues that mediated selectivity within the pore region, H180 and F56A, were defined for AQP1 (10). In another investigation, cysteine mutations within the pore-forming domains of AQP1 were examined. These mutations abolished water channel function, and sucrose gradient analyses established that the absence of channel function was due to an inability of the mutant channel to tetramerize. Thus the
residues that are critical for AQP assembly and selectivity could be defined (81).

Another important application of the yeast system was to characterize 10 AQP2 mutations that are known to cause nephrogenic diabetes insipidus. These mutants fell into three categories: those that are functional, those that are partially functional, and those that are completely inactive (112). Together, these studies have contributed significantly to our overall understanding of several aspects of AQP biology, including differences between AQP family members, the structure-function relationships regarding the selectivity filter, the importance of intermolecular contacts required for tetramer formation, and a molecular characterization of disease-causing mutants.

**Signaling and Other Molecules**

Serum/glucocorticoid-induced kinase and 3-phosphoinositide-dependent kinase 1. The yeast expression system has been used to characterize kidney-resident proteins that are involved in signaling and other activities. One of these proteins is serum/glucocorticoid-induced kinase 1 (SGK1), a cytosolic kinase of the AGC (PKA, PKG, PKC) family (74). SGK1 is activated in response to a variety of stimuli, including aldosterone. Aldosterone, among other stimuli, transcriptionally regulates SGK1, which then either directly or indirectly regulates various channels and transporters, including ENaC, the Na⁺-K⁺-2Cl⁻ cotransporter, Kir1.1, and the Na⁺-K⁺-ATPase (66, 74), each of which was discussed above.

Yeast encode two kinases, Ypk1 and Ypk2, whose catalytic domains share 55% sequence identity with the catalytic domain of SGK1. When expressed in yeast, SGK1 was able to restore the viability of yeast lacking the genes encoding both Ypk1 and Ypk2, making SGK-dependent functional assays possible in this mutant background. Like the ion channels discussed in the preceding section (i.e., ENaC and CFTR), mutant yeast strains were then used to identify factors that modulated SGK1 levels. It was discovered that SGK1 turnover required the E2 ubiquitin-conjugating enzymes, Ubc6 and Ubc7, the E3s Hrd1 and Doa10, and the proteasome (2). Therefore, even though SGK1 is a cytosolic protein, it can interact with the cytoplasmic face of the ER and is degraded by the ERAD pathway. By analogy, 3-phosphoinositide-dependent kinase 1 (PDK1) is a constitutively active kinase that regulates AGC kinases, including SGK1 (3). Yeast encode two PDK1 homologs, Pkh1 and Pkh2, and the expression of human PDK1 can rescue the growth of yeast strains in which the genes encoding Pkh1 and Pkh2 are deleted (21, 74). This result indicates that the requirements for the functional expression of PDK1 can ultimately be defined in the yeast system.

**Rh glycoprotein kidney and trehalase.** The characterization of two other renal proteins, Rh glycoprotein kidney (RhGK) and trehalase (TREH), has benefited from the development of yeast expression systems. RhGK is a poorly characterized protein, but, by expressing RhGK in yeast, it was demonstrated that it functions as an ammonium transporter (80). TREH, a glycoprotein expressed in small intestines and renal brush borders, is responsible for metabolizing trehalose to glucose. The presence of TREH in the urine is a marker of kidney damage. Yeast have three TREHs, Ath1, Nth1, and Nth2, each possessing a unique function. Gene complementation studies in yeast deleted for the endogenous TREHs demonstrated that the human enzyme is not required for general metabolism (i.e., metabolizing trehalose to glucose), but may instead be a stress response protein that might be important for renal function (92).

**Conclusions**

As discussed in this review, *S. cerevisiae* is an affordable, facile, and genetically amenable system to model renal disease, but a discussion of any system is incomplete without mentioning its limitations. Two of the major limitations to studying renal proteins in the yeast system are that laboratory strains of yeast are single-cell organisms and that yeast cells are not polarized, except during bud emergence. Therefore, yeast cannot be used to model the important cell-to-cell interactions that occur within an organ, like the kidney. Also, studies to examine the residence of proteins to distinct polarized areas in the cell cannot be interpreted in yeast. Nevertheless, this simple system has facilitated studies that have led to the isolation of factors required for the degradation, folding, and transport of critical resident proteins in the kidney. The yeast system has also provided a robust means to perform structure-function analyses of these proteins. New functions and the effects of disease-causing mutants in specific proteins of renal origin have been established in yeast. This simplified system can also be utilized to more completely view the essential cellular pathways that impact metabolic and cellular processes. We have attempted to highlight both the diversity of the proteins (Fig. 1) that have already been investigated in yeast, and the diversity of techniques this model system offers (Table 1). The use of yeast will undoubtedly continue to provide new information and may ultimately provide a fast track to understand the molecular basis of select kidney diseases.

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**DISCLOSURES**

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**REFERENCES**

5. Beitz E, Wu B, Holm LM, Schultz JE, Zeuzhen T. Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea,


