Original Article

Ribosomal protein L3 mediated the transport of digoxin in *Xenopus laevis* oocyte

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ABSTRACT — Ribosomal protein L3 (RPL3) is known to be an indispensable and essential component for the peptidyltransferase center. In the present study, we found a novel function of RPL3 using a *Xenopus laevis* oocyte expression system. When expressed in *X. laevis* oocytes, RPL3 mediated the high affinity transport of [³H]digoxin ($K_m = 213.3 \pm 46.8$ nM) in a time-, concentration-, and sodium-dependent manners. The maximum velocity of the transport of [³H]digoxin via RPL3 produced at physiological pH. However, we did not observe RPL3-mediated transport of several organic solutes such as [¹⁴C]androstenedione, [³H]dexamethasone, [³H]dehydroepiandrosterone sulfate, [³H]L-tryptophan, [¹⁴C]L-ascorbic acid, [¹⁴C] α -ketoglutarate, [¹⁴C]glutarate, [³H]methotrexate, [³H]bumetanide, [³H]probenecid, [¹⁴C]salicylic acid, [¹⁴C]theophylline and [³H]valproate. Our results suggest that RPL3 functions as a drug carrier protein and may be involved in the digoxin toxicity in the human body.

Key words: Ribosomal protein, RPL3, Drug transporter, Organic solutes, Digoxin toxicity

INTRODUCTION

Ribosomes are highly conserved nucleoprotein and composed of four RNA species with eighty different gene products. Ribosomal protein holds a unique position from a toxicological and pharmacological point of view. Among them, ribosomal protein L3 (RPL3) is known to be an indispensable and essential component for the peptidyltransferase center (Meskauskas and Dinman, 2007), and appears to be extremely important because it is widely distributed and highly conserved across species (Meskauskas and Dinman, 2007). RPL3 also acts as a binding site for a ribosome inhibitory protein (Meskauskas and Dinman, 2007). In addition, RPL3 has a role in drug resistance (Meskauskas and Dinman, 2007), suggesting that this protein may be involved in the regulation of drug clearance.

Therapeutic achievement of drug concentration in target organs regulates several sequential steps; uptake, intracellular processing, elimination, excretion, and metabolism. Although the idea of these processes has been intensively researched during the past decades in the human liver, kidney, brain and placenta; the uptake step is

one of an important process because there are many different kinds of membrane-anchored proteins called transporters are expressed in these organs (Klein *et al.*, 1999; Saier, 2000; Van Aubel *et al.*, 2000; Leonard *et al.*, 2003; Leonessa and Clarke, 2003; Rizwan and Burckhardt, 2007).

The adenosine 5'-triphosphate (ATP)-binding cassette (ABC) transporters are involved in the transport of a wide range of lipophilic and ionic compounds across plasma membranes and function as barriers against the entry of toxic chemicals. Among them, ABCB1/P-glycoprotein (Meissner *et al.*, 2002), ABCC5/multi-drug resistant protein 5 (MRP5) (McAleer *et al.*, 1999), and ABCC9/SUR2 (Shi *et al.*, 2005; Melaine *et al.*, 2006) are predominantly expressed in endothelial cells and act as a functional barrier between blood and cardiomyocyte resulting the reduction of the intracellular concentration of organic compounds. On the other hand, organic anion transporting polypeptide-B (OATP-B[*SLC21A9/SLCO21A9*]) (Tamai *et al.*, 2000), Fly-like putative transporter 1 (FLIPT1[*SLC22A15*]) and FLIPT2[*SLC22A16*] (formerly known as hOCT6[hCT2]) (Eraly and Nigam, 2002), human organic cation transporter 1 (hOCT1[*SLC22A1*])

(Zhang *et al.*, 1997) and 3 (hOCT3[*SLC22A3*]) (Wu *et al.*, 1998a), human organic cation transporter novel 2 (hOCTN2[*SLC22A5*]) (Wu *et al.*, 1998b), human multidrug and toxin extrusion 1 (hMATE1) (Otsuka *et al.*, 2005) transporters have been also known to be expressed in the heart; however, none of these transporters functions as the uptake carrier protein for organic solutes. In this respect, Grube *et al.* (2006) have revealed that OATP2B1(*SLC21A9*[*SLCO2B1*]) is a high-affinity uptake transporter for atorvastatin. Because secondary structural signature of these transporters have been predicted to be several membrane spanning domains, these findings lead us to hypothesize that membrane-bound protein signature is an indispensable structural feature(s) to stimulate the drug transport. Taking these findings into consideration, RPL3 may have function as a drug carrier protein.

In the present study, therefore, we focused on RPL3 and tested the transport characterization using a *X. oocyte* expression system. Although RPL3 plays an important role in the protein synthesis, our results indicate that RPL3 may function as a drug carrier protein. Our findings would provide new insights into a novel function of ribosomal protein, especially RPL3.

MATERIALS AND METHODS

Materials

[³H]Digoxin (40 Ci/mmol), [³H]dehydroepiandrosterone sulfate (74 Ci/mmol), [¹⁴C]salicylic acid (55.5 mCi/mmol) and [¹⁴C]L-ascorbic acid (4 mCi/mmol) were purchased from Perkin Elmer Life Sciences, Inc. (Boston, MA, USA). [¹⁴C]Glutarate (50 Ci/mmol), [¹⁴C]androstenedione (55 Ci/mmol), [³H]bumetanide (20 Ci/mmol), [¹⁴C]theophylline (52 mCi/mmol), [³H]methotrexate (40 Ci/mmol) and [³H]L-tryptophan (20 Ci/mmol) were purchased from ARC Inc. (St. Louis, MO, USA). [³H]Dexamethasone and [³H]probenecid were purchased from Amersham Biosciences (Buckinghamshire, UK). [¹⁴C]α-Ketoglutarate (54.5 mCi/mmol) was purchased from NENTM Life Science Product, Inc. (Boston, MA, USA). [³H]Valproate (55 Ci/mmol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). Deoxycytidine [5'-α-³²P]triphosphate (dCTP) (111 TBq/mmol) was obtained from Muromachi Yakuhin Kaisha, LTD. (Tokyo, Japan). All other chemicals not listed here were of the highest grade commercially available.

Construction of cDNA library and isolation of RPL3

Human heart aorta poly (A)⁺ RNA was purchased from BD Biosciences Clontech (Palo Alto, CA, USA). This

mRNA was used for construction of cDNA Library and the uptake experiments. A nondirectional cDNA library was prepared from human heart aorta poly (A)⁺ RNA using the Superscript Choice System (Life Technologies, Gaithersburg, MD, USA) and was ligated into a phage vector λZipLox *Eco*RI arms (Life Technologies). An Expressed Sequence Tag (EST) data base searched for "Query SLCO2A1" (NM_005630) and an EST clone (AI709009) was identified. After PCR amplification of this EST clone (left primer, 5'-GGATGGCATGGAT-AGTCAGG-3'; right primer, 5'-GACAGAAGCCAAC-GAAGGAG-3'), the PCR product was labeled with [5'-α-³²P]dCTP by random priming (¹⁷Quick Prime Kit, BD Biosciences Clontech (Palo Alto, CA, USA)), and the library was screened with an EST clone as a probe under low stringency conditions. Replicate filters of a phage library were hybridized overnight in a hybridization solution (50% formamide; 5x standard saline citrate (SSC); 3x Denhardt's solution; 0.2% sodium dodecyl sulfate (SDS); 10% dextran sulfate; 0.3 μg/ml denatured salmon sperm DNA; 2.5 mM sodium pyrophosphate; 25 mM 4-morpholineethane sulfonic acid (MES); 0.03% Antifoam A; pH 6.5) at 37°C overnight. The filters were washed in 3 x SSC and 0.5% SDS at 37°C. cDNA inserts in positive λZipLox phages were recovered in a plasmid pZL1 vector by *in vitro* excision.

cDNA Sequence

Double-stranded cDNA of isolated clones were sequenced in both directions. Deleted clones, obtained by a KiloSequence deletion kit (Takara, Tokyo, Japan), and specifically synthesized oligonucleotide primers were used for sequencing RPL3 cDNA, which was sequenced by the dye terminator method using a dye primer cycle sequencing kit (ver. 3.1., Applied Biosystems, Foster City, CA, USA) and automated Applied Biosystems 310 DNA sequencer. The sequence was analyzed using DNASIS[®]-Pro. Ver. 2.02 (HITACHI Software Engineering, Yokohama, Japan).

cRNA Synthesis and functional characterization of RPL3 in *Xenopus laevis* oocytes

Selection and isolation of stage V and VI defolliculated *X. oocytes* was performed as described elsewhere (Kobayashi *et al.*, 2002). Collagenase A (Boehringer Mannheim, Mannheim, Germany) was used to remove the follicular layer at a final concentration of 2.0 mg/ml in a oocyte Ringer 2 (OR2) solution (83 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 5 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), pH 7.5). The RPL3 cDNA was linearized with *Hind*III, and capped cRNA

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was transcribed *in vitro* by T7 RNA polymerase. Defolliculated oocytes were microinjected with 50 ng of *in vitro* transcribed cRNA and incubated for two to three days in a modified Barth's solution containing gentamicin (50 µg/ml) at 18°C. Uptake experiments of radiolabeled substrates, as indicated in each experiment, were performed in ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.4) at room temperature. Oocytes were incubated in 150 µl of the same solution containing radiolabeled substrates for 1 hr at room temperature. For Na⁺-dependent transport experiments, we used ND96 buffer containing lithium chloride, choline choloride, mannitol, and N-metyl-D-glucamine with an equivalent amount of sodium chloride. Concentration-dependent uptake experiments of [³H]digoxin in oocytes expressing RPL3 were performed at final concentrations of 1, 10, 20, 50, 100, 150 and 300 nM. The compound was incubated with expressing RPL3 oocytes for 1 hr at room temperature, stopped with ice-cold ND96 solution and washed 5 times as described above. Three oocytes were transferred to one scintillation vial and dissolved in 200 µl 10% SDS. A scintillation cocktail was added, and radioactivity was counted. Counts in cRNA minus uninjected oocytes were subtracted from the counts in RPL3 cRNA-injected oocytes. Data are presented as mean ± S.E.M., except for kinetic constants for which the error represents the error of the fit. K_m indicates the Michaelis-Menten constant (nM). The experiments were repeated with oocytes from at least five to six frogs.

Statistical analysis

Kinetic data from experiments measuring the uptake of radiolabeled substrates were fit to the Michaelis-Menten equation by nonlinear least-squares regression analysis with standard errors derived from these curves (GraphPad Prism Version 4.01). Comparisons of data measuring initial rates of uptake of radiolabeled substrates in the presence and absence of inhibitors were performed by the unpaired Student's *t* test (GraphPad Prism Version 4.01).

RESULTS

Because of the human prostaglandin transporter (hPGT) (*SLC2A1/SLCO2A1/OATP2A1*) (NM_005630) gene is expressed ubiquitously in the human body (<http://www.bioparadigms.org/slc/menu.asp>), we chose this gene for screening and an EST clone (AI709009) was identified. After multiple rounds of screening using [³²P]dCTP-labeled EST clone under low stringency condition, we isolated several positive clones. These clones had over-

lapping identical sequences, identical to RPL3. The nucleotide and deduced amino acid sequences of RPL3 are shown in Fig. 1A. RPL3 cDNA is 1255 nucleotides long and codes for a protein of 351 amino acids with a calculated molecular mass of 40,265.2 Da (GenBank™/EMBL/DDBJ accession number: AB26619). As shown in Fig. 1A, there is one consensus sequence for *N*-glycosylation sites (Thr²⁵⁰) (●). Kyte and Doolittle hydropathy analysis (Kyte and Doolittle, 1982) of RPL3 protein and revealed that this protein has at least one hydrophobic stretches long enough to span the membrane as α -helices (Fig. 1B).

When we submitted the RPL3 sequence to Genbank, a similar sequence from humans had been deposited to GenBank (GenBank™/EMBL/DDBJ: NM_000967, Homo sapiens RPL3 transcript variant 1). The nucleotide sequence of RPL3 is 99% identical to that of RPL3 except for 1 base: (T at position 986 to C) resulting in no amino acid changes. We also identified several human gene products (GenBank™/EMBL/DDBJ: XR_019077, XR_016455, XR_018187, XR_017039, XR_016530, XR_018016, XR_018281, XR_017333, XM_936228) and RPL3 transcript variant 2 (GenBank™/EMBL/DDBJ: NM_001033853) that exhibited approximately 85% and 99%, respectively, predicted amino acid identity.

Because RPL3 predicted to have at least one membrane spanning domain, we assumed that RPL3 can transport organic solutes like CD98/4F2hc (Wells *et al.*, 1992; Kanai *et al.*, 1998). Therefore, *X. laevis* oocytes injected with RPL3 cRNA were used for the transport characterization. As shown in Table 1, oocyte expressing RPL3 exhibited the transport of [³H]digoxin to about 4 fold that of the control oocytes. However, RPL3 cRNA-injected oocytes exhibited no transport of [¹⁴C]androstenedione, [³H]dexamethasone, [³H]dehydroepiandrosterone sulfate, [³H]L-tryptophan, [¹⁴C]L-ascorbic acid, [¹⁴C] α -ketoglutarate, [¹⁴C]glutarate, [³H]methotrexate, [³H]bumetanide, [³H]probenecid, [¹⁴C]salicylate, [¹⁴C]theophylline and [³H]valproate, indicating that [³H]digoxin would be a substrate of RPL3.

On the basis of these results, we subsequently examined the pharmacological characterization of this clone. As shown in Fig. 2A, the transport activity of [³H]digoxin via oocyte expressing RPL3 increased linearly until 2.5 hr of incubation. The concentration-dependent transport of [³H]digoxin via RPL3 is shown in Fig. 2B. The RPL3-mediated uptake of [³H]digoxin showed saturable kinetics and could be modeled by the Michaelis-Menten equation. Nonlinear regression analyses yielded K_m values of 213.3 ± 46.8 nM. The effect of extracellular cation on the uptake of [³H]digoxin was studied (Fig. 2C). The uptake

Fig. 1. A, cDNA and deduced amino acid sequences of RPL3. Putative *N*-glycosylation sites are indicated by closed circles (●). B, Kyte and Doolittle hydropathy plot of RPL3 (window 9). Hydrophobic peaks, which seem to be membrane spanning domain, is numbered 1 (TMD1). The *abscissa* indicates amino acid number. The *ordinate* is hydropathy.

of [³H]digoxin via RPL3 was affected by the replacement of extracellular sodium with choline, lithium, mannitol, and N-methyl-D-glucamine (NMDG), indicating that RPL3 is a Na⁺-dependent. The effect of pH on the uptake of [³H]digoxin by RPL3-expressing oocytes was

subsequently examined (Fig. 2D). There was a change in [³H]digoxin transport with a pH-dependent manner up to pH 7.5; however, the activity was decreased by pH 8.5. Thus, the uptake of [³H]digoxin mediated by RPL3 is sensitive to pH, and maximum velocity of the transport

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Table 1. Uptake of various ^{14}C - and ^3H -labeled compounds by RPL3-expressing oocytes

Tracer	Concentration	Non-injected	RPL3	Fold increase
^{14}C]Androstenedione	2 μM	12.43 $\pm$ 0.11	11.88 $\pm$ 0.16	0.96
^3H]Dexamethasone	20 nM	14.92 $\pm$ 0.64	16.38 $\pm$ 0.69	1.10
^3H]DHEA-S	10 nM	2.47 $\pm$ 0.03	2.80 $\pm$ 0.15	1.13
^3H]L-Tryptophan	50 nM	65.40 $\pm$ 1.96	63.56 $\pm$ 2.05	0.97
^{14}C]L-Ascorbic acid	25 μM	0.81 $\pm$ 0.05	0.90 $\pm$ 0.11	1.11
^{14}C] α -Ketoglutarate	5 μM	1.14 $\pm$ 0.07	1.10 $\pm$ 0.08	0.96
^{14}C]Glutarate	5 μM	4.06 $\pm$ 0.26	4.25 $\pm$ 0.22	1.05
^3H]Methotrexate	100 nM	5.92 $\pm$ 0.29	6.84 $\pm$ 0.29	1.16
^3H]Digoxin	50 nM	3.17 $\pm$ 0.18	12.24 $\pm$ 0.61*	3.86
^3H]Bumetanide	100 nM	2.97 $\pm$ 0.07	3.40 $\pm$ 0.55	1.14
^3H]Probenecid	2 μM	1.05 $\pm$ 0.02	1.06 $\pm$ 0.04	1.01
^{14}C]Salicylate	5 μM	2.26 $\pm$ 0.04	2.33 $\pm$ 0.04	1.03
^{14}C]Theophylline	2 μM	3.46 $\pm$ 0.06	3.59 $\pm$ 0.08	1.04
^3H]Valproate	50 nM	23.6 $\pm$ 1.63	27.5 $\pm$ 1.53	1.17

After two days incubation, uptake experiments were performed in a ND96 solution containing for 1 hr. Values are mean $\pm$ S.E.M. of 9-15 oocyte determinations. The significance between control (non-injected) and RPL3-cRNA-injected oocytes was determined by the unpaired *t* test (**p* < 0.05). The units of measurement were femtomoles per oocyte per hour for ^3H tracers and picomoles per oocyte per hour for ^{14}C tracers. Other experimental conditions and methods are described in *Materials and Methods*. DHEA-S, dehydroepiandrosterone sulfate.

activity is approximately physiological pH.

Because RPL3 is known to be widely distributed and highly conserved across species (Meskauskas and Dinman, 2007), we subsequently searched for a regulator or activator on the transport of ^3H]digoxin via RPL3. The transport activity was compared with the oocytes injected with RPL3 cRNA alone or with RPL3 cRNA plus human heart aorta mRNA. The result is shown in Fig. 3. As indicated in Fig. 3, however, we did not observe any significant differences in RPL3-mediated uptake of ^3H]digoxin between RPL3-cRNA-injected oocytes and RPL3-cRNA plus human heart aorta mRNA-injected oocytes.

DISCUSSION

In the present study, we reports here with a novel function of RPL3. We found that the ribosomal protein L3 (RPL3) can transport of digoxin in a time-, concentration-, sodium-, and pH-dependent manners.

To date, several kinds of organic solute carrier protein have been reported (<http://www.bioparadigms.org/slc/menu.asp>), and these transporters are predicted to

have several membrane spanning domains (Fujieda *et al.*, 2009; Sekine and Endou, 2009). For example, the OAT (*SLC22A*), the OCT (*SLC22A*) and the OATP (*SLC21A/SLC21A*) families predict to have 10 to 12 membrane spanning domains. Similarly, ABC transporters also predicted to have 6 to 12 membrane spanning domains (Solbach *et al.*, 2006). Based on these findings, Kyte and Doolittle hydrophathy plot (window 9) analysis was firstly examined to examine whether RPL3 have membrane spanning domain(s). We observed that RPL3 is likely to have one membrane spanning domains. To confirm this, TMPred-Prediction of Transmembrane Regions and Orientation program (http://www.ch.embnet.org/software/TMPRED_form.html) also used and predicted that the first 27 amino acids (amino acid sequence from 23 to 40) is a transmembrane domain; therefore, at least, the mature RPL3 protein would have one membrane spanning domain. Taking these findings together with the published papers (Fujieda *et al.*, 2009; Sekine and Endou, 2009), we assumed that, like the organic anion transporter (OAT) family and the lambda light chain of human immunoglobulin surface antigen-related gene (IgLC-rG) (Kobayashi *et al.*, 2010),

Fig. 2. Functional characterization of RPL3 in *Xenopus laevis* Oocytes. A, Time -dependent uptake of [³H]digoxin in RPL3-expressing oocytes. The uptake of 50 nM digoxin in oocytes expressing RPL3 was measured during 2.5 hr of incubation. B, Concentration-dependence of RPL3-mediated uptake of [³H]digoxin. The uptake rate of digoxin by the control and RPL3-expressing oocytes for 1 hr were measured at variable concentrations. C, Effect of extracellular cation on [³H]digoxin uptake in *Xenopus laevis* oocytes expressing RPL3. The uptake rates of [³H]digoxin (50 nM) by control oocytes or RPL3-expressing oocytes for 1 hr were measured in the presence or absence of extracellular Na⁺. Extracellular Na⁺ was replaced with an equimolar concentration of choline, lithium, mannitol, and NMDG. The significance between control and RPL3-cRNA-injected oocytes was determined by the unpaired *t* test (**p* < 0.01). D, Effect of pH on [³H]digoxin transports in RPL3-expressing oocytes. One-hour uptakes of 50 nM [³H]digoxin was measured in Na⁺ buffers adjusted to pH values ranging from 5.5 to 8.5. Other experimental conditions and details are described under "Materials and Methods" Data are mean $\pm$ S.E.M. (*n* = 7-12 oocytes).

RPL3 can transport of several organic solutes; therefore, we subsequently examined the transport assays using a *X. laevis* oocyte expression system.

When expressed in *X. laevis* oocytes, it was of interest that RPL3 mediates the high affinity transport of [³H]digoxin ($K_m = 213.3 \pm 46.8$ nM). However, we did not observe the RPL3-mediated transport of several organic solutes ([¹⁴C]androstenedione, [³H]dexamethasone, [³H]dehydroepiandrosterone sulfate, [³H]L-tryptophan, [³H]probenecid,

[¹⁴C]L-ascorbic acid, [¹⁴C] α -ketoglutarate, [¹⁴C]glutarate, [³H]methotrexate, [³H]bumetanide, [³H]probenecid, [¹⁴C]salicylic acid, [¹⁴C]theophylline and [³H]valproate). These findings indicate that, at least in part, [³H]digoxin is a substrate of RPL3.

Digoxin is a potent selective inhibitor of Na⁺,K⁺-ATPase, which is located on the surface of smooth muscle cells, and is clinically used for the treatment of heart failure and resulted in the reduction of the rate of patient's

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Fig. 3. Functional co-expression of RPL3 cRNA and human heart aorta mRNA in *Xenopus Laevis* Oocytes. Uptake of [³H]digoxin (50 nM) was compared between *Xenopus laevis* oocytes injected with RPL3 cRNA (50 ng) (labeled as “mRNA”, Red column; labeled as “cRNA”, yellow column; both RPL3 cRNA (50 ng) and human aorta poly(A)⁺ RNA (5 ng) (labeled as “cRNA + mRNA”, blue column). The significance between control and mRNA, cRNA and/or cRNA + mRNA was determined by the unpaired *t* test (**p* < 0.01). The transport activity of the cRNA + mRNA co-expression slightly increased.

hospitalization (Li-Saw-Hee and Lip, 1998). Because digoxin has a narrow therapeutic range and interacts with many kinds of clinically used drugs when concomitantly administered, it is difficult to control and maintain the serum digoxin level specifically in renal failure patients (Li-Saw-Hee and Lip, 1998). Concerning the drug transporters in the human heart, OCT1[*SLC22A1*] (Slitt *et al.*, 2002), hOCTN1[*SLC22A4*] (Slitt *et al.*, 2002), hOCTN2 (human organic cation transporter novel 1) [*SLC22A5*] (Wu *et al.*, 1998a), hMATE1[*SLC47A2*] (Otsuka *et al.*, 2005), and hPGT (*OATP2A1/SLC02A1*) (Lu *et al.*, 1996) have known to be expressed; however, none of these transporters are involved in the digoxin transport to date. Taking these findings and our uptake experiments into consideration, RPL3 may play an important role in the heart handling of digoxin. In this respect, further study is needed.

Kanai *et al.* (1998) revealed that L-type amino acid transporter 1 (LAT1[*SLC7A5*]) requires the heavy chain of 4F2 cell surface antigen (CD98). Ballatori *et al.* (2005) revealed that when organic solute transporter α and β (Ost α and Ost β) are coexpressed in *X. oocytes*, they are able to transport of digoxin. Based on their findings,

we tested whether the RPL3 protein functions is needed to form the maximum transport activity. Because we isolated RPL3 cDNA from a human heart aorta cDNA library, both RPL3 cRNA and human heart aorta mRNA are microinjected and tested transport assay. Interestingly, we found that the synergic augmentation of the transport of [³H]digoxin uptake was not detected by expression with RPL3 cRNA alone, suggesting that the transport of [³H]digoxin via RPL3 may be a single transport system. Further detailed study will be required as to whether this protein is functioning as a transport activator or a regulator. It would be interesting to elucidate whether RPL3 is coupled to other ribosomal protein unit or unidentified proteins.

RPL3 cDNA and deduced amino acid sequences exhibited similar to RPL3 variant 1. Although RPL3 is highly conserved among eukaryotes and prokaryotes, it has been known that some ribosomal proteins and its mutations contribute to human diseases such as Turner syndrome (Meskauskas and Dinman, 2007). When we submitted RPL3 cDNA to GenBank SNPs data base (http://www.ncbi.nlm.nih.gov/SNP/snp_blastByOrg.cgi), there is many SNPs found in RPL3 cDNA; therefore, genetic polymorphism(s) of RPL3 in relation to human diseases need to be investigated.

In the present study, we found a novel function of RPL3. Although RPL3 have been investigated from a biochemical point of view, we discovered that RPL3 may function as a drug carrier presumably digoxin as a substrate of this clone. Our results, therefore, may provide new insights into the toxicological and pharmacological action of RPL3-mediated digoxin transport and may contribute to the elucidation of molecular mechanism(s) of the digoxin toxicity in the human body.

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