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Two NCS1 transporters of the moss Physcomitrella patens share substrate specificities with other members of the NCS1 family but express novel phenotypes and distinct transport profiles

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Abstract
The two genes PpNCS1A and PpNCS1B of the moss Physcomitrella patens are nucleobase transporters and putative members of the purine-related transporter (PRT) or nucleobase:cation symporter 1 (NCS1) family. Previously characterized members of this family include the uracil transporter (FUR 4) of Saccharomyces cerevisiae, the adenine-guanine-hypoxanthine-cytosine transporter (FCY2) of S. cerevisiae and the recently characterized adenine-guanine-uracil transporter (AINCS1) of Arabidopsis thaliana. The two NCS1 genes of P. patens were cloned into yeast expression vectors and expressed in yeast strains lacking their own NCS1 genes. Transport profiles of the two P. patens NCS1 genes were discovered by radiolabeled nucleobase uptake and competition assays, and toxic analog growth studies. Interestingly, the two genes exhibit overlapping yet distinct solute specificities. PpNCS1A transports adenine, cytosine and uracil, but not guanine, while PpNCS1B transports adenine, cytosine and guanine, but not uracil. Future work will confirm the results presented here by yeast growth studies on media containing a sole nitrogen source, and kinetic parameters will be determined by radionucleobase homologous and/or heterologous competition assays.

Introduction
Nucleobases are the building blocks of nucleic acids, which constitute one of the four major classes of biological molecules. Nucleobases may be synthesized de novo, or derived via salvage pathways; pathways involved in nucleobase metabolism are ancient, highly compartmentalized, and often conserved. Crucial to the proper function of these pathways are nucleobase transporters, which facilitate the movement of nucleobases and their derivatives across selective membranes. Nucleobase transporters have variable substrate specificities and transport profiles, which are often representative of the transporter family to which they belong. Among plants, for example, members of the Purine Related Transporter (PRT) or Nucleobase: Cation Symporter 1 (NCS1) family are known to transport adenine, guanine, uracil and cytosine. The current study seeks to determine the transport profile of two putative members of the NCS1 family found in the moss Physcomitrella patens.

Materials and Methods
S. cerevisiae strains RG191 (Met a leu2A met15A ura3Δ fcy2Δ) and ATTC: 4003158 (Mat a his3Δ leu2A) were transformed using the Li/Al/SS carrier method with the plasmids pRH561 (PpNCS1A in vector pRG399) and pRH573 (PpNCS1B in vector pRG399). The strains were allowed to grow overnight in liquid Synthetic Complete medium (.6% yeast nitrogen base, 2% glucose, .002% each his, met, ura) at 30°C and allowed to reach an O.D.600 value of .6. Strains were then plated on solid SC media containing increasing concentrations of toxic nucleobase analogs and allowed to grow for two days at 30°C. Yeast expressing no NCS1 transporter gene, PpNCS1A or PpNCS1B was incubated for 5 minutes with .25 μM [³H] Adenine, 1 μM [³H] Guanine, 1 μM [³H] Uracil, .5 μM [³H] Hypoxanthine or 1 μM [³H] Xanthine; cell and isotope solution was filtered, washed with sterile ddH2O, and radioactivity of filters was measured by a scintillation counter.

Toxic Analog Growth Assays

Yeast transformed with plasmid pRH561 (PpNCS1A) or plasmid pRH573 (PpNCS1B) exhibits sensitivity to the toxic nucleobase analogs 8-azaadenine, 8-azaguanine and 5-fluorocytosine.

Substrate Specificities and Affinities

Yeast expressing PpNCS1A exhibits significant uptake of [³H] Adenine (p=.0005), [³H] Guanine (p=.0001), [³H] Uracil (p=.0004), [³H] Hypoxanthine (p=.0001) and [³H] Xanthine (p=.0004); Yeast expressing PpNCS1B exhibits significant uptake of [³H] Adenine (p=.0022), [³H] Guanine (p=.0288), [³H] Uracil (p=.0001), [³H] Hypoxanthine (p=.0001) and [³H] Xanthine (p=.0005).

Radionucleobase Uptake

Uptake of [³H] Adenine by yeast expressing PpNCS1A or PpNCS1B decreased in the presence of cold adenine, guanine, cytosine and hypoxanthine competitors; uptake of [³H] Uracil by yeast expressing PpNCS1A or PpNCS1B decreased in the presence of cold uracil and xanthine competitors. The presence of cold urate did not substantially decrease uptake of [³H] Uracil.

Conclusion

The putative NCS1 genes belonging to the moss P. patens exhibit identical solute specificities, but differing affinities for those compounds. Additionally, their transport profiles do not exactly match any of the transport profiles of NCS1 family gene members previously characterized.