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Galectin 9 is the sugar-regulated urate transporter/channel UAT

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UAT, also designated galectin 9, is a multifunctional protein that can function as a urate channel/transporter, a regulator of thymocyte-epithelial cell interactions, a tumor antigen, an eosinophil chemotactic factor, and a mediator of apoptosis. We review the evidence that UAT is a transmembrane protein that transports urate, describe our molecular model for this protein, and discuss the evidence from epitope tag and lipid bilayer studies that support this model of the transporter. The properties of recombinant UAT are compared with those of urate transport into membrane vesicles derived from proximal tubule cells in rat kidney cortex. In addition, we review channel functions predicted by our molecular model that resulted in the novel finding that the urate channel activity is regulated by sugars and adenosine. Finally, the presence and possible functions of at least 4 isoforms of UAT and a closely related gene hUAT2 are discussed.
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There is now abundant evidence that galectin 9 is a protein that likely serves a variety of functions *in vivo*. Galectin 9 was independently cloned in 1997 by 3 groups investigating disparate issues, first by our group [1] and then 2 others [2,3]. This fascinating protein has been shown to be a urate channel/transporter [1,4–8], a regulator of thymocyte-epithelial cell interactions [9], a tumor antigen [3], an eosinophil chemotactic factor [10], and a mediator of apoptosis [9,11]. Our studies have focused on the role of galectin 9 in urate transport.

Urate is the product of purine metabolism and is excreted from the body by the kidney. Elevated serum urate levels, which are most often consequent to renal urate underexcretion [12], are clearly associated with gout. Hyperuricemia is also common in hypertension [13], and often precedes and predicts its development in adults [14] and adolescents [15], suggesting a causal relationship. Indeed, recent studies demonstrate that the induction of hyperuricemia in rats results in hypertension [16]. Hyperuricemia and precocious gout may also play a causal role in a familial form of renal failure [17–24]. Hyperuricemia has also been associated with progression of IgA nephropathy [25], and results in increased fibrosis in a rat model of inflammatory renal disease [16]. Finally, hyperuricemia may be an independent risk factor for cardiovascular disease [26–30], although this finding is controversial and a mechanism for uric acid-induced pathogenesis is as yet unproven [31–33].

Mammalian urate transport occurs predominately in the renal proximal tubule [6,34–36]. Two molecular mechanisms of urate transport have been described in membrane vesicles derived from renal proximal tubule cells. Studies from our group demonstrated that renal proximal tubule cell membranes contain an electrogenic urate transporter that is inhibitable by oxonate, a specific inhibitor of the enzyme uricase [37,38], while those of others demonstrated the presence of a very recently cloned urate/anion exchanger [39–43]. Further experiments in our laboratory documented that urate transport can be significantly inhibited by a polyclonal antibody to purified uricase [36]. These data showing inhibition of transport by the specific uricase inhibitor oxonate and anti-uricase antibodies suggested that the urate transporter and uricase share significant homology. Therefore, a rat renal cDNA expression library was screened with our anti-uricase antibody and a single clone was identified that was designated UAT [1]. As predicted, there was a region of high homology between UAT and the urate binding site of uricase [8]. Subsequently, due to its homology to other members of the galectin family, UAT was also designated galectin 9.

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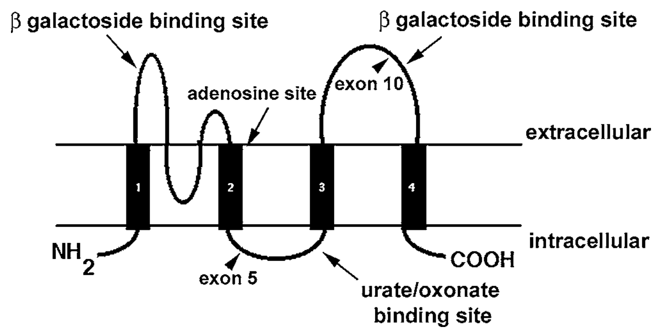

Figure 1. Molecular model of UAT derived from computer modeling and lipid bilayer studies indicating the 4 transmembrane domains numbered 1–4, and the intracellular amino and carboxy termini. The unique linker portion of UAT is comprised of transmembrane domains 2 and 3 and the intracytoplasmic loop, and contains the urate and adenosine binding sites that are important for the transporter/channel function of the protein.

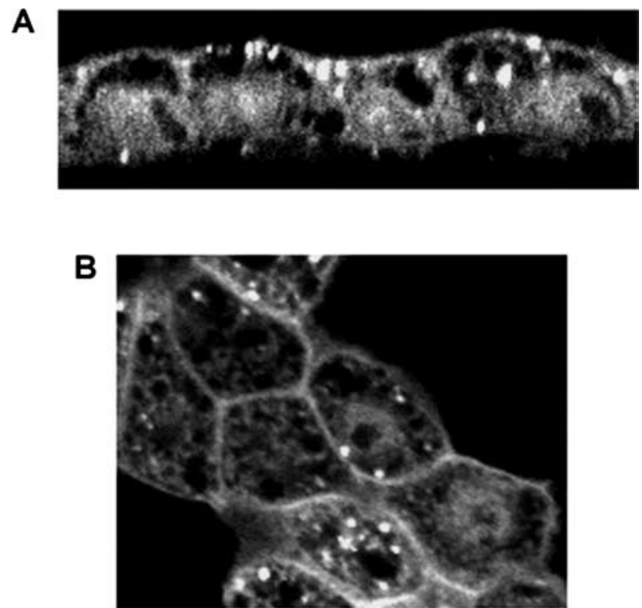

Figure 2. Confocal microscopy of stably transfected LLC-PK1 cells expressing EGFP fused to the carboxy terminus of the urate transporter (hUAT-EGFP). (a) Sagittal confocal image and (b) horizontal confocal image of cells grown for 6 days on Transwell clear permeable filter supports. Republished with the permission of The Journal of Clinical Investigation from "Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter." Lipkowitz *et al.*, *J Clin Invest* **107**, 1103–115 (2001); permission conveyed through Copyright Clearance Center, Inc.

The generally held view is that most galectins are secreted proteins that mediate cell-cell interactions [10,44–48]. However, hydropathy plots and more detailed modeling as well as the results of lipid bilayer experiments detailed below led us to develop the model depicted in (Figure 1) for UAT. This model contains 4 transmembrane domains that have predicted α helices long enough to span the membrane, with domains 1–3 homologous to the transmembrane domains of uric acid/xanthine permease, bacterial rhodopsin, and cytochrome c oxidase, respectively [8]. Further this model was predicted to have a urate/xanthine/oxonate binding site on the intracellular side of the membrane on the basis of homology to the enzyme uricase, both lectin binding sites on the extracellular surface of the membrane, an extracellularly-exposed domain homologous to the adenosine A1/A3 receptors, and intracellular amino and carboxy termini.

This model was validated in part by epitope tag studies and in part by reconstituting the channel in a lipid bilayer system. In the epitope tagging studies, both rat and human UAT proteins were fused with green fluorescent protein on the amino or carboxy termini and transfected into LLC-PK1 porcine renal tubule epithelial or MDCK (Madin-Darby canine kidney) cells [5,7]. Confocal micrographs of the cells demonstrate GFP expression that appears to be both in the apical and lateral portions of the cell membrane (Figure 2) [5,7]. There is also intracellular expression in the endoplasmic reticulum and golgi; however this may be due to overexpression of the protein by transfection, and varies over time being intense soon after transfection relative to membrane expression, but diminishing significantly relative to membrane expression at later time points. To determine whether the amino and carboxy termini of UAT were intracellular as predicted in our model (Figure 1), rat and human UAT were expressed with amino or carboxy-terminus FLAG epitopes, after which immunocytochemistry was performed with anti-FLAG antibodies using cell-permeabilizing or non-permeabilizing conditions. As can be seen in (Figure 3) [5,7], the FLAG epitope was only detected in permeabilized cells, demonstrating that both amino and carboxy terminus of hUAT are intracellular, and that secreted protein if present is not detected by our assay. To verify that the expressed protein was indeed spanning the cell membrane and not intracellular, cells were extensively washed after which the cell surface was biotinylated with the impermeant reagent sulfo-NHS-SS-biotin, cells were lysed, and biotinylated proteins captured on streptavidin beads. The captured proteins were then visualized on immunoblots [5,7]. As depicted in (Figure 4), GFP-tagged hUAT was detected by these studies (lanes 1,2), while GFP alone, which has a cytoplasmic distribution, was not detected (lane 3). These studies therefore demonstrated that hUAT must span the cell membrane at least twice, since both amino and carboxy termini are intracellular, while a portion of the protein is exposed to the biotinylating reagent on the cell surface. The inability to detect amino or carboxy termini without permeabilization excluded the possibility that secreted UAT is bound to the cell surface, while the extensive washing prior to cell surface biotinylation assured that the biotinylation reagent bound to a protein exposed at the cell surface rather than free secreted protein. Additional epitope tagging experiments failed to further identify other intracellular and extracellular domains as epitopes in predicted intracellular and extracellular domains

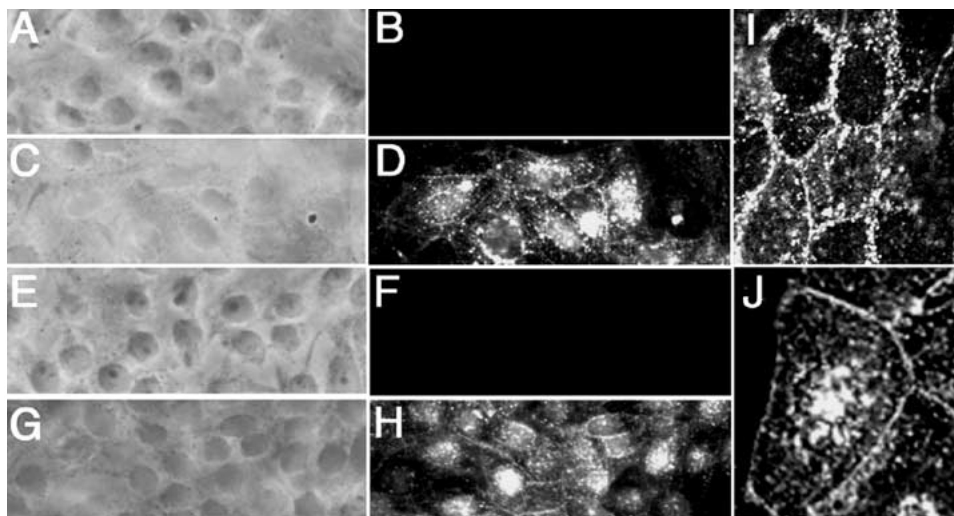

Figure 3. Brightfield, fluorescent, and confocal microscopy of non-permeabilized and permeabilized stable pools of LLC-PK1 cells expressing hUAT/FLAG chimeric proteins. A–D and I, cells transfected with constructs containing FLAG on the amino terminus of hUAT. E–H and J, cells transfected with constructs containing FLAG on the carboxy terminus of hUAT. A, C, E, and G are brightfield images of non-permeabilized (A and E) and permeabilized (C and G) cells. B, D, F, and H are fluorescent images of non-permeabilized (B and F) and permeabilized (D and H) cells. I and J are confocal images of permeabilized cells. Republished with the permission of The Journal of Clinical Investigation from "Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter." Lipkowitz *et al.*, *J Clin Invest* **107**, 1103–115 (2001); permission conveyed through Copyright Clearance Center, Inc.

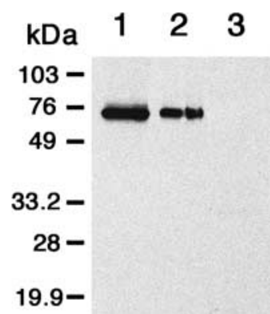

Figure 4. Western blot of surface biotinylated proteins harvested from stable pools of LLC-PK1 cells expressing hUAT/EGFP chimeric proteins or EGFP without hUAT. Lanes 1–3 contain eluates from LLC-PK1 cells transfected with constructs containing EGFP on the amino terminus of hUAT (lane 1), the carboxy terminus of hUAT(lane 2), and EGFP alone (lane 3). Lanes 1 and 3 contain 5 ul eluate; lane 2 contains approximately 0.3 ul eluate. Republished with the permission of The Journal of Clinical Investigation from "Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter." Lipkowitz *et al., J Clin Invest* **107**, 1103–115 (2001); permission conveyed through Copyright Clearance Center, Inc.

were not accessible to antibodies, most likely due to protein structure/folding.

Since we published this data, studies of others have also demonstrated that galectin 9 is detectable at the cell surface [49], although they have not confirmed that galectin 9 is a transmembrane protein.

Studies were also performed to confirm that UAT functions as a urate transporter/channel by reconstituting recombinant rat and human UAT in a lipid bilayer system. For these studies a lipid bilayer consisting of a 1:1 mix of phosphatidylserine:phosphatidylethanolamine was formed over a 20–50 μm hole separating two fluid filled chambers (Figure 5A). This system allows the control of the ion concentrations on each side of the bilayer and the voltage across the bilayer, and measures the currents of ions moving across the bilayer. After a bilayer was formed, recombinant UAT protein was sonicated with the same lipids to form proteoliposomes, which were then added to one chamber and subsequently fused with the bilayer (Figure 5B). The ratio of protein:lipid and the quantity of proteoliposomes added were adjusted with the goal of inserting a single ion channel into the bilayer. If necessary, unfused proteoliposomes and free protein were removed by changing the solutions in the chamber. Fusion of these UAT proteoliposomes with a lipid bilayer in the presence of urate resulted in ion channel activity [1,4,5,7,8] as depicted in (Figure 6A). There was no channel activity if an irrelevant protein (albumin) was sonicated into proteoliposomes, and there was no channel activity in the absence of urate (traces similar to Figure 7-ribose) indicating that the ion that was passing through the channel was urate, and that UAT was the channel protein. With urate in equal concentrations on both sides of the bilayer, there was clearly evident channel activity (6A) that resulted in a symmetrical current voltage relationship and a channel conductance of approximately 4 picoSiemens (6B). In the presence of a urate gradient across the bilayer, the chemical gradient of urate creates a urate current in the absence of an applied voltage (Figure 6C,

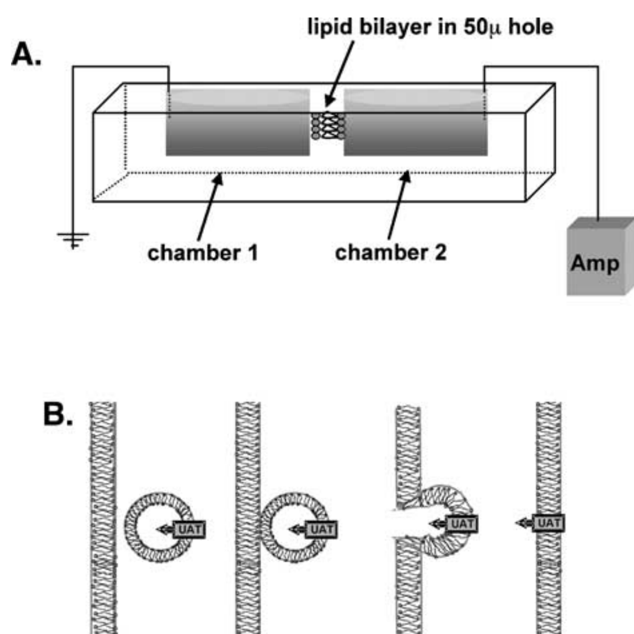

Figure 5. (a) A schematic drawing of the lipid bilayer chamber. Solutions with defined ion concentrations are placed in chambers 1 and 2 which are separated by a lipid bilayer that covers a 20–50 μ hole. An amplifier (Amp) applies voltage to chamber 2 (chamber 1 is grounded) and monitors current between the chambers. (b) A schematic of the fusion process for proteoliposomes conaining individual UAT channels with the bilayer.

$V = 0$ mV). The reversal potential (voltage required to null this current) shifted to appropriately -20 mV, which indicates that the current detected in the bilayer is a urate current, and that the channel is >100-fold more selective for urate than for other ions in the solutions.

Once it was verified that UAT was indeed a urate transporter/channel, further studies were performed to determine whether its properties were similar to the transporter we had described using brush border membrane vesicles [37,38,50]. As can be seen in Table 1, transport was electrogenic (driven by membrane potential) in membrane vesicles, which is consistent with the transporter being an anion channel. As with vesicles, the channel was inhibited by the specific inhibitors of urate transport oxonate and pyrazinoate [1,4,8], and was inhibited by the same antibody (anti-uricase) that inhibited transport in vesicles and was used to clone UAT [1,4,8,36]. Finally, transport in membrane vesicles was stimulated by copper [37,38], and in preliminary data we have shown that copper significantly increases the open probability of the channel in the bilayer system [51].

It should be noted that studies of others have confirmed that recombinant pig UAT is an electrogenic urate transporter: transfection of pig UAT into 293 cells resulted in electrogenic urate transport when compared to cells transfected with a control plasmid [52]. Other specific properties of the electrogenic transporter were not tested in these studies.

Our molecular model of the transporter predicted additional properties of the channel that had not been tested in membrane vesicles. First, homology to adenosine A1/A3 receptors was detected, and was confirmed to be functionally significant in that both urate channel activity in the bilayer [4,8] and urate transport into cells in culture [5] were inhibited by adenosine.

Second, since UAT is a galectin we predicted that channel activity might be regulated by specific sugars. While ligand dependent gating of ion channels is a well known phenomenon, direct regulation of channels by sugars has not previously been reported. We therefore tested this hypothesis by examining channel activity in the presence of sugars [4]. (Figure 7) depicts urate channel activity in the absence of sugar and after the sequential addition of ribose (50 mM), glucose (20 mM), and lactose (100 μ mol) to the extracellular side of the channel. As can be seen, there was virtually no channel activity in the absence of sugar as depicted in the current traces in Figure 7A. The observed current was 0 (Figure 7B), and the open probability approximated 0 (Figure 7C). In data not depicted, there was also minimal channel activity after addition of sugars to the intracellular face of the bilayer. Ribose added to the extracellular side of the channel had no effect on channel activity. However, addition of glucose to the extracellular side of the bilayer significantly increased the open probability of the channel to 13% (7C) with a current of about 0.3 picoAmperes (pA) (7B). After the addition of lactose open probability increased dramatically to approximately 63% (7C), and the most commonly observed current doubled to about 0.6 pA (Figure 7B).

Table 1. Comparison of urate transport by membrane vesicles from rat kidney, recombinant UAT in lipid bilayers, and by cells in culture

	Membrane vesicles	*Recombinant rUAT/hUAT*	*Cells in culture*
Electrogenic transport	X	X	X
Sugar Dependence	NT	X	NT
Oxonate Inhibition	X	X	NT
Pyrazinoate inhibition	X	X	NT
Adenosine inhibition	NT	X	X
Copper dependence	X	X	NT
Anti-uricase inhibition	X	X	NT

X = effect present; NT = not tested.

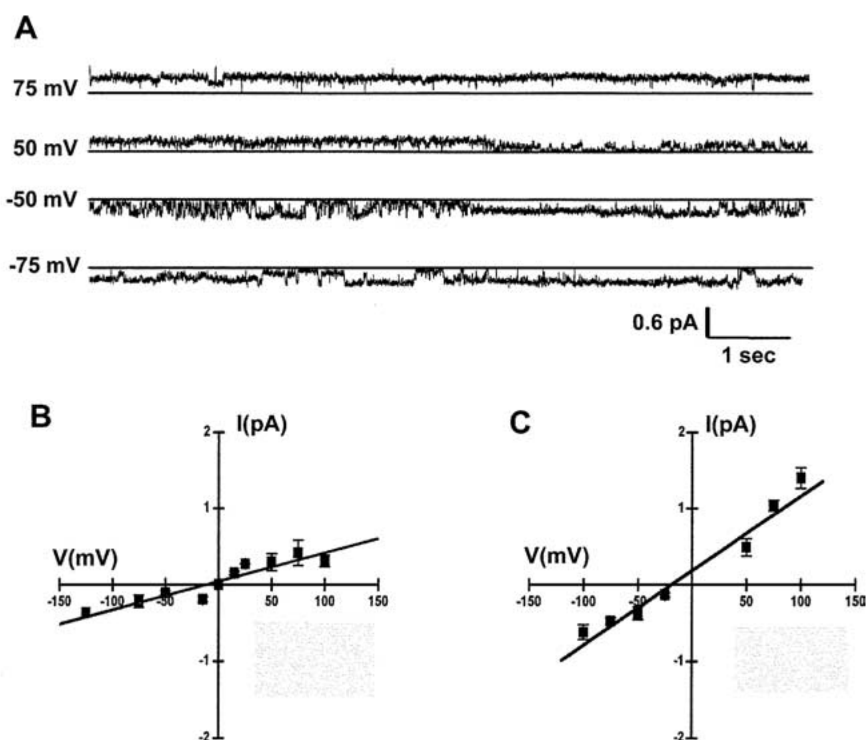

Figure 6. Representative traces and current/voltage relationship of hUAT channel activity in lipid bilayers. (a) Channel activity at various holding potentials (mV) in symmetrical urate solutions of 2.5 mM urate, 220 mM Cs_2SO_4, and 10 mM HEPES-NaOH at pH 7.4 after hUAT containing proteoliposomes fused with the planar lipid bilayer. Solid horizontal lines indicate the closed state. (b) Current/voltage (I/V) relationship of the hUAT channel in symmetrical urate solutions. Data represent the mean ± S.E. in 11 experiments. The solid line represents the best fit by linear regression analysis ($r = 0.97$) of G, the slope conductance in picoSiemens (pS). (c) Current/voltage (I/V) relationship of the hUAT channel in the presence of a 10:1 urate gradient (2.5 mM trans:0.25 mM cis). Data represent the mean ± S.E. in 4 experiments. The solid line represents the best fit by linear regression analysis ($r = 0.97$) of G, the slope conductance in picoSiemens (pS). Republished with the permission of The Journal of Clinical Investigation from "Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter." Lipkowitz *et al.*, *J Clin Invest* **107**, 1103–115 (2001); permission conveyed through Copyright Clearance Center, Inc.

A number of conclusions can be derived from these and other findings [4]. First, like other functions of galectins, channel activity of hUAT/galectin 9 is regulated by sugars. Second, since lactose is a much more potent stimulator of channel activity than glucose at 200-fold lower concentration, it appears that the affinity of the channel for lactose is much greater than that for glucose, consistent with the findings of others for sugar affinity of lectin binding sites [53]. While it has been noted that the full 130 amino acids of the carbohydrate recognition domains (CRD) of galectins are required for proper folding and sugar recognition, our model predicts that only 85–95 amino acids are exposed in the extracellular loops of UAT. Since UAT maintains both sensitivity and selectivity for sugars, we suggest that the stucture of UAT in the membrane substitutes for the availability of the entire CRD for folding. Finally the finding that lactose induces an increase in the urate channel's conductance that is an integer multiple of basal channel conductance suggests that exposure to lactose may result in multimerization of the channel. Two other pieces of evidence are consistent with this hypothesis. First, transmembrane domain 1 of UAT contains a region of homology to a dimerization motif in glycophorin A. Second, studies of UAT monomers fused with different epitopes and transfected into cells demonstrated that monomers with different tags co-immunoprecipitate, confirming the predicted multimerization [5]. Whether lactose results in a conformational change in hUAT that promotes multimerization, or whether binding of galactose and glucose to different lectin binding sites crosslinks individual monomers remains to be determined.

Regulation of UAT by sugars is a novel and important finding. While ligand dependent gating of ion channels has been reported, this is the first ion channel that is directly regulated by sugars. Further, there are reported abnormalities of urate homeostasis when blood sugar levels are dysregulated in diabetes. Modest elevations in blood glucose levels in diabetic patients [54] are associated with hyperuricemia. Since UAT is expressed in most tissues [1,2,5,7,9] we hypothesize that this hyperuricemia may occur via stimulation of systemic urate efflux from cells. When blood glucose rises to higher levels (at which glucose is excreted in the urine) patients may develop increased rates of urate excretion in the urine and concomitant hypouricemia [55,56], perhaps due to stimulation of excessive

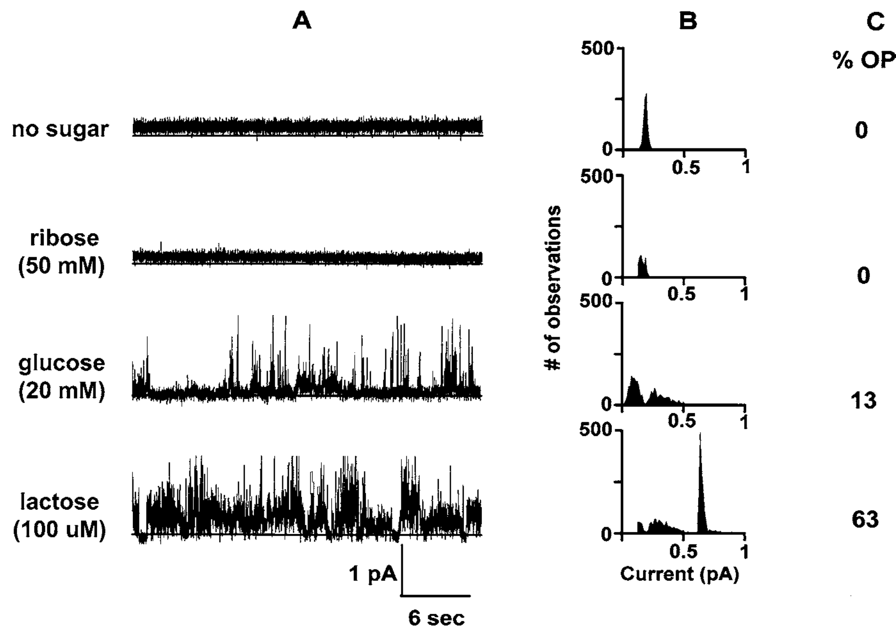

Figure 7. Representative traces of hUAT channel activity in lipid bilayers, current, and open probability. (A) Channel activity at 50 mV holding potential in symmetrical urate solutions of 2.5 mM urate, 220 mM Cs_2SO_4, and 10 mM HEPES-NaOH at pH 7.4 after hUAT containing proteoliposomes fused with the planar lipid bilayer. Solid horizontal lines indicate the closed state. Activity is recorded as picoamperes of current (pA). Traces were recorded in the absence of sugar followed by sequential addition of D-ribose (50 mM), D-glucose (20 mM) and α-lactose (100 μ mol). (B) histogram of observed currents in individual traces. (C) Channel open probability (percent of time in the open state, %OP).

urate secretion by glucose in the lumen of the renal proximal tubule, which by immunocytochemistry is very rich in UAT [6,36,55,56]. These transport abnormalities are reversed by correction of blood sugar levels [55].

The results of the lipid bilayer studies also confirm elements of our molecular model of UAT that could not be confirmed by epitope tagging. In particular oxonate and xanthine (which were predicted to act at the urate binding site in the intracellular loop, Figure 1) act at opposite sides of the bilayer from sugars and adenosine (predicted to act from the extracellular side, Figure 1). Combined with the cell culture data indicating that both amino and carboxy termini of UAT are intracellular, these findings are only possible if there are at least 4 transmembrane domains. Of note, in this model the unique functions of UAT as a urate transporter derive from amino acids that are in or adjacent to the linker region of the protein, which has little homology to other galectins or other proteins (Figure 1) [1,52].

Several studies have now demonstrated that hUAT/galectin 9 is expressed in cells as a number of isoforms. As depicted in (Figure 8), PCR of full length hUAT from colon and leukocytes (PBL) results in at least 4 isoforms whose expression levels differ in different cell types. Sequencing of these isoforms indicates that there is expression of at least 4 variants, one containing all 11 exons, one with exon 5 deleted, one with exon 10 deleted, and one with both exons 5 and 10 deleted. Additionally, deletion of exon 6 has been reported by others [52]. Our model was derived from studies performed on the most commonly expressed isoform, that with exon 5 deleted.

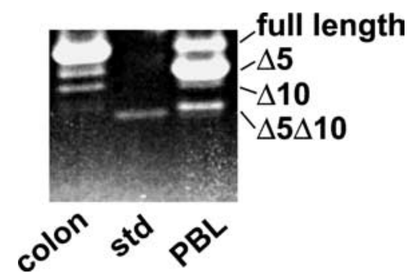

Figure 8. Ethidium stained polymerase chain reaction products of colon and peripheral blood lymphocyte (PBL) cDNA amplified with primers that specifically amplify a 1300 base pair fragment of hUAT encompassing both start and stop codons and separated in a 2% agarose gel. The 4 bands detected were verified by sequencing to be full length hUAT, exon 5 deletion (Δ5), exon 10 deletion (Δ10), and both exons 5 and 10 deleted (Δ5Δ10). STD = 1 kBP size standard.

Of interest, exon 5 is predicted to contain a second urate binding site, while exon 6 abuts the urate binding site described in our model. The isoforms with exon 10 deletions result in a frame shift in the translation of the protein, and early truncation before the second lectin binding site and 4th transmembrane domain. We have performed preliminary studies to determine whether these isoforms generate stable transmembrane proteins. When fused to GFP on their amino termini, all of the isoforms are expressed in cell culture as determined by GFP fluorescence and western blot of cell lysates; however while the full length and exon 5-deleted isoforms are transmembrane proteins, cell

surface biotinylation studies did not detect either of the exon 10 deleted isoforms, implying that these are not transmembrane proteins. Studies of others indicate that the various isoforms may subserve disparate functions in different cells. As an example of this, the full length protein may exhibit decreased urate transport as compared to the exon 5 deleted isoform, although expression of these isoforms was not normalized in the reported studies [52]. In contrast, this same full length protein may exhibit greater chemotactic activity than shorter isoforms [49]. No data are as yet available regarding the function of the exon 10 deleted isoforms, but the loss of membrane localization as well as one of the lectin binding sites suggests that this function could be quite disparate from any of the currently known functions of this protein.

In addition to the expression of multiple isoforms of UAT/galectin 9, our previous studies identified a 95% homologous gene/protein, hUAT2, that is expressed, albeit at significantly lower levels, in a number of tissues [7]. We have not yet tested the functions of this protein either as an ion channel or a chemoattractant.

The data reviewed in this paper indicate that UAT/galectin 9 is a protein that may serve a variety of functions that may differ in different organs. Our group has focused on the role of UAT as a urate ion channel, and we are particularly excited about the identification of UAT as one of the first examples of a sugar-regulated ion channel. Future directions will encompass elucidation of the mechanisms of channel multimerization as regulated by the lectin binding sites as well as assessment of the channel activity of the different isoforms of the protein and of hUAT2. In addition, preliminary data from our laboratory indicate that membrane content of the channel can be both up and down regulated by protein-protein interactions. Evaluation of the pathways that are involved in this regulation are likely to be extremely important as there is data that at least one genetic disorder of renal transport, Liddle's syndrome, is due to dysregulation of cell membrane content of the epithelial sodium channel resulting from a mutation in a protein interaction domain [57].

Acknowledgments

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References

1 Leal-Pinto E, Tao W, Rappaport J, *et al*., Molecular cloning and functional reconstitution of a urate transporter/channel, *J Biol Chem* **272**, 617–25 (1997).

2 Wada J, Kanwar YS, Identification and characterization of galectin-9, a novel beta-galactoside-binding mammalian lectin, *J Biol Chem* **272**, 6078–86 (1997).

3 Tureci O, Schmitt H, Fadle N, Pfreundschuh M, Sahin U, Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease, *J Biol Chem* **272**, 6416–22 (1997).

4 Leal-Pinto E, Cohen BE, Lipkowitz MS, Abramson RG, Functional analysis and molecular model of the human urate transporter/channel, hUAT, *Am J Physiol Renal Physiol* **283**, F150–63 (2002).

5 Rappoport JZ, Lipkowitz MS, Abramson RG, Localization and topology of a urate transporter/channel, a galectin, in epithelium-derived cells, *Am J Physiol Cell Physiol* **281**, C1926–39 (2001).

6 Hyink DP, Rappoport JZ, Wilson PD, Abramson RG, Expression of the urate transporter/channel is developmentally regulated in human kidneys, *Am J Physiol Renal Physiol* **281**, F875–86 (2001).

7 Lipkowitz MS, Leal-Pinto E, Rappoport JZ, Najfeld V, Abramson RG, Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter, *J Clin Invest* **107**, 1103–15 (2001).

8 Leal-Pinto E, Cohen BE, Abramson RG, Functional analysis and molecular modeling of a cloned urate transporter/channel, *J Membr Biol* **169**, 13–27 (1999).

9 Wada J, Ota K, Kumar A, Wallner EI, Kanwar YS, Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin, *J Clin Invest* **99**, 2452–61 (1997).

10 Matsumoto R, Matsumoto H, Seki M, *et al*., Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes, *J Biol Chem* **273**, 16976–84 (1998).

11 Saita N, Goto E, Yamamoto T, *et al*., Association of galectin-9 with eosinophil apoptosis, *Int Arch Allergy Immunol* **128**, 42–50, AA&action=render&rendertype=fulltext&uid=IAA.iaa28042 (2002).

12 Steele TH, Urate excretion in man, normal and gouty. In Handbook Experimental Pharmacology, edited by Kelley WN, Weiner IM. (Springer-Verlag, Berlin, Germany, 1978), vol. 51 pp. 257–86.

13 Saggiani F, Pilati S, Targher G, *et al*., Serum uric acid and related factors in 500 hospitalized subjects, *Metabolism* **45**, 1557–61 (1996).

14 Jossa F, Farinaro E, Panico S, *et al*., Serum uric acid and hypertension: The Olivetti heart study, *J Hum Hypertens* **8**, 677–81 (1994).

15 Goldstein HS, Manowitz P, Relation between serum uric acid and blood pressure in adolescents, *Ann Hum Biol* **20**, 423–31 (1993).

16 Mazzali M, Hughes J, Kim YG, *et al*., Hyperuricemia causes hypertension and renal diseae via a novel crystal-independent mechanism, *J Am Soc Nephrol* **11**, 337A (2000).

17 Moro F, Ogg CS, Simmonds HA, *et al*., Familial juvenile gouty nephropathy with renal urate hypoexcretion preceding renal disease, *Clin Nephrol* **35**, 263–9 (1991).

18 McBride MB, Raman V, Ogg CS, *et al*., Renal urate hypoexcretion preceding renal disease in a new kindred with familial juvenile gouty nephropathy (FJGN), *Adv Exp Med Biol* 191–4 (1991).

19 Yokota N, Yamanaka H, Yamamoto Y, *et al*., Autosomal dominant transmission of gouty arthritis with renal disease in a large Japanese family, *Ann Rheum Dis* **50**, 108–11 (1991).

20 Saeki A, Hosoya T, Okabe H, *et al*., Newly discovered familial juvenile gouty nephropathy in a Japanese family, *Nephron* **70**, 359–66 (1995).

21 McBride MB, Rigden S, Haycock GB, *et al*., Presymptomatic detection of familial juvenile hyperuricaemic nephropathy in children, *Pediatr Nephrol* **12**, 357–64 (1998).
22 Massari PU, Hsu CH, Barnes RV, *et al*., Familial hyperuricemia and renal disease, *Arch Intern Med* **140**, 680–4 (1980).
23 Puig JG, Miranda ME, Mateos FA, *et al*., Hereditary nephropathy associated with hyperuricemia and gout, *Arch Intern Med* **153**, 357–65 (1993).
24 Reiter L, Brown MA, Edmonds J, Familial hyperuricemic nephropathy, *Am J Kidney Dis* **25**, 235–41 (1995).
25 Syrjanen J, Mustonen J, Pasternack A, Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy, *Nephrol Dial Transplant* **15**, 34–42 (2000).
26 Langford HG, Blaufox MD, Borhani NO, *et al*., Is thiazide-produced uric acid elevation harmful? Analysis of data from the hypertension detection and follow-up program, *Arch Intern Med* **147**, 645–9 (1987).
27 Bengtsson C, Lapidus L, Stendahl C, Waldenstrom J, Hyperuricaemia and risk of cardiovascular disease and overall death. A 12-year follow-up of participants in the population study of women in Gothenburg, Sweden, *Acta Med Scand* **224**, 549–55 (1988).
28 Freedman DS, Williamson DF, Gunter EW, Byers T, Relation of serum uric acid to mortality and ischemic heart disease. The NHANES I Epidemiologic Follow-up Study, *Am J Epidemiol* **141**, 637–44 (1995).
29 Alderman MH, Cohen H, Madhavan S, Kivlighn S, Serum uric acid and cardiovascular events in successfully treated hypertensive patients, *Hypertension* **34**, 144–50 (1999).
30 Johnson RJ, Kivlighn SD, Kim YG, Suga S, Fogo AB, Reappraisal of the pathogenesis and consequences of hyperuricemia in hypertension, cardiovascular disease, and renal disease, *Am J Kidney Dis* **33**, 225–34 (1999).
31 Culleton BF, Larson MG, Kannel WB, Levy D, Serum uric acid and risk for cardiovascular disease and death: The framingham heart study [see comments], *Ann Intern Med* **131**, 7–13 (1999).
32 Dobson A, Is raised serum uric acid a cause of cardiovascular disease or death? *Lancet* **354**, 1578 (1999).
33 Johnson RJ, Tuttle KR, Culleton B, Levy D, Much ado about nothing, or much to do about something? The continuing controversy over the role of uric acid in cardiovascular disease, *Hypertension* **35**, E10–3 (2000).
34 Abramson RG, Levitt MF, Micropuncture study of uric acid transport in rat kidney, *Am J Physiol* **228**, 1597–1605 (1975).
35 Abramson RG, Lipkowitz MS, Evolution of the uric acid transport mechanisms in vertebrate kidney. In edited by Kinne RKH. *Basic Principles in Transport* (Basel Switzerland, Karger, 1990), vol 3., pp. 115–53.
36 Knorr BA, Lipkowitz MS, Potter BJ, Masur SK, Abramson RG, Isolation and immunolocalization of a rat renal cortical membrane urate transporter, *J Biol Chem* **269**, 6759–64 (1994).
37 Abramson RG, King VF, Reif MC, Leal-Pinto E, Baruch SB, Urate uptake in membrane vesicles of rat renal cortex: Effect of copper, *Am J Physiol* **242**, F158–70 (1982).
38 Abramson RG, Lipkowitz MS, Carrier-mediated concentrative urate transport in rat renal membrane vesicles, *Am J Physiol* **248**, F574–84 (1985).
39 Guggino SE, Martin GJ, Aronson PS, Specificity and modes of the anion exchanger in dog renal microvillus membranes, *Am J Physiol* **244**, F612–21 (1983).
40 Kahn AM, Aronson PS, Urate transport via anion exchange in dog renal microvillus membrane vesicles, *Am J Physiol* **244**, F56–63 (1983).
41 Roch-Ramel F, Werner D, Guisan B, Urate transport in brush-border membrane of human kidney, *Am J Physiol* **266**, F797–805 (1994).
42 Blomstedt JW, Aronson PS, pH gradient-stimulated transport of urate and p-aminohippurate in dog renal microvillus membrane vesicles, *J Clin Invest* **65**, 931–4 (1980).
43 Enomoto A, Kimura H, Chairoungdua A, *et al*., Molecular identification of a renal urate anion exchanger that regulates blood urate levels, *Nature* **417**, 447–52 (2002).
44 Barondes SH, Gitt MA, Leffler H, Cooper DN, Multiple soluble vertebrate galactoside-binding lectins, *Biochimie* **70**, 1627–32 (1988).
45 Gitt MA, Massa SM, Leffler H, Barondes SH, Isolation and expression of a gene encoding L-14-II, a new human soluble lactose-binding lectin, *J Biol Chem* **267**, 10601–6 (1992).
46 Hughes RC, Secretion of the galectin family of mammalian carbohydrate-binding proteins, *Biochim Biophys Acta* **1473**, 172–85 (1999).
47 Oda Y, Herrmann J, Gitt MA, *et al*., Soluble lactose-binding lectin from rat intestine with two different carbohydrate-binding domains in the same peptide chain, *J Biol Chem* **268**, 5929–39 (1993).
48 Hirashima M, Ecalectin as a T cell-derived eosinophil chemoattractant [In Process Citation], *Int Arch Allergy Immunol* **120**, 7–10 (1999).
49 Chabot S, Kashio Y, Seki M, *et al*., Regulation of galectin-9 expression and release in Jurkat T cell line cells, *Glycobiology* **12**, 111–8 (2002).
50 Knorr BA, Beck JC, Abramson RG, Classical and channel-like urate transporters in rabbit renal brush border membranes, *Kidney Int* **45**, 727–36 (1994).
51 Leal-Pinto E, Lipkowitz MS, Cohen BE, Abramson RG, Activity of rat and human urate transporters, rUAT and hUAT, is regulated by copper, *J Am Soc Neph* **11** (Program and Abstracts), 45A (2000).
52 Spitzenberger F, Graessler J, Schroeder HE, Molecular and functional characterization of galectin 9 mRNA isoforms in porcine and human cells and tissues, *Biochimie* **83**, 851–62 (2001).
53 Ali N, Salahuddin A, Isolation and characterization of soluble beta-galactoside-binding lectins from mammalian liver, *Biochim Biophys Acta* **992**, 30–4 (1989).
54 Whitehead TP, Jungner I, Robinson D, *et al*., Serum urate, serum glucose and diabetes, *Ann Clin Biochem* **29**, 159–61 (1992).
55 Gonzalez-Sicilia L, Garcia-Estan J, Martinez-Blazquez A, *et al*., Renal metabolism of uric acid in type I insulin-dependent diabetic patients: Relation to metabolic compensation, *Horm Metab Res* **29**, 520–3 (1997).
56 Magoula I, Tsapas G, Paletas K, Mavromatidis K, Insulin-dependent diabetes and renal hypouricemia, *Nephron* **59**, 21–6 (1991).
57 Lifton RP, Gharavi AG, Geller DS, Molecular mechanisms of human hypertension, *Cell* **104**, 545–56 (2001).