The 26th JSSX Young Investigator's Award (Nov. 17, 2011)

Identification of Transporters Involved in Drug Disposition and Application for Drug Development and Therapy

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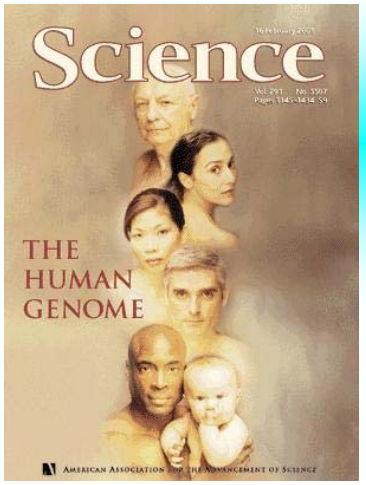
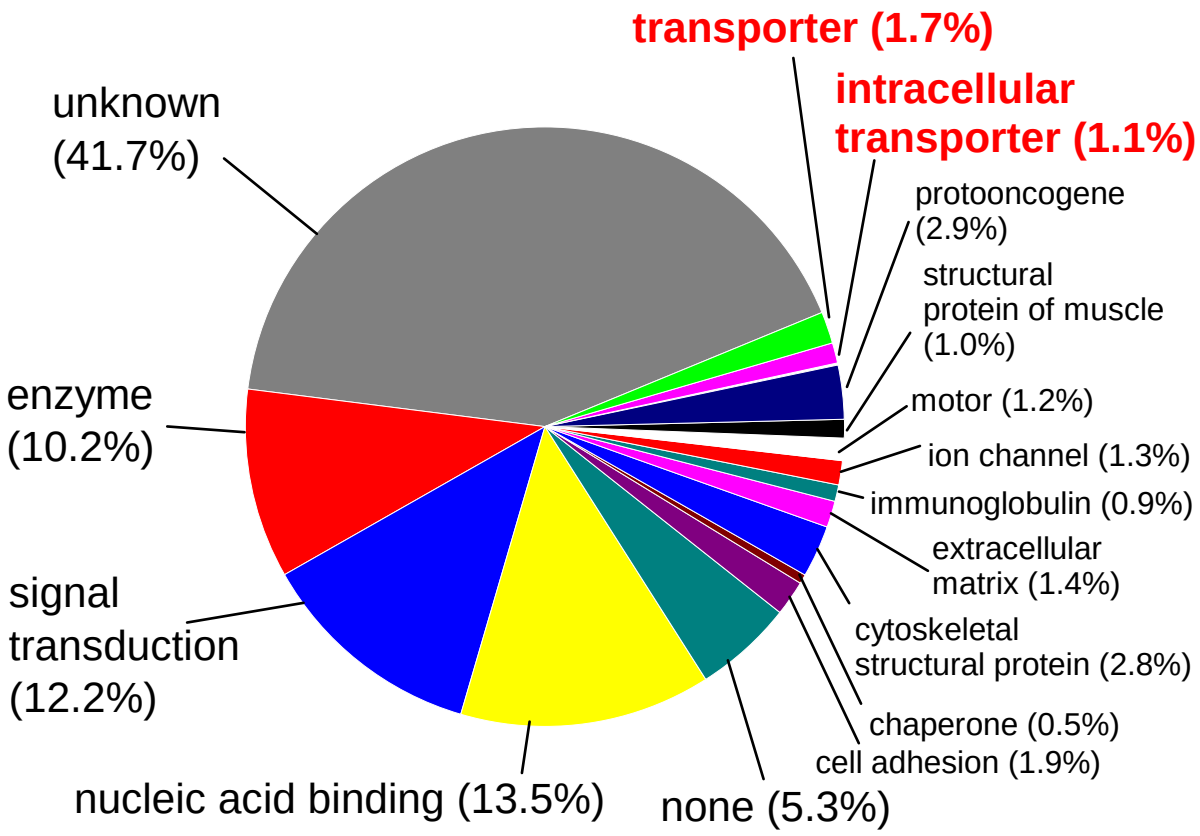


Distribution of the Molecular Functions of 26,383 Human Genes

Science, 291, 5507, 1304-1351 (2001)

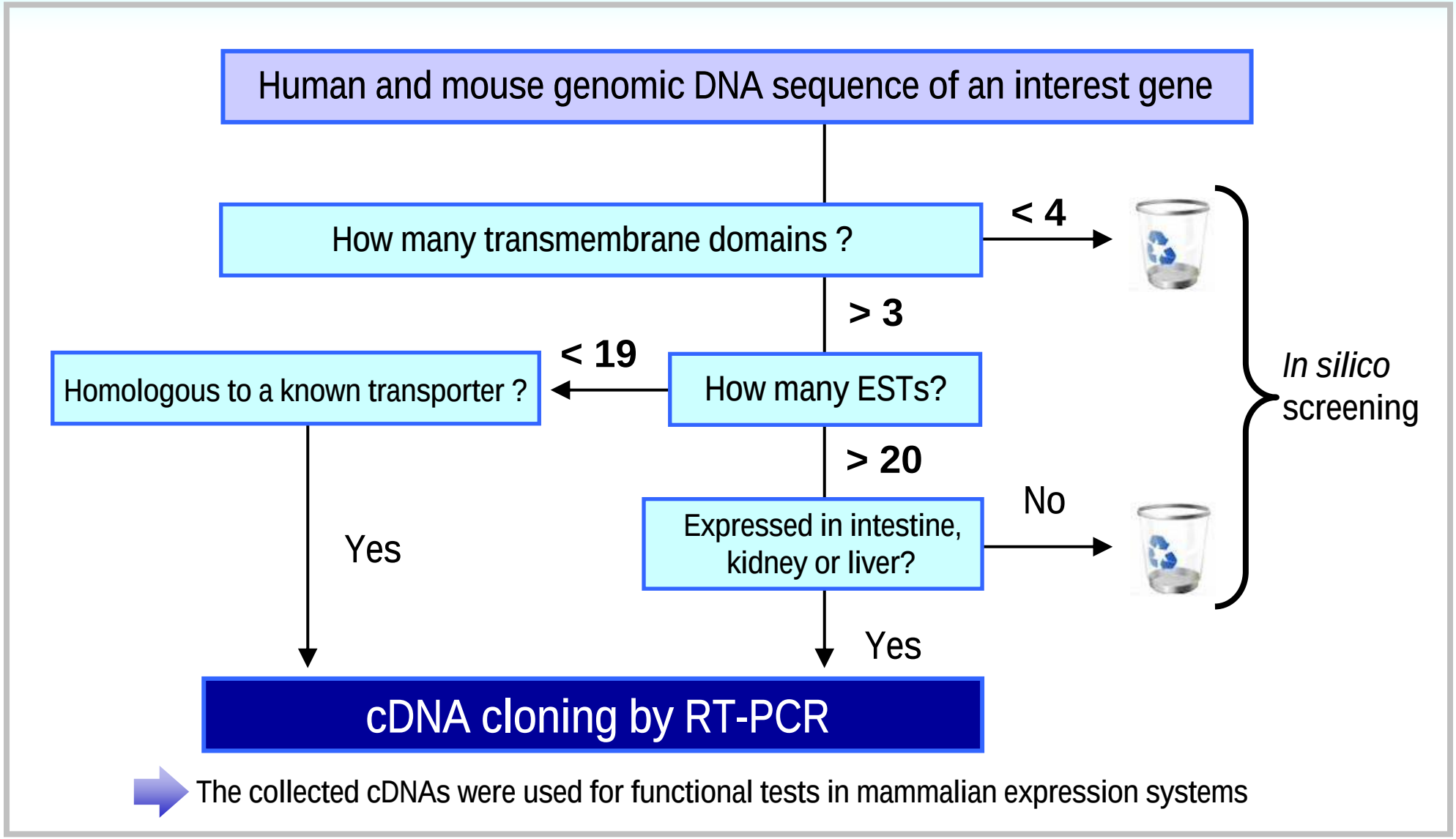


Species	Genome size (Mbp)	No. of genes (estimated)
Human	**3,000**	**26,383**
Drosophila	**80**	**13,000**
C. elegans	**97**	**19,099**

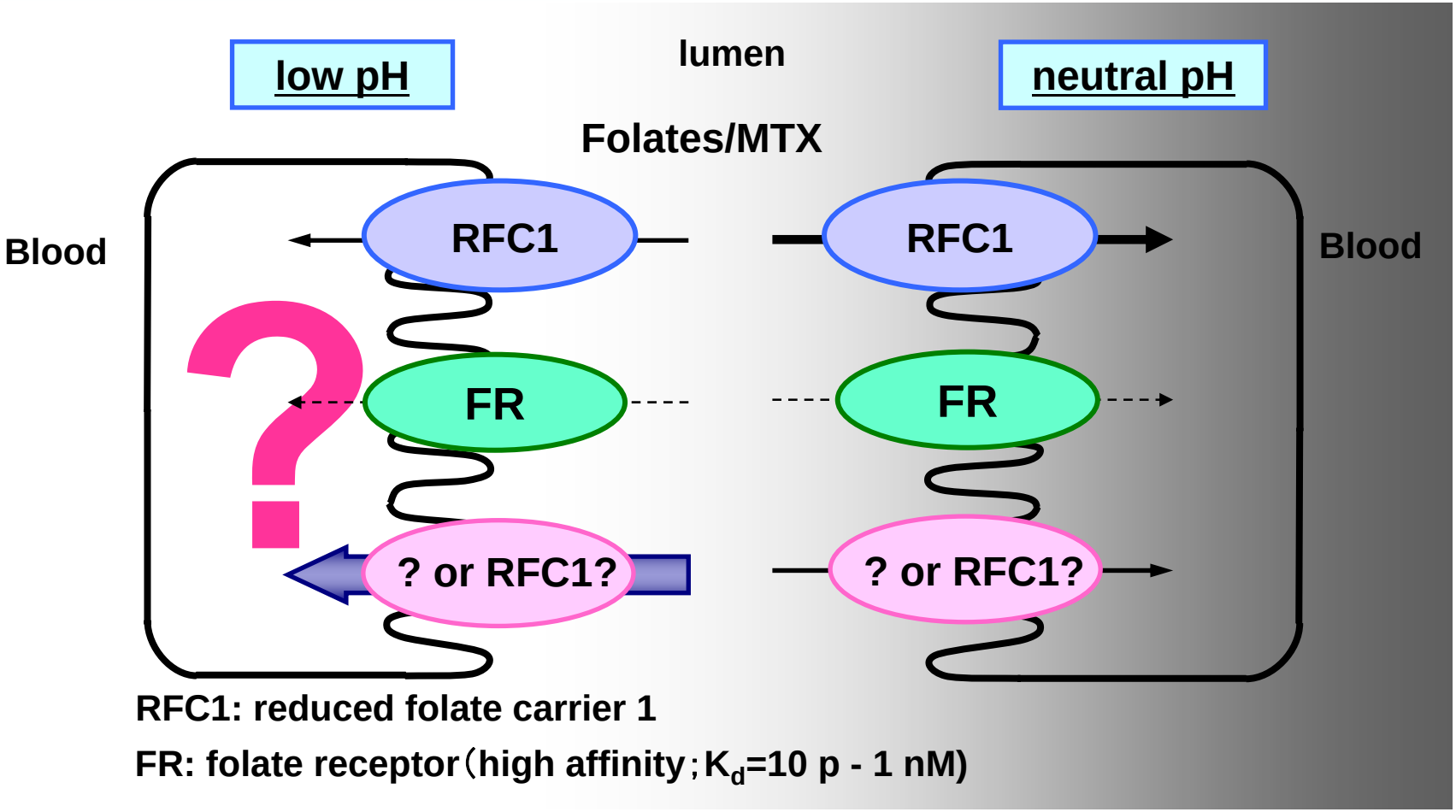
Category of molecular function	No. of genes
Unknown function	12,809
Nucleic acid binding	4,158
Signal transduction	3,775
Enzyme	3,142
Protooncogene	902
Cytoskeletal protein	876
Cell adhesion	577
Transporter	533

*Based on the first draft of the human genomic sequence

Strategy for the Identification of Genes Involved in the Disposition of Drugs and Nutrients



Mechanism of Intestinal Absorption of Folates



RFC1: reduced folate carrier 1

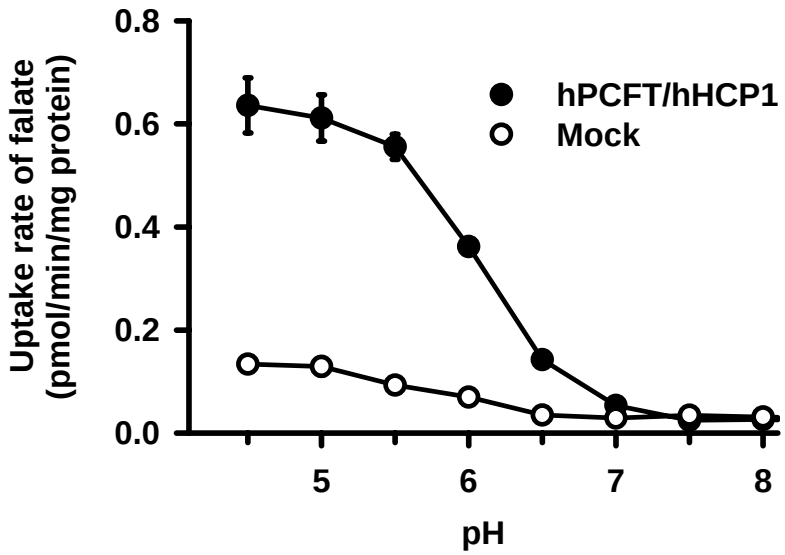
FR: folate receptor（high affinity；K_d=10 p - 1 nM)

➡ Is there an unidentified specific transporter for folates?

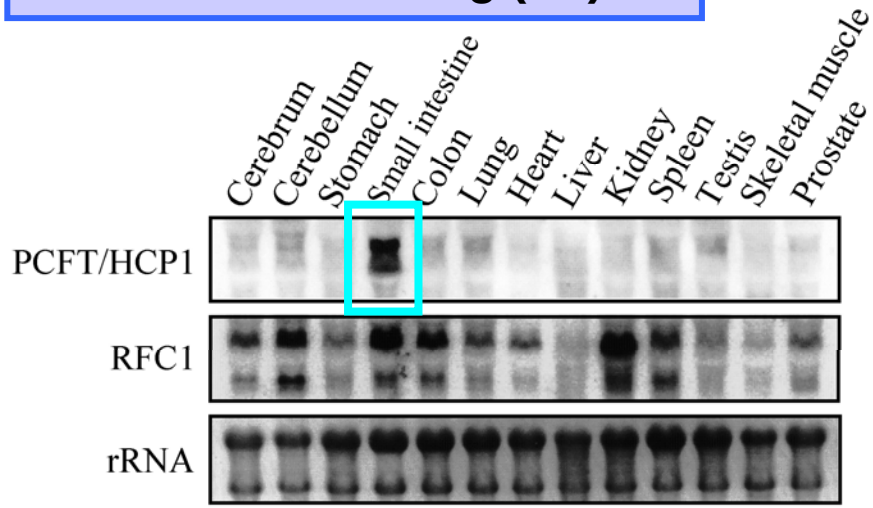
Said HM and Mohammed ZM, *Curr Opin Gastroenterol*, 22, 140-162 (2006)

Functional Characteristics of Proton-Coupled Folate Transporter/Heme Carrier Protein 1(PCFT/HCP1)

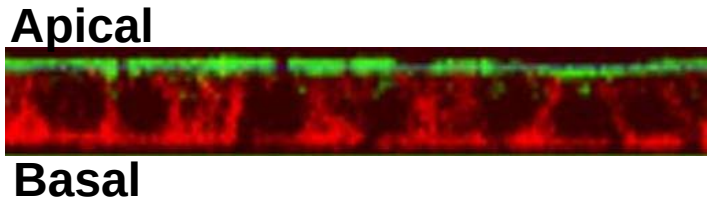
pH-Dependence



Northern blotting (rat)



Cellular localization



Concentration-dependence

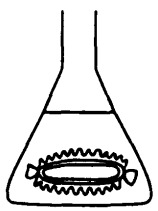
Substrate	K_m (μM)	V_{max}(pmol/min/mg protein)
Folate	1.67 ± 0.10	70.0 ± 2.7

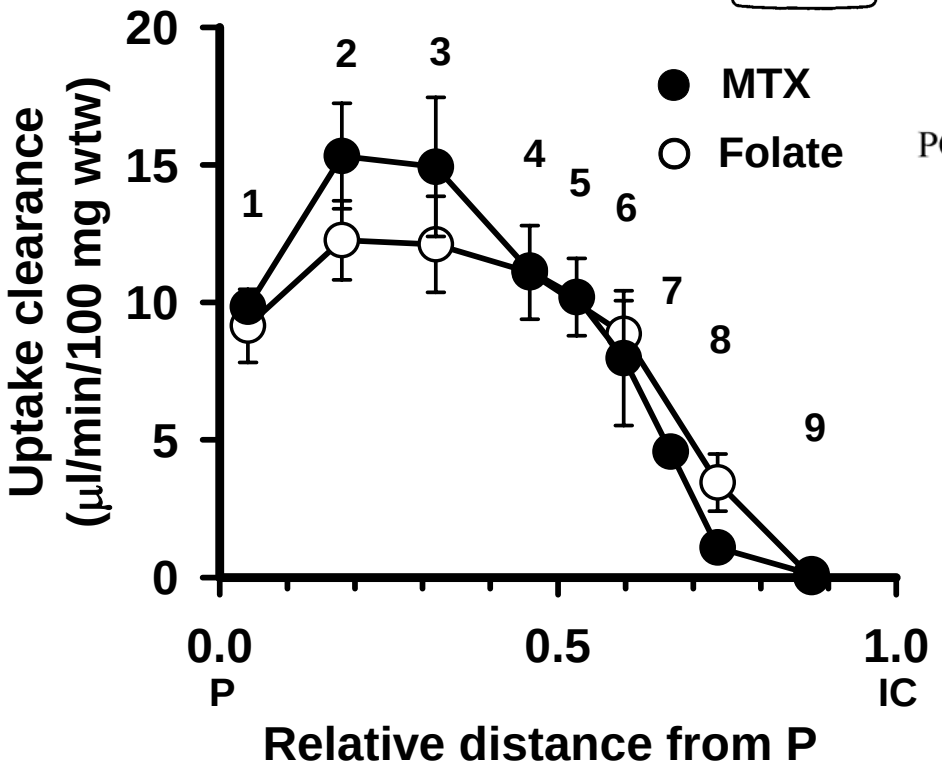
hPCFT/HCP1 is responsible for the intestinal absorption of folates and also methotrexate.

Nakai Y, et al, *J Pharmacol Exp Ther*, 322, 469 - 476, 2007; Inoue K, et al, *Am J Physiol*, 294, G660 - G668, 2008.

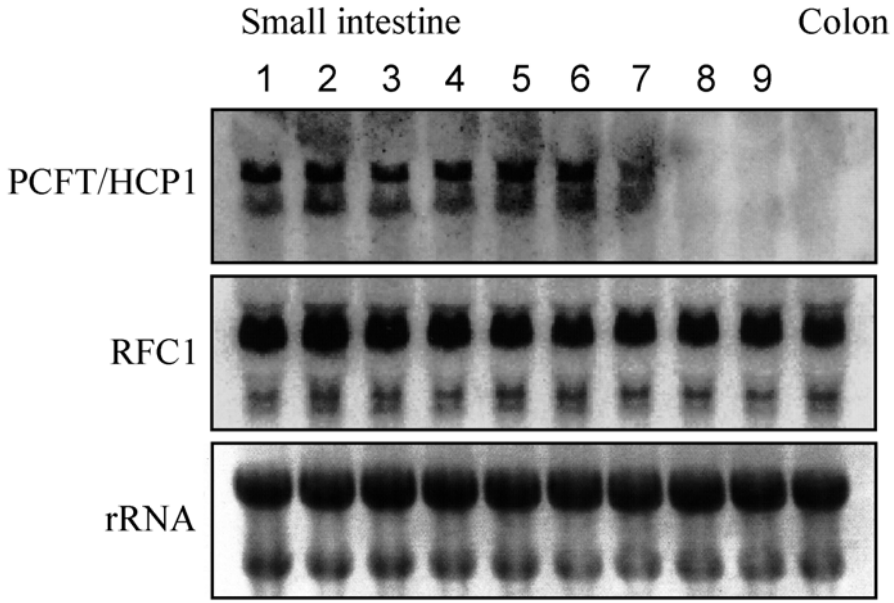
Distribution of Folate Transport Activity and mRNA of rPCFT/HCP1 in Rat Small Intestine

Folate and MTX uptakes by rat everted sacs





Northern blotting



Distribution profile of the activity of folate transport system was in agreement with that of the mRNA of rPCFT/HCP1

Inoue K, et al, *Am J Physiol*, 294, G660 - G668, 2008.

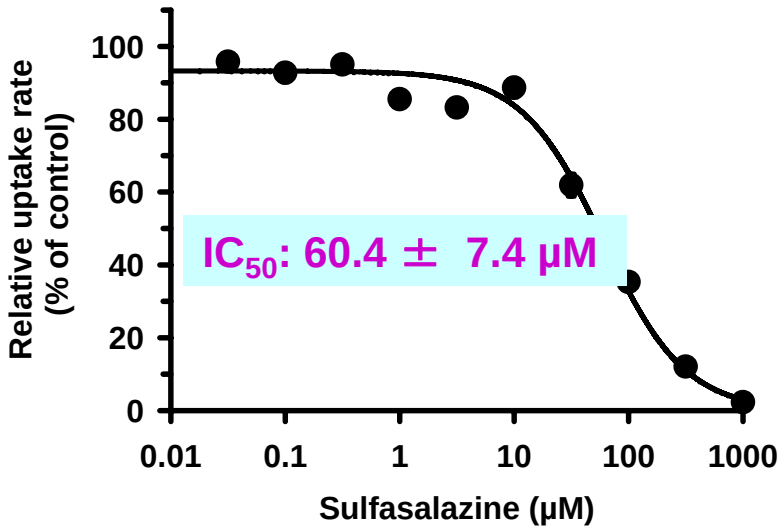
rPCFT/HCP1 is the molecular entity of the folate transport system.

Effect of NSAIDs and DMARDs on MTX Uptake Mediated by hPCFT/HCP1

Inhibitor (NSAIDs)	Concn. (mM)	Uptake rate (% of control)
Acetylsalicylate	0.2	92 ± 4
Diclofenac	0.2	64 ± 5 *
Flufenamate	0.2	93 ± 7
Flurbiprofen	0.2	97 ± 4
Ibuprofen	0.2	93 ± 4
Indomethacin	0.2	49 ± 2 *
Ketoprofen	0.2	99 ± 4
Loxoprofen	0.2	103 ± 5
Mefenamate	0.2	102 ± 7
Naproxen	0.2	94 ± 3
Phenylbutazone	0.2	94 ± 4
Salicylamide	0.2	98 ± 3
Salicylate	2	82 ± 3

Inhibitor (DMARDs)	Concn. (mM)	Uptake rate (% of control)
Aurothiomalate	0.2	99 ± 4
Azathioprine	0.2	96 ± 1
Dexamethazone	0.2	99 ± 2
Prednisolone	0.2	97 ± 2
Prednisone	0.2	101 ± 2
Sulfasalazine	0.2	19 ± 2 *

MTX, methotorexate; **NSAIDs**, non-steroidal anti-inflammatory drugs; **DMARDs**, disease modifying anti-rheumatic drug; * $p < 0.05$



Nakai Y, et al, *J Pharmacol Exp Ther*, 322, 469 - 476, 2007

Guideline for Treatment of Rheumatoid Arthritis (RA)

American College of Rheumatology (ACR), ***N Engl J Med***, 350, 2591-2602, 2004

Begin patient education
Start DMARD therapy within 3 mo of diagnosis
Consider NSAID
Consider local or low-dose systemic corticosteroids
Start physical therapy or occupational therapy

↓

Inadequate response (after 3 months of therapy)

↓

***DMARDs**, disease modifying anti-rheumatic drug
Preferred initial DMARD: MTX
Second-line DMARD: salfasaslzine

Change or add DMARDs*

No previous MTX treatment

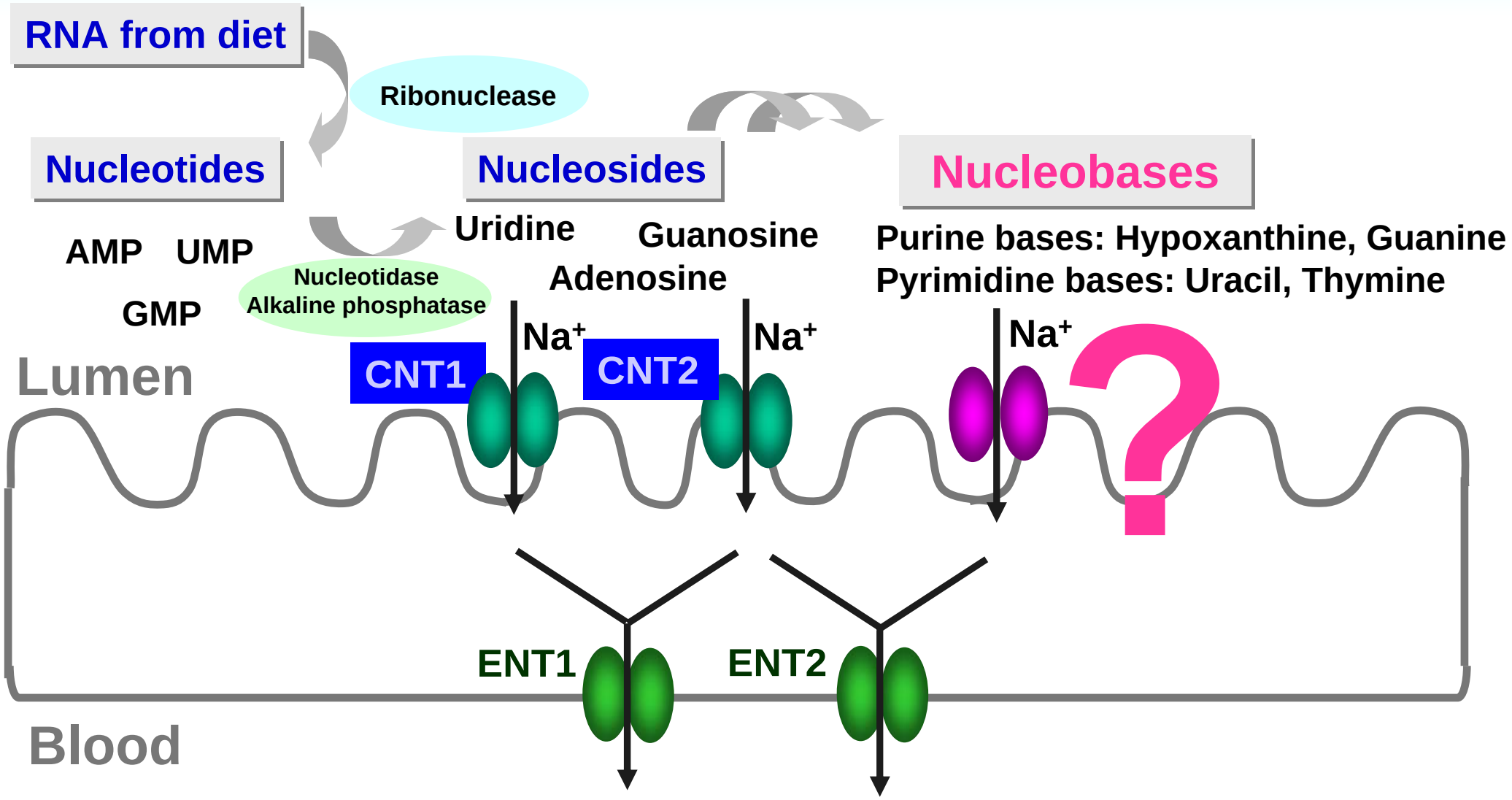
- **MTX**
- Other mono-therapy
- Combo

Suboptimal response to MTX

- Combo
- Other mono-therapy
- Biologic DMARDs

➡ **The simultaneous use of MTX and sulfasalazine should better be avoided.**

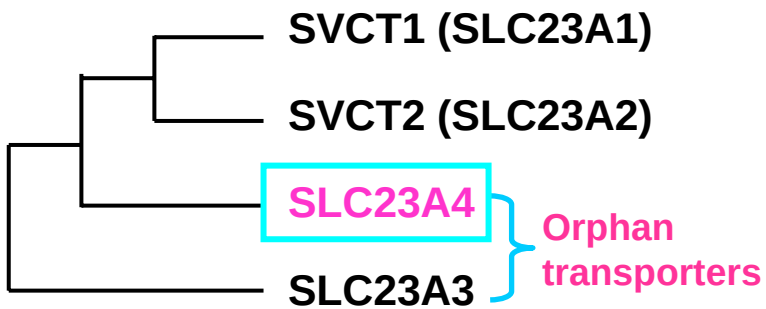
Absorption Mechanism of Dietary Nucleic Acids in Gastrointestinal Tract



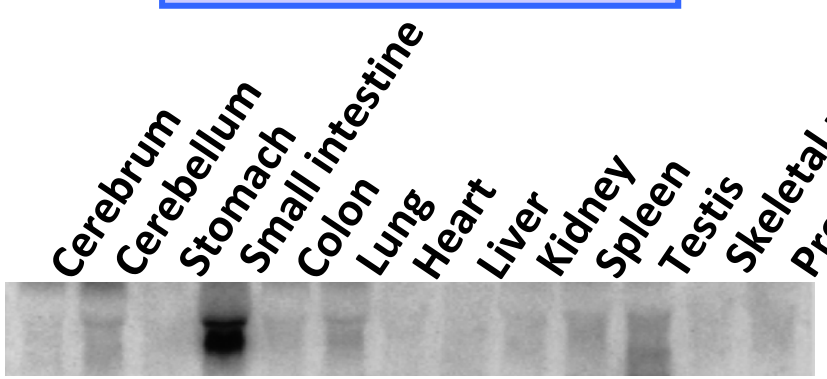
CNTs: concentrative nucleoside transporters, ENTs: equilibrative nucleoside transporters

Identification of Rat Sodium-Dependent Nucleobase Transporter 1 (SNBT1)

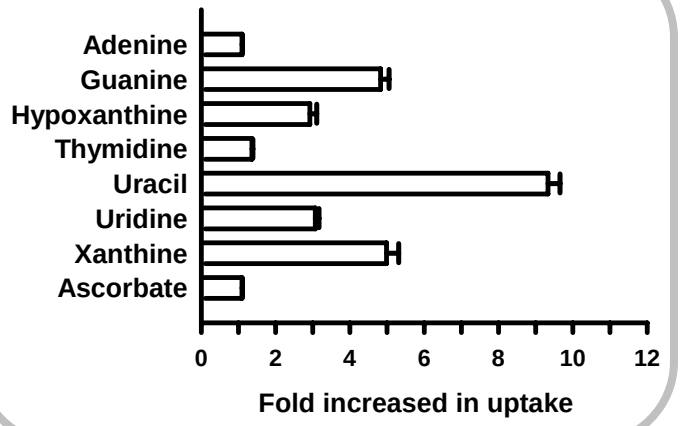
Phylogenetic tree



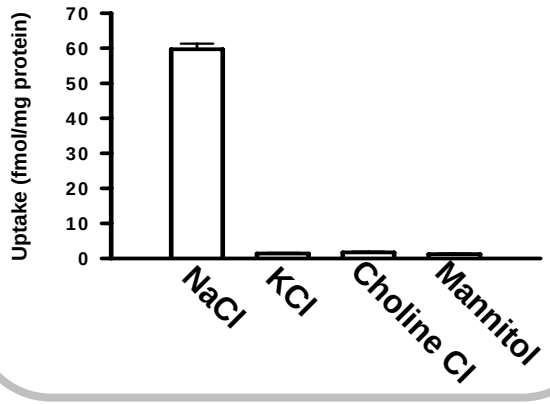
Tissue Distribution



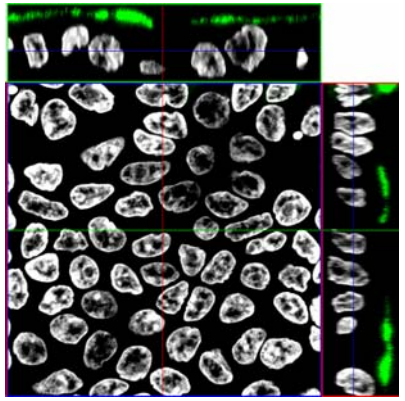
Substrate specificity



Ion dependence



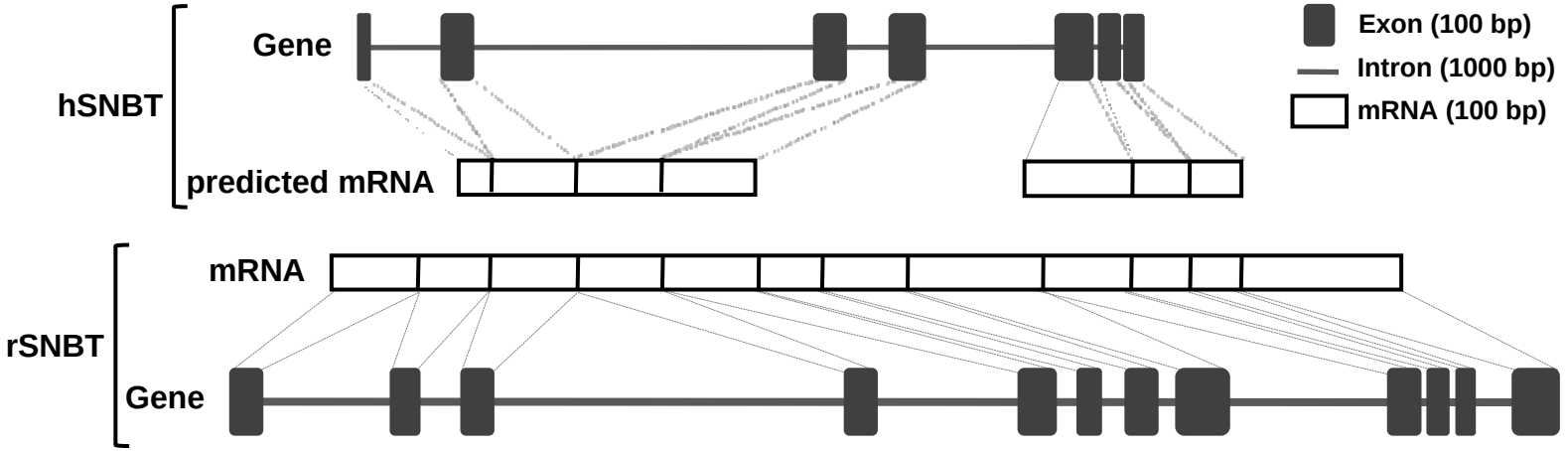
Cellular localization

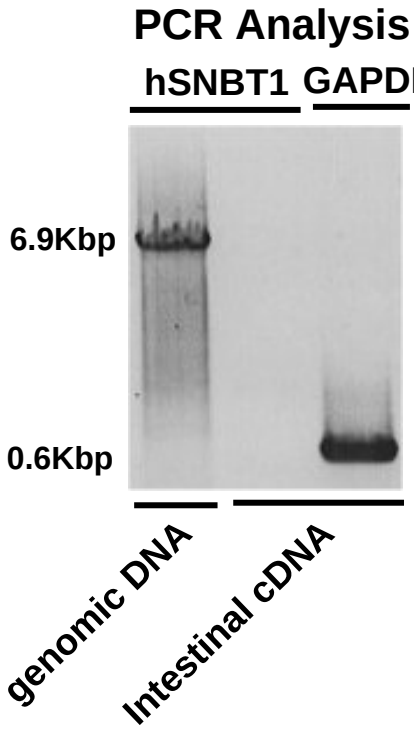


Yamamoto S, et al,
J Biol Chem, 285, 522-531, 2010.

rSNBT1 seemed to be the molecular entity responsible for the intestinal absorption of nucleobases.

Genetic Defect of Human SNBT1 Gene





SNBT1 gene is a pseudogene in human

There is a genetic species difference in the handling of nucleobases between human and non-primate mammals.

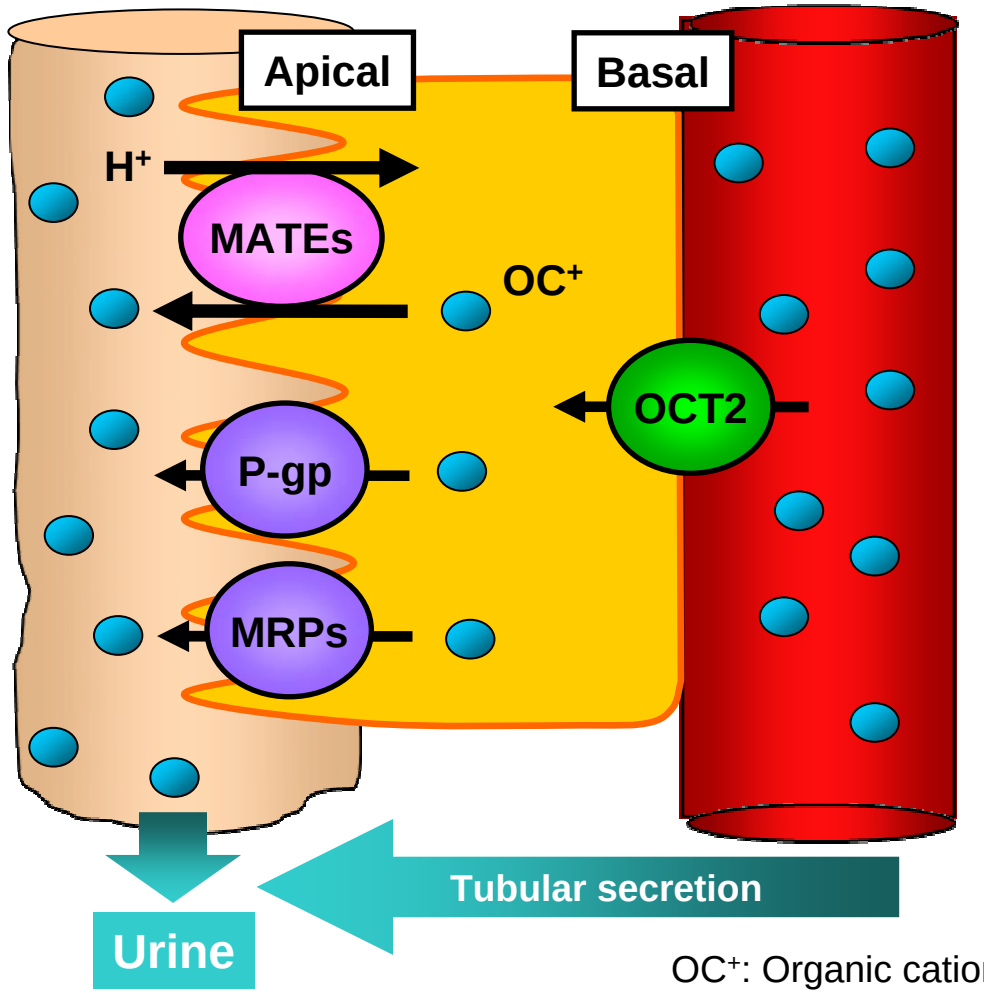
Multidrug and Toxin Extrusion Proteins (MATEs)

Transport substrate

Cationic compounds:
Tetraethylammonium (TEA)
Procainamide,
1-Methyl-4-phenylpyridinium (MPP)
Cimetidine
N-Methylnicotinamide
Metformin
Creatinine
etc

Zwitterionic compounds:
Norfloxacin
Levofloxacin
etc

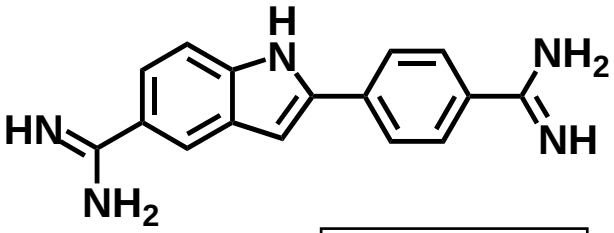
Role of MATEs in renal proximal tubular cells



OC^+: Organic cation

DAPI as a Unique Fluorescence Probe Substrate of MATEs

DAPI
(4',6-diamidino-2-phenylindole)



M.W. 277.3
$\lambda_{excitation}$ 358nm
$\lambda_{emission}$ 461nm

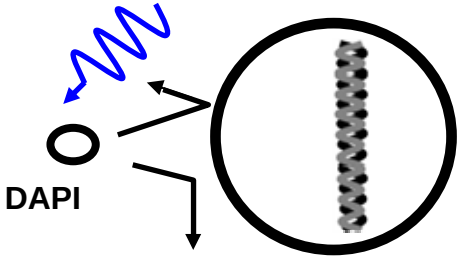
DAPI : nuclear staining agent
: cationic and impermeable through the plasma membrane

Unique nature of DAPI
It can emit fluorescence only when intercalated into DNA.
Its irreversible DNA binding with high capacity enables its high accumulation in the cells.

Advantage in fluorometric detection
Real-time visualization

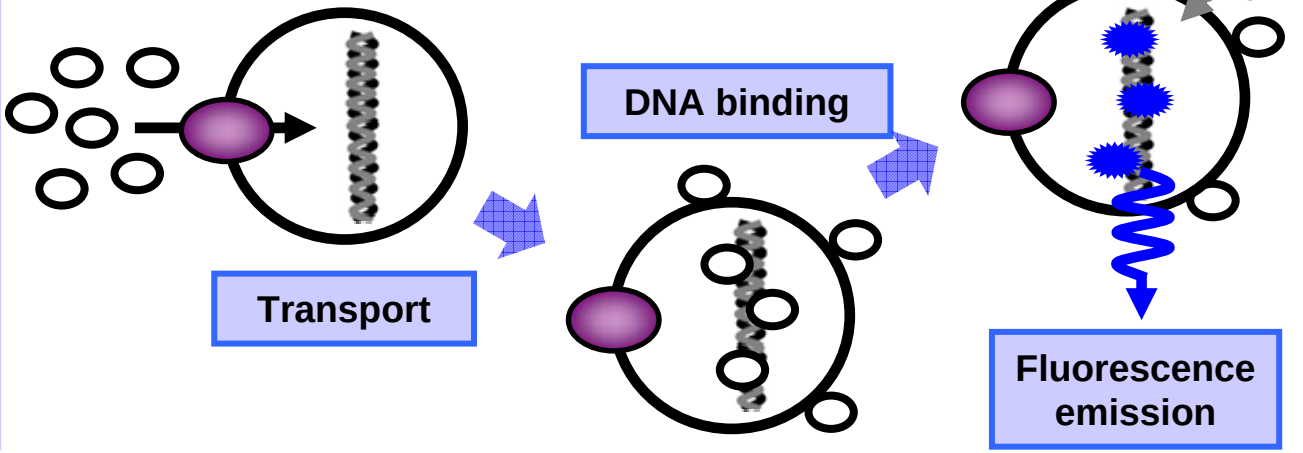
Regular cells without DAPI transporters

No fluorescence



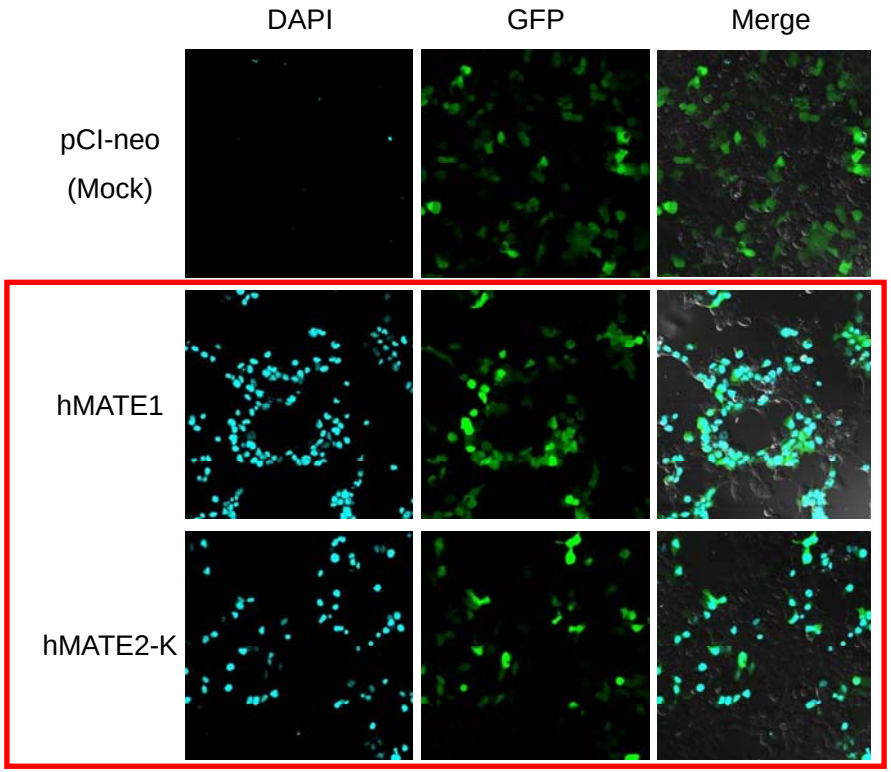
No penetration

Cells having MATEs



DAPI Transport by hMATEs

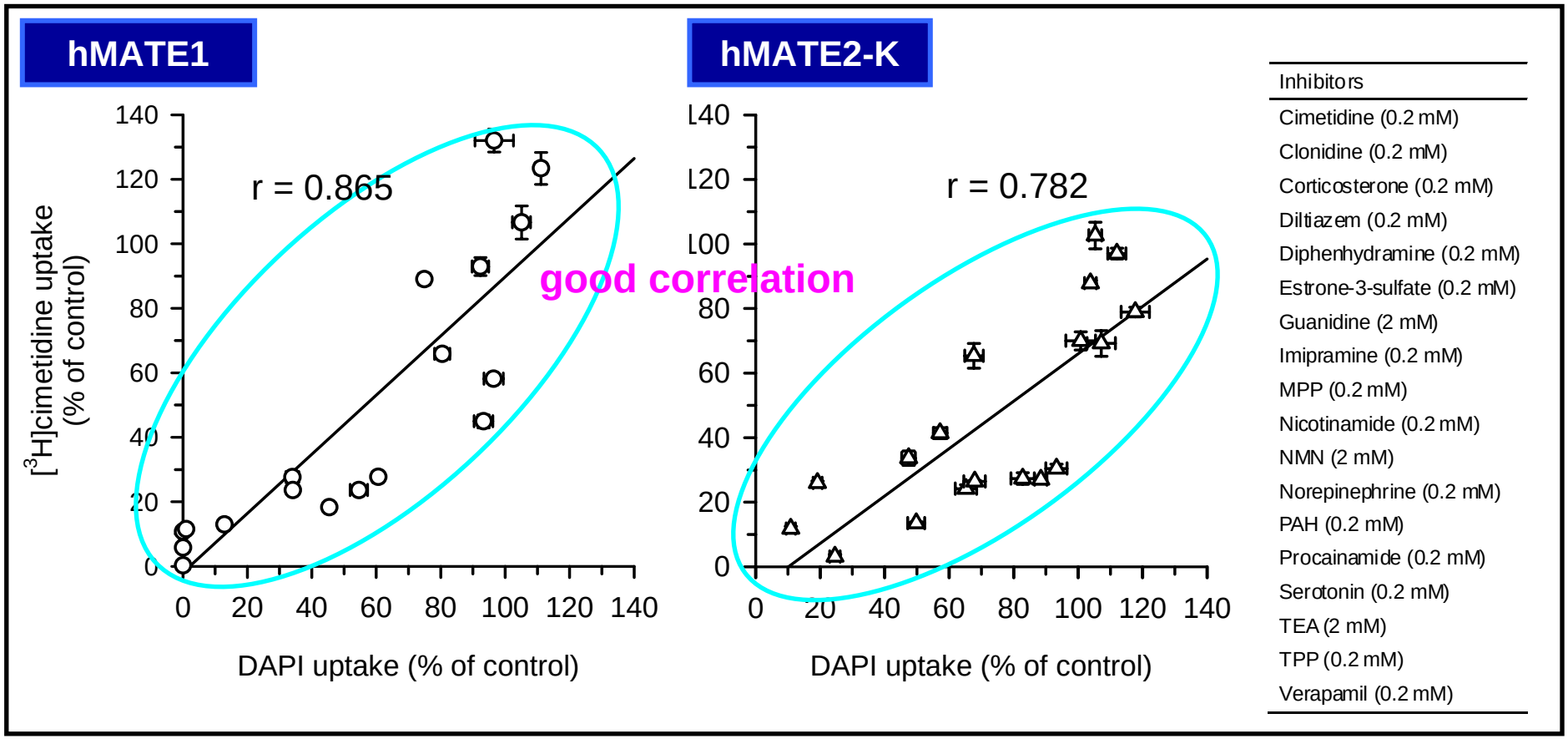
Fluorescent microscopic images



Human MATE1 and MATE2-K can transport DAPI

Yasujima T, et al, *Drug Metab Dispos*, 38, 715-721, 2010

The Performance of DAPI Uptake Assay in Identifying Inhibitors of hMATEs



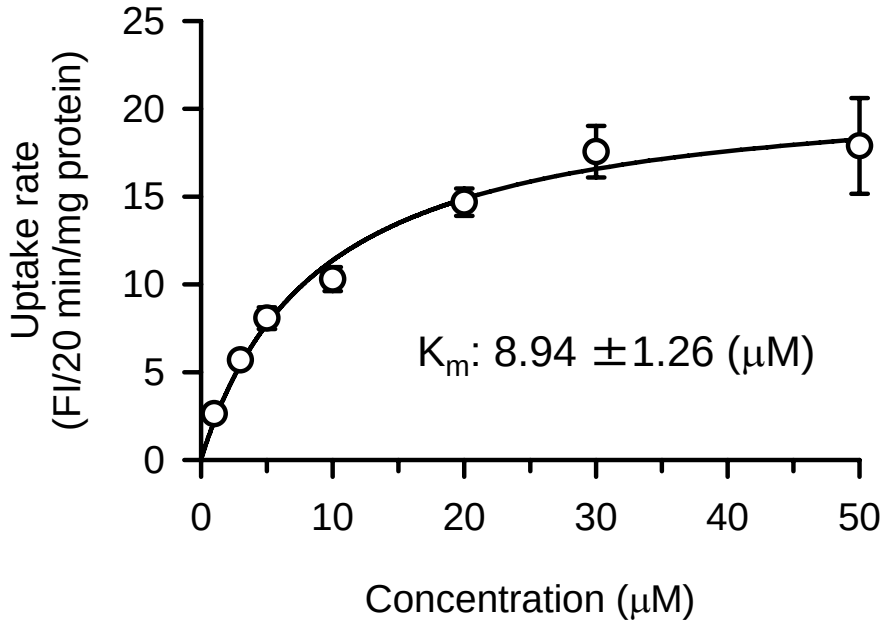
Inhibitors
Cimetidine (0.2 mM)
Clonidine (0.2 mM)
Corticosterone (0.2 mM)
Diltiazem (0.2 mM)
Diphenhydramine (0.2 mM)
Estrone-3-sulfate (0.2 mM)
Guanidine (2 mM)
Imipramine (0.2 mM)
MPP (0.2 mM)
Nicotinamide (0.2 mM)
NMN (2 mM)
Norepinephrine (0.2 mM)
PAH (0.2 mM)
Procainamide (0.2 mM)
Serotonin (0.2 mM)
TEA (2 mM)
TPP (0.2 mM)
Verapamil (0.2 mM)

DAPI can be an alternative probe substrate that enables fluorometric rapid assays of the functionality of both hMATEs.

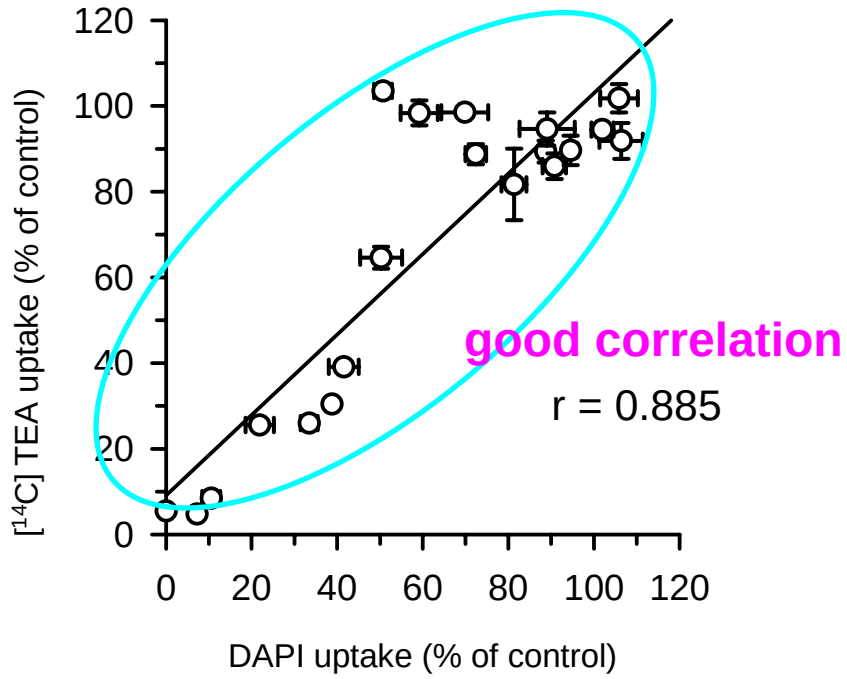
Yasujima T, et al, *Drug Metab Dispos*, 38, 715-721, 2010

DAPI Transport by hOCT1 and DAPI Uptake Assay

Concentration dependence



Correlation



DAPI uptake assay is also effective for evaluation of the functionality of hOCT1.

Yasujima T, et al, *J Pharm Sci*, 100, 4006 - 4012, 2011.

MATEs and OCT1 Picked Up as Important Drug Transporters in International Transporter Consortium White Paper

International Transporter Consortium White Paper

Transporter/alias (Gene)	Selected substrates	Selected inhibitors	Organs/cells	Comments
OATP1B1/OATP-C, OATP2, LST-1 (*SLCO1B1*)	Bromosulphophthalein, oestrone-3-sulphate, oestradiol-17β-glucuronide, statins*, repaglinide*, valsartan, olmesartan*, bilirubin glucuronide, bilirubin, bile acids	Saquinavir, ritonavir*, lopinavir*, rifampicin*, cyclosporine*	Hepatocytes (sinusoidal)	• Has a role in disposition and excretion • Has clinically relevant polymorphisms • Has a role in clinical drug–drug interactions
OATP1B3/OATP-8 (*SLCO1B3*)	Bromosulphophthalein, cholecystokinin 8, statins*, digoxin, fexofenadine, telmisartan glucuronide, telmisartan*, valsartan, olmesartan, oestradiol-17-β-glucuronide, bile acids	Rifampicin*, cyclosporine*, ritonavir, lopinavir*	Hepatocytes (sinusoidal)	• Has a role in disposition and excretion
OAT1 (*SLC22A6*)	Para-aminohippurate, adefovir, cidofovir, zidovudine*, lamivudine*, zalcitabine*, acyclovir*, tenofovir*, ciprofloxacin*, methotrexate*	Probenecid*, novobiocin	Kidney proximal tubule, placenta	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OAT3 (*SLC22A8*)	Oestrone-3-sulphate, non-steroidal anti-inflammatory drugs, cefaclor, ceftizoxime, furosemide*, bumetanide*	Probenecid*, novobiocin	Kidney proximal tubule, choroid plexus, blood–brain barrier	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OCT2 (*SLC22A2*)	*N*-Methylpyridinium, tetraethylammonium, metformin*, pindolol, procainamide, ranitidine amantadine, amiloride, oxaliplatin, varenicline*	Cimetidine*, pilsicainide, cetirizine*, testosterone, quinidine	Kidney proximal tubule, neurons	• Has a role in disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions
OATP1A2/OATP-A (*SLCO1A2*)	Oestrone-3-sulphate, dehydroepiandrosterone sulphate, fexofenadine*, bile salts, methotrexate, bromosulphophthalein, ouabain, digoxin, levofloxacin, statins*	Naringin, ritonavir, lopinavir, saquinavir, rifampicin*	Brain capillaries endothelia, cholangiocytes, distal nephron	• Has role in disposition and excretion
OCT1	Oestrone-3-sulphate, bromosulphophthalein, taurocholate, *statins, fexofenadine, glyburide, taurocholate	Rifampicin, cyclosporine*	Hepatocytes (sinusoidal), endothelia	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
OCT1 (*SLC22A1*)	Tetraethylammonium, *N*-methylpyridinium, metformin*, oxaliplatin	Quinine, quinidine, disopyramide	Hepatocytes (sinusoidal), intestinal enterocytes	• Has a role in disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions
PEPT1 (*SLC15A1*)	Glycylsarcosine, cephalexin, cefadroxil, bestatin, valacyclovir, enalapril, aminolevulinic acid, captopril, dipeptides, tripeptides	Glycyl-proline	Intestinal enterocytes, kidney proximal tubule	• Has a role in absorption, disposition and excretion • Has a role in clinical drug–drug interactions
MATE1, MATE2-K		fenopril, fosinopril	Kidney proximal tubule, choroid plexus, lung	• Has a role in excretion
MATE1 (*SLC47A1*)	Metformin, *N*-methylpyridinium, tetraethylammonium	Quinidine, cimetidine, procainamide	Kidney proximal tubule, liver (canalicular membrane), skeletal muscle	• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions
MATE2-K (*SLC47A2*)	Metformin, *N*-methylpyridinium, tetraethylammonium	Cimetidine, quinidine, pramipexole	Kidney proximal tubule	• Has a role in disposition and excretion

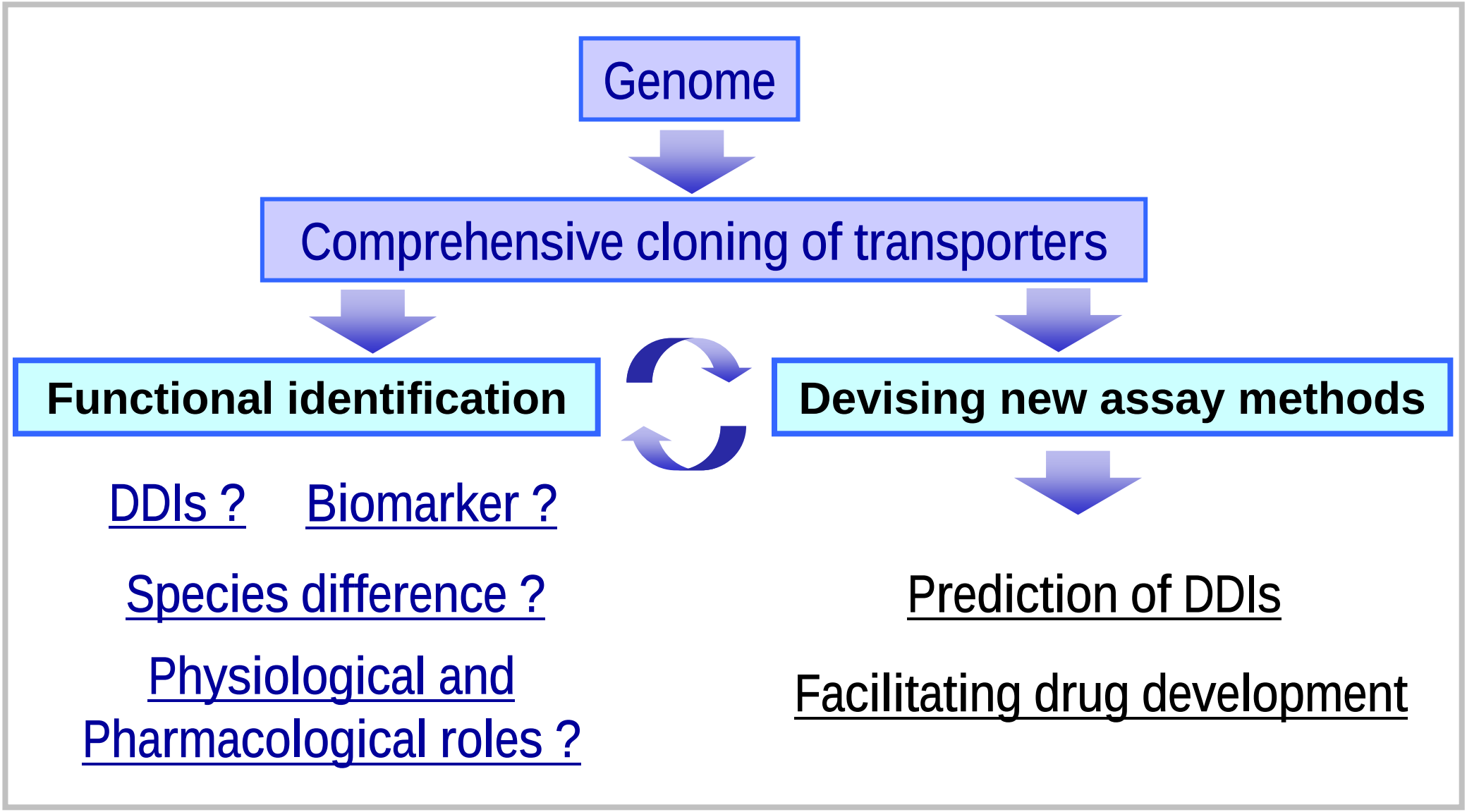
Importance of drug transporters in drug development

It is recommended
- to identify substrates and inhibitors
- to devise strategies to avoid DDIs

DAPI uptake assay is very useful !

International Transporter Consortium, *Nat. Rev Drug Discov*, 9, 215 - 236, 2010.

Our Future Direction of Transporter Studies



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村田 友昭 (Murata, Tomoaki)
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渥美 亮 (Atsumi, Ryou)

熊本大・薬（Kumamoto Univ.）

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