



Research article

The nucleobase cation symporter 1 of *Chlamydomonas reinhardtii* and that of the evolutionarily distant *Arabidopsis thaliana* display parallel function and establish a plant-specific solute transport profile



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ABSTRACT

The single cell alga *Chlamydomonas reinhardtii* is capable of importing purines as nitrogen sources. An analysis of the annotated *C. reinhardtii* genome reveals at least three distinct gene families encoding for known nucleobase transporters. In this study the solute transport and binding properties for the lone *C. reinhardtii* nucleobase cation symporter 1 (CrNCS1) are determined through heterologous expression in *Saccharomyces cerevisiae*. CrNCS1 acts as a transporter of adenine, guanine, uracil and allantoin, sharing similar – but not identical – solute recognition specificity with the evolutionary distant NCS1 from *Arabidopsis thaliana*. The results suggest that the solute specificity for plant NCS1 occurred early in plant evolution and are distinct from solute transport specificities of single cell fungal NCS1 proteins.

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1. Introduction

Nucleobase biochemistry serves diverse needs during a plant's life cycle. Purines and pyrimidines are central to nucleic acid biochemistry, ATP synthesis, carbohydrate, glycoprotein and phospholipid metabolism, as well as the biosynthesis of many secondary metabolites such as cytokines and caffeine [1,2]. Nucleobase biochemistry is comprised of an intricate balance of *de novo* synthesis, catabolism and salvage pathways. A shared aspect is the highly compartmented nature of these pathways. In turn, a myriad of transport systems has evolved to serve the movement of nucleobase compounds – both within and between cells. Purine and pyrimidine biochemistries involve plastidic and cytoplasmic exchange [2,3]. Nucleobases are also moved between adjacent cells as seen in the generation of ureides (purine derivatives) in soybean nodule bacteroid-containing and nonbacteroid-containing cells [4]. Longer distance nucleobase transport involving different tissues is key to nitrogen source–sink relationships as seen in endosperm to cotyledons nucleobase movement in germinating castor bean seedlings [5].

In plants a surprising number of nucleobase transporters fill the needs of nucleobase movement – no less than five distinct gene families encoding nucleobase transporters are present in the *Arabidopsis* genome. Two transporter families are unique to plants. *Arabidopsis* contains 21 purine permeases (PUP) some of which transport adenine, cytosine and cytokinins [6–8]. Of the eight ureide permeases (UPS) in *Arabidopsis*, three are known to transport allantoin, uracil, uric acid and xanthine [9–11]. The remaining three nucleobase transporter families have orthologs in both prokaryotic and eukaryotic taxa. The nucleobase-ascorbate transporters (NATs) are ubiquitous and cooperatively transport a large array of solutes including the oxidized purines xanthine and uric acid, uracil and ascorbate [12,13]. None of the twelve *Arabidopsis* NAT loci have been functionally characterized, however, the maize leaf permease 1 is known to transport xanthine and uric acid [14,15]. The AzgA-like transporter family in *Arabidopsis* transports adenine, guanine, and uracil [16]. The lone nucleobase cation symporter 1 in *Arabidopsis* (AtNCS1) facilitates the movement of adenine, guanine, and uracil [17]. Genes that encode for NCS1 proteins are found throughout the plant kingdom [17,18].

Most fungi contain multiple NCS1 as exemplified by the canonical *Saccharomyces cerevisiae* FCY2-subfamily – transporting adenine, guanine, cytosine and hypoxanthine – and the FUR4-like subfamily – transporting uracil, uridine, allantoin and other metabolites [13]. In single cell microbes, robust nucleobase transport systems are mainly dedicated to scavenging and importing

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		↓		↓	■ □
AtNCS1	MVSNCLSLSLHLNHPKHNRHLSLRSRTKAKLYQHSVFTDSSHKSSTYSCVSTFDIQRKSSKHVELGKHSFSPILPGDNLVLSRSGVIRPRLSAMTG				
CrNCS1	MQOQLR-STSIARAAAAPGARVPGLVRPAPCGP--RRAPFTS-----PCFTPLPSAP---FTR-GPAPAGLGMGMS				
ScFUR4	MPDNLSLHLSGSSKRLNSRQLMESSNETFAPNNVDLEKEYKSSQSNITTEVY-----EASSFEKVSSEK--PQYSSFWKKIYYEVVV				
ScDAL4	MANDALSAIFSNPSRKGVQPSSTIVSYTNEDDIIIDVENGKFNKNKNINTNVYV-----DNSSIEESEVVL--PETKSIWKKIYYDFIV				
ScFUI1	MPVSDSGFDNSSKTMKDDTIPTDEYIEITKESEMGGATKITSKIDANVIEKK-----DTSENNITIAQD--DEKVSWLQRVVEFEV				
AnFURA					MSAIKRWIKKLEV
AnFURD					MRFGRFHLRV
AnFCYB	MAGAFD-----FDLEKNPPVQSTADNSSDGAVPG-----ETFTYG-----DSTYAK---				
ScFCY2	MLEEGNNVYEQDLEKSPVIGSSLENEKKVAAS-----ETFTATSE---D---DQYIVESS				
MHP1					MNSTP
			I *		II
AtNCS1	SEINDHG-----YDESQFDPSLTNDLKPPTPSQRT-FSWLDMSSLWGLVGVPTYYLAGS-LVDLGMMAWQGIATVVTANLILLVPLVL				
CrNCS1	DPITAR-----PPTNPDPSLINEDFSPTQDKRT-FDITDITATWITLVISITTYILAAS-LVDLGMSSWQGIATVVTANLILLVPLVL				
ScFUR4	VDKSI-----LGVSLDSFMYNQDLKPVKEKRRV-WSWYNICYFWLAECFNINTWQIAAT-GLQLGLNWWQCWITTIWIGYGVGAFVVL				
ScDAL4	LDKTT-----LNVSLKESFLYNRDLKPVEEERRC-WSWFNYLYFWLADCFNINTWQIAAT-GLQLGLNWWQCWITTIWIGYGVGAFVVL				
ScFUI1	KNDSTDLADHKPENPIRTFKDLQESLSTYLTNDLRPEAKRRRT-WTWKQYIFFWISGSFNVTWQISAT-GLQLGLNWWQWICIVWGYTFVAFVVL				
AnFURA	ESDPG-----LNTQLMLTNHDLRPVEPDRQ-RRWYNFIFFWIADSNINTWMISS-MIVDGLSWQSWLGVWFGYFLAACFVCL				
AnFURD	EQSRS-----AFASGNARWTNLDLPVPRAGR-VGFLSFISYHISDAFNAATWQFASS-IIAVGLSWRESLGLVALSFFIISFVIAA				
AnFCYB	-----IQRLAELNIEQRGIERVPAEQTDTSVFNIGSMWLAANMVSSFAIGVLGKSVYSLGFVDAILTVLFFNLLGIMTVCF				
ScFCY2	EATKLSW-----FHKFFASLNAETKGVPEVTEDEKDDSLNAAASWFSANWIASYALGALGPMVFGINFGQSLVLIIFFNIMGLIFVAF				
MHP1	IEEAR-----SLL-NPSNAPTRYAERS-VGPFSLAAITFAMALQVAIFIAAGQ--MTSSFCVWQVIVAIAGCTIAVILLFF				
		▼ *	III		IV
AtNCS1	TAQPGTLYGISFPVLARSSFGIRGAHIPTLLRALVCGGYGIGTWIGGEAIFLLFGHIK----KSALSHTLFWLGTSPLEFSCFVFWLAQLCIVWRG				
CrNCS1	NAHPGTKYGVFPVLARASFGIQGANPLSLRAIVACVFGITWIGGSSIFQMLMAVTG-----GAVAAAPIAWLGISLPELLCFLGFWAAQVWVIVRG				
ScFUR4	ASRVGSAYHLSFPPISSRA-SFGIFFLSWPINRVMAIVYAVYAYIAATPVSLMLKSI-FG--KDLQKIPDHFGSPNATTYEFMCFFFIWFVQPFLLVP				
ScDAL4	NSRFGSAYHLSFPPIVTRAS-FGIFFSMWPIINRVMAIVYAVYAWLGATPVALMLKSI-FG--KNLEDRIPNHFGSPNSTTTFEFCFFIWFVWVIVPFLVA				
ScFUI1	GSKVGNHYHSFPIISRVSEFGIYFSIWIWVIRNVMAIVYAVYAYIAGSQCVQLMLKAIFG--TNLNTRIKDTIKNPNLTNFEFCMFCMFWWACLPFLWFF				
AnFURA	TGRIGAVYHISFPVCRSSFGVWGLWPVLNRTVMAIITVYGVGYIGGCITLMIRAIWPSYESLPNGIPE---SSGVDTKNFLSFFLFWLLSLPALWFF				
AnFURD	NGAVGSIYHIPFPVIARAS-WGFWGSYIATISRVILATIFWFAINVNGANAVKAMISAIWPSFLSMKNTIPQ---DQGIETNTMIAYMIFWIVQMPFLCIH				
AnFCYB	FSCFCF-PFGLRQMVFSRLWFGWYVTKGFAVLNIALCLGSAANAIVGAQMLHAVNSD-----VPGFAALILIISCTLLTVTFAG				
ScFCY2	FSVFGAELGLRQMLSRYLGVNVTARIFSLINVIACVGVIVTSVSAQLLNMVNEGSGH-----VCPIWAGCLIIIGGTVLVTFVFG				
MHP1	TQSAAIRWGINFTVAARMPPGIRGLSIPITLKALLSLFWFGFOTWLGALALDEITRLLTG-----FTNLPLWIVIFGAIQVVTTFYFG				
		V		VI	▼
AtNCS1	MDGIRKLEKYSAPILISLTSCLLAWSYLKAGFGHMLSLSSKLT-----AQFWTLFFPSLTANISFWATLALNIEDFSRFAKSQTDQIIQG-VGLPVFM				
CrNCS1	MESIRILEKYSAPILIGLSLALMGWAVTAGGFGPMLSTSPSQFVGMPKQGQFWSVFWPAVTANVGYATLSLNIEDFTRYAKSKQDQVMGQAIGLPLFM				
ScFUR4	PHKIRHIFTYKAVLVFPFASFGFLIWAIRRAHGRIALGSLTDVQ---PHGSAFSAWFLRSLMGCMANSTMTVINADFSRFSKPNPSALWSLVCIPFLF				
ScDAL4	PHKIRHIFTYKALIFPFAAFGLIWAIRKSHGKIELGLTLDVQ---PHGSEFSWIFVRSMLMACVNAALIIINADFGFRFAKNQASLWPCQLVAILPFLF				
ScFUI1	PDKLRHIFALKSAITPFAAFGLIWTICAKAGHLALGSLNDNGGA--ISKTVLAWSVIRAIMSALDNSTLILNADPFRFGTKYKSSVYSQALIALPVCY				
AnFURA	VHQIRHIFTYKSIYSPIAAIAFFAWAISRANG---LGPVHQSHIT--VHGSTLAWAVKALMSCLGNALALIMNDEDFSRFARKPKDALWAQLLTIPIGF				
AnFURD	PNKVRWLFATKSVLPAAWIAIILWAFVAEGK---GALIEQRAT--VSGSQYSVWVLASMTSVLGNATLSVNSQDSFRYSRVSARKOLLYIPLLPVIF				
AnFCYB	-YKVVHLYEYWSNIPTFIVFMILGLTFAHSGD---FQNIEMVG---TSEMGSVLSFGSAVYGFATGTSYAADTYVQYQANRSKRRIFLSTWLGLIVPL				
ScFCY2	-YSVIHAYEKMSWVPNFVFLVIAQLSRSGK---FKGGEWVG---ATTAGSVLSFGSSIFGFAAGTTTYAADTYVYMPKSTNKYKIFFSVLVAGLAFPL				
MHP1	ITFIRWMNVFASPVLLAMGVYVYMLDGAQVS--LGEVMSMG-----GENPGMPFSTAIMIFVGGIIVVVSIIHIVKECKVDPNASREGQTKADARY				
		VII		VIII	▽▽*▽
AtNCS1	GLFTTFVGVAVTSSTSIIFG-----RVISNPIELLGQIGGL-----ATLLAIVGISLATLTNIAANVVA-PANALVNLNPKFTFTRGCAFLTAVLG				
CrNCS1	ALFTFLGLAVTSATVVIYG-----EAIIDPVQLLGMEGL-----VPIICISLFLGLMWATLTNIAANVVA-PANAFVNCAPKWIIFSEAGGILTAVLG				
ScFUR4	SITCLIGLITVTAAGYIYG-----INVMSPLDVLEKFLQTTYNKCTRAGVPLISFVFAVQOLGTNISANSLS-CGTDMSAIFPKFINIKRGSIFCAAMA				
ScDAL4	AITCLIGIIVTAAGYHLYG-----VNYMSPLDVLGQFLETTYTRGTRAGVFLISFVFALAQOLGTNISANSLA-CGADMTALLFPRIINIRGSLFCVAMA				
ScFUI1	AITSLIGILSVSAAYPLYG-----VNYMSPLDILNRYLDN-YTSCNIRAGVPLISFIFAFDOLCANLSGNSIP-AGDITALLPKFINIRRGGSYICALIS				
AnFURA	GITSFIGIIASSSAVIFCG-----DAWNPDLDLGRFLEG-ASSAEFRGVFIIALGFALAQOLGTNISANSVS-AGTDMTALLPRYITIRRGSYICAAIG				
AnFURD	TFISFIGIAVSSAGWTRYNT-PIPDPIELISHWDSR-----AARFFGAFSALASLGNISANIS-ANDMLALFPYVVDLRGQIICGVIS				
AnFCYB	LFVEMLEGVAVMTATDK-CKSKY---DVGYATSGNGGLIAAVLQPL--LGGFGDFCLVILALSTIVANNCPNFYSVALTVQVLSRYAQRVPRFIWTLFCTGV				
ScFCY2	FFTMLGAASAMAA-LNDPTW---KAYYDKNAMGVYIAILVPSNLNGFGQCCVLLALSTIANNI-PMYTVALSAQALWAPLAKIPRVVWTMAGNAA				
MHP1	ATAQWLGMPASIIIFGFIKAASMLVGBNPNVIAITEVVG---VSIEMAILFQVFLVLLATWSTNPAANLLS-PAYTLCSTFRVRVFTFKTKGVIVSAVVG				
		X		XI	
AtNCS1	IVFCFWRLKSSSESFVYTWLIGYSALLGPICGIIIVDYILIKKMKLNIGDLYLSLSPSGEYIF--SKGYNVAALVALVAGIIP--VVPGLFLHKIS-ALSKI				
CrNCS1	LIMCPFWNLVSSTHGFVNTWLIGYSALLGPVIGVMSDYFIVRQRLDIDSLYSKGDKIYNY--KGTNPAAALWAILIGVLP--TLPGFLSTIG-VLSGL				
ScFUR4	LICIPFWNLMTSSKFT-MALSAYAIFLSSIAGVVCSDYFVVRRGYIKLTHIYSHQKGSFYMYGNRECNWRAALAAVLCGVAP--CLPGFIIEVGAIPAIV				
ScDAL4	LICIPFWNLMTSSKFT-SALGAYAIFLSSIAGVICADYFVVRRGYIKLTHLFLAQKGSFYMYGNRECNWRAAFVAYICGIAP--NLPGFIIDVGVAPKITV				
ScFUI1	LICIPFWDLSSSSKFT-TALAAVAVFLSAIAGVISADYFIVRKGYVNIHFCYTDKPGSYMY-NKYCTNWRRAVAYIFGIAP--NFAGFLGSVG---VSV				
AnFURA	LAMCPFWNLSDSNQFT-TYLSAYSIFLSAIAGVMICDYVVRKGYLIVKDLYSGEKDSAYRF--NYGFSWQAYASVLSGLLI--NIVGFAVAG---RDV				
AnFURD	WALVPWKILSASNFL-NFMSAYAIFLGPIAAIMLDFWLLIKNRKYDTVALYQPDTPYRFRN-AWLVNWRRAVAVFLVGVIP--SLPGLSNVSN--SRIQ				
AnFCYB	SIAIAIPGYSHFETVLENFMNFIAYWLAIYSAIIMDFVFKRG---FSGYVVENFDKREK---LPVGIATIAFGFGVAG--MITGMSQFWY-VGPIA				
ScFCY2	TLGISIPATYIFDGFENFMDSIGYLLAIYIAISCEHFIYSG---FSAYNIDDWDNWEH---LPIGIAGTAALIAISGAFG--VALGMCTYV-VGEIG				
MHP1	LMMFWQFAGVLTNFTL--NLLASAL--GFLAGIMISDYFLVRRRISLDHLYRTKGIYTYNR---GVNVAALAVYAVAVLAVSFLTPDLMFVTGLIALLI				
		XII			
AtNCS1	SNGFVVVDNALFFSPIIAGFVYWIIMS-RLGRQSSLSSSSHPL				
CrNCS1	PPIFGCLYDLAWFVGVAVSSVYVYCLMRGAPGAYKSGGDPFNGVGGGAAPGGATI				
ScFUR4	SDGAMKLYLSYVWVGYGLSFSSYTALCYFFFPVGC-P--VNIIKDKGWQFQ--WANVDDFEEEWKDTIERDDLVDNDSIVYEHEHEKFTI				
ScDAL4	SEGAMRLYYLGPVGVFFISAVIYLILCYFFFPVGP-T--VTNFLTKEGWQFQ--WAYVEDFEQDWKNELRRDDLDDTVSIYDGTTEEKIVY				
ScFUI1	PVIGAMKYYIYNVYVGYLLAALSYCYLVFYFPIKGI-P--GDAKITDRKWL--WVEVEEFGTER-----EAFEEYGGVSTGYEKIRIY				
AnFURA	PVGQYIYNVNYLSGPIVSFVYFYIITRLCPAATSDTWEVNTDLEDTGHDIDAEDHTGKPIGFTSEPRDEYKAGAGASV				
AnFURD	VGVGIHPIYQFGWLLGVGTSLVYIALSYGFPVREAL-IERAVLSDEVYEGR--EVEGEGVEEGRE---ELGESKREGVGKEKGFVAVY				
AnFCYB	RHA--AGGDVGFLGLFAAFASYLCLRP-----FEIKFFGR				
ScFCY2	RLIGKYGGDIGFELGASWAFIYINILRP-----LKLKYFGR				
MHP1	LHIPAMRWAKTFPLFSEAESRNEDYLR--PIGPVAPADESATANTKEQNQPAGGRGSHHHHHH				

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