centre for ecology, evolution and environmental changes

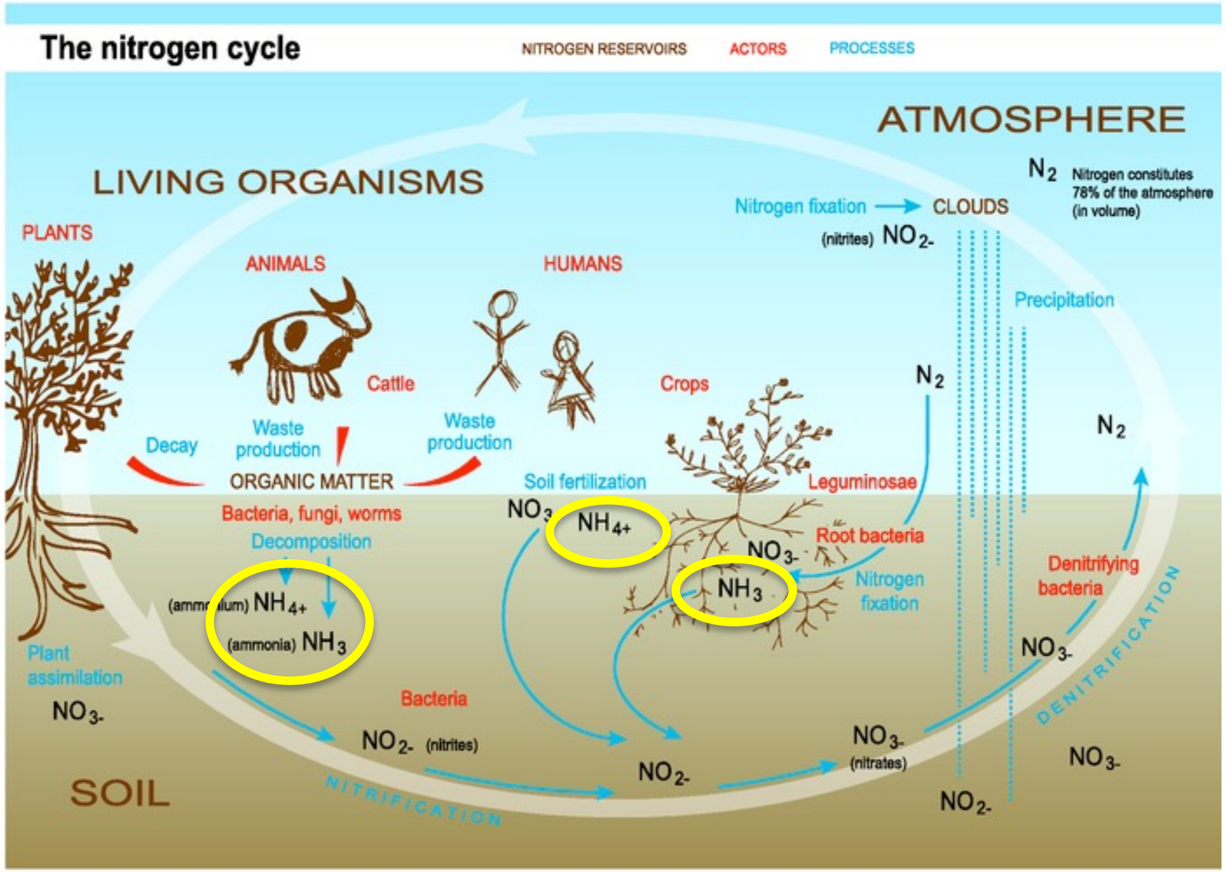
Ciências ULisboa

How do Ammonium transporters work?

(transport NH_4^+ or NH_3?)

– Teresa Dias –

What is NH_4^+ / NH_3? The N cycle



High Affinity Transport System (HATS)

[N] from 0 to 0.5-1 mM

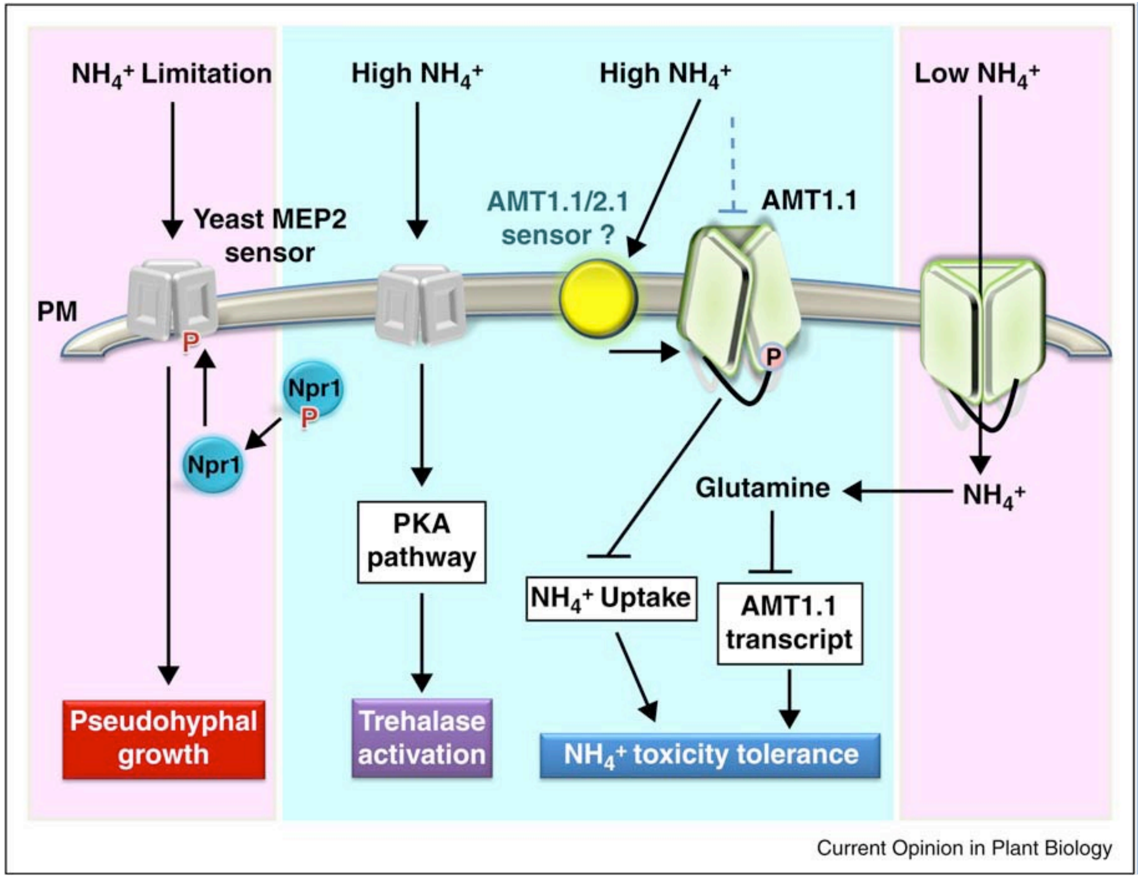
MEP, AMT: Km values in µM range

Rh: Km values in mM range

Low Affinity Transport System (LATS)

[N] from 0 to 0.5-1 mM

NSCC (e.g., K+ channels), Aquaporins



Ammonium transporter family - distribution

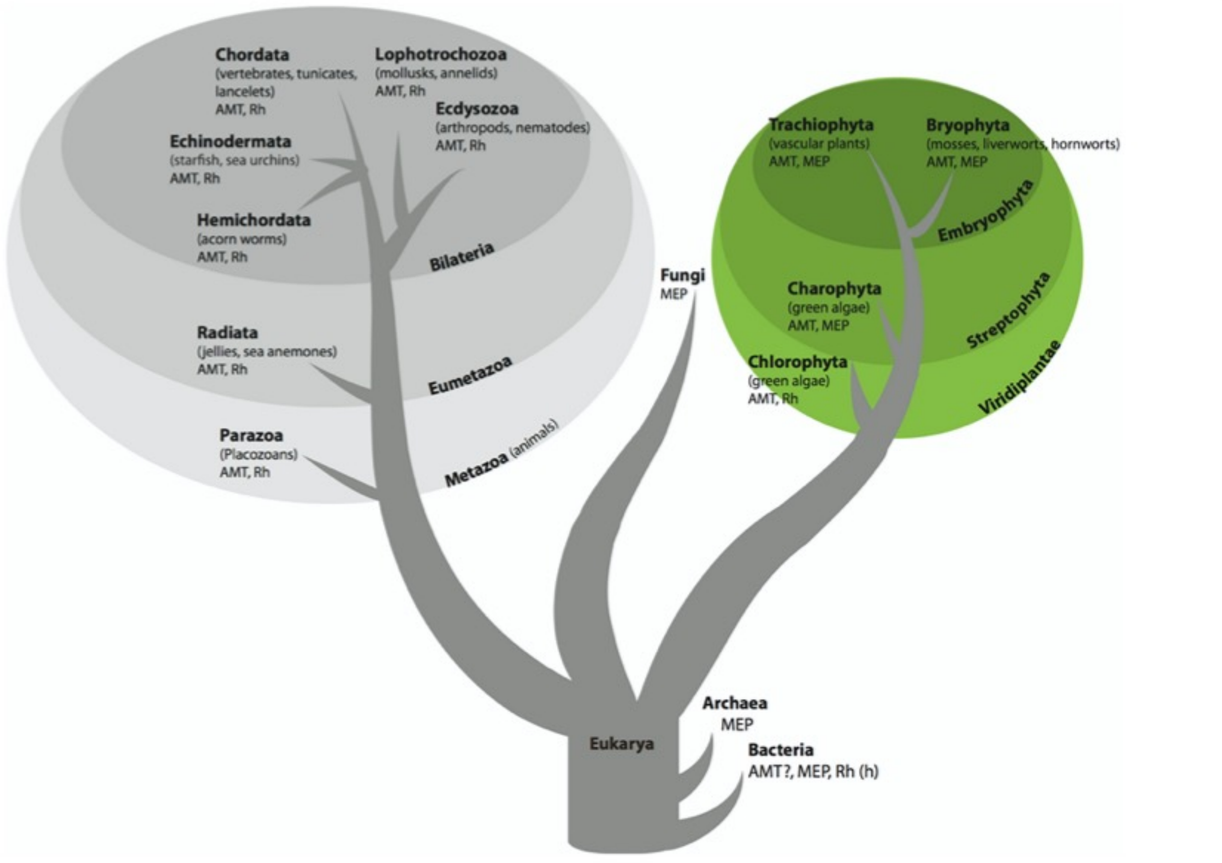
Genes in the ammonium transporter family are found in almost all **prokaryotic** and **eukaryotic** lineages.

The gene family consists of three major clades, the **AMT** (*ammonium transporters*), **MEP** (*methylammonium/ammonium permeases*), and **Rh** (*Rhesus*) and we refer to the family as the AMT/MEP/Rh family.

Although the AMT/MEP/Rh proteins are structurally similar, their function differs. In:

- microorganisms and plants is to **uptake** ammonium/ammonia, which for these organisms is an important nutrient used in the synthesis of N-containing metabolites such as amino acids

- animals is to **excrete** ammonium/ammonia, a by-product which is toxic to animal cells



McDonald and Ward 2016

Vol 456 | 20 November 2008 | doi:10.1038/nature07518 nature

ARTICLES

A role for Rhesus factor Rhcg in renal ammonium excretion and male fertility

Sophie Biver[1]*, Hendrica Belge[2]*, Soline Bourgeois[3,4]*, Pascale Van Vooren[1], Marta Nowik[3], Sophie Scohy[1]†, Pascal Houillier[4], Josiane Szpirer[1], Claude Szpirer[1], Carsten A. Wagner[3], Olivier Devuyst[2] & Anna Maria Marini[1]

The kidney has an important role in the regulation of acid–base homeostasis. Renal ammonium production and excretion are essential for net acid excretion under basal conditions and during metabolic acidosis. Ammonium is secreted into the urine by the collecting duct, a distal nephron segment where ammonium transport is believed to occur by non-ionic NH_3 diffusion coupled to H^+ secretion. Here we show that this process is largely dependent on the Rhesus factor Rhcg. Mice lacking Rhcg have abnormal urinary acidification due to impaired ammonium excretion on acid loading—a feature of distal renal tubular acidosis. *In vitro* microperfused collecting ducts of *Rhcg*$^{-/-}$ acid-loaded mice show reduced apical permeability to NH_3 and impaired transepithelial NH_3 transport. Furthermore, Rhcg is localized in epididymal epithelial cells and is required for normal fertility and epididymal fluid pH. We anticipate a critical role for Rhcg in ammonium handling and pH homeostasis both in the kidney and the male reproductive tract.

Ammonium is a principal nitrogen source for microorganisms and plants, whereas in animals it is best known for its cytotoxic effects that may lead to hepatic encephalopathy for instance[1]. Because more than 98% of ammonium is in the NH_4^+ form at physiological pH, through-

ago[13], the physiological role of Rhesus-type proteins remains largely unknown. Human Rhesus factors comprise the blood-group antigens (RHCE and RHD)[14,15], their associated glycoprotein (RHAG)[16], and two non-erythroid members (RHBG and RHCG)[17–19]. The Rhbg

Ammonium transporter family - structure

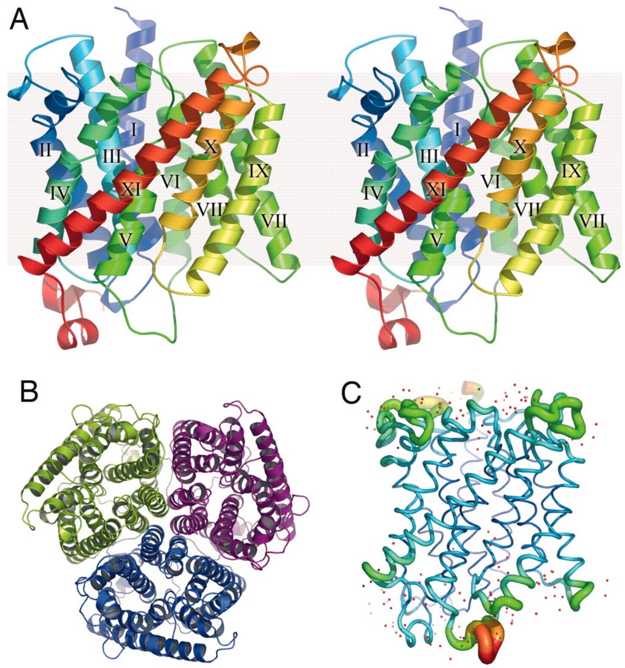
The structures of AMT/MEP/Rh proteins have been elucidated

(e.g., *Escherichia coli* and *Archaeoglobus fulgidus* indicate that these proteins have 11 transmembrane domains that fold into a pore; Rh proteins have demonstrated that these proteins have 12 transmembrane domains and likewise trimerize in the membrane to form a triple pore)

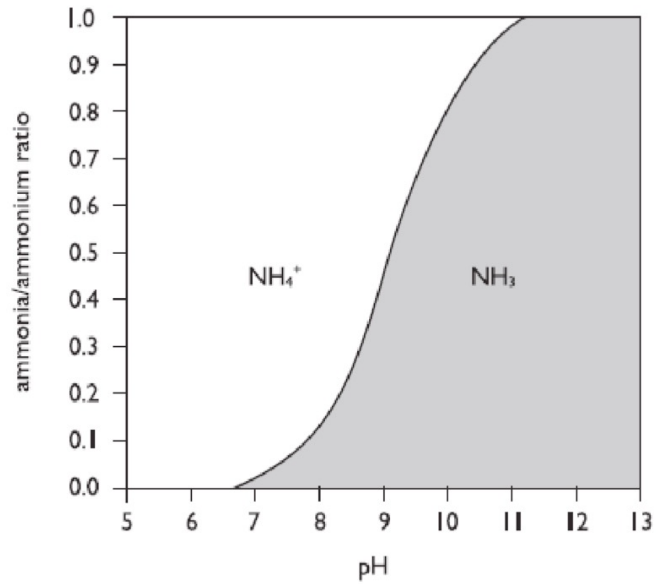
BUT

the transport mechanism and **the substrate (NH_4^+ or NH_3) of these proteins remain hotly debated topics**

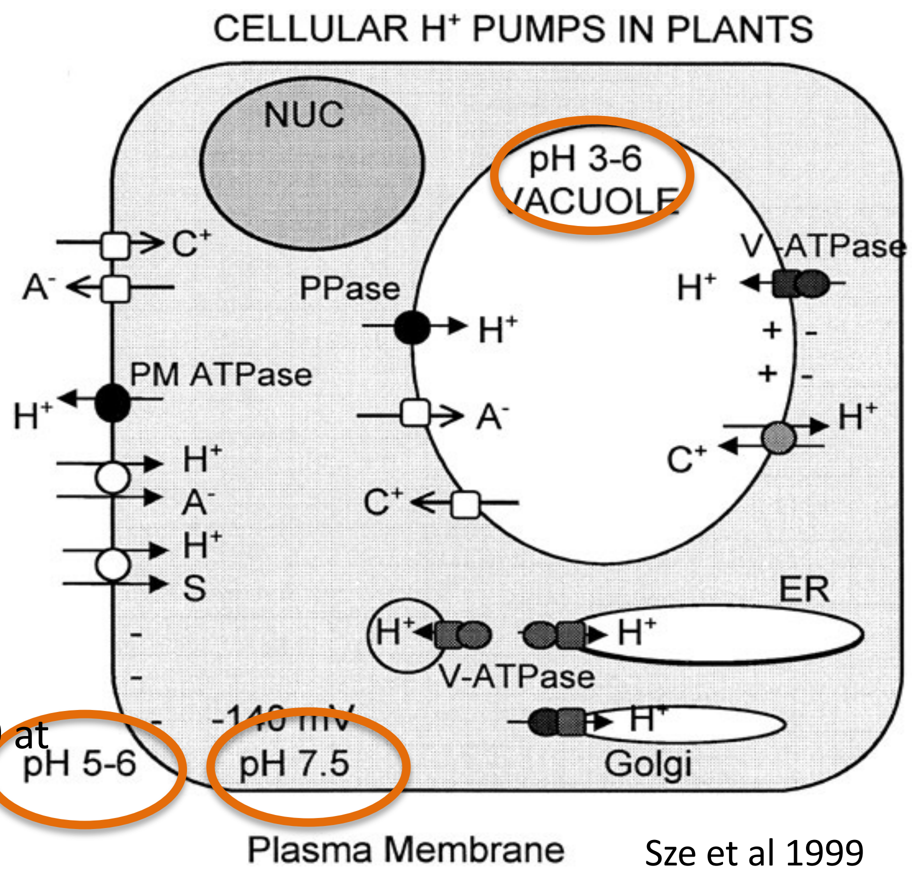
Crystal structure of Archaea MEP transporters



What is the difference between NH_4^+ and NH_3?



Conversion of NH_4^+ to NH_3 depends on pH and temperature, with a p*K*a (where *K*a is the acid dissociation constant) ≈ 9.25 at 25°C (p*K*a ≈ 9.09 at 30°C); at pH <7, more than 99% is NH_4^+



Sze et al 1999

Ammonium transporter family – HOW DOES IT WORK? What is transported?

At least three distinct transport mechanisms have been reported for proteins in the AMT/MEP/RH family.

MEP

AmtB from *E. coli* and Amt1 from the archaean *A. fulgidus* are both found in the MEP clade. Crystal structures indicate that these proteins carry **NH_3** through the pore. An ammonium ion docks in an extracellular vestibule, the proton is stripped, the ammonia molecule transverses the pore, and the molecule is reprotonated on the intracellular side. As no charge is transported across the membrane, this transport is **electroneutral**.

Rh proteins

are generally understood to be passive channels transporting either CO_2 or **NH_3**, or perhaps both. From the crystal structure of the Rh from *Nitrosomonas europaea* this protein most likely transports either NH_3 or CO_2. The function of the human RhBG has been studied by radioactive methylammonium uptake and electrophysiology and determined to be **electroneutral**.

Ammonium transporter family – HOW DOES IT WORK? What is transported?

Some proteins encoded by the AMT/MEP/Rh family

have been demonstrated to facilitate the movement of **NH_4^+** across the membrane, either as NH_4^+ uniporters or as NH_3/H^+ co-transporters. Because a net charge crosses the membrane, this type of transport is **electrogenic**.

It is unclear whether all members of the large AMT/MEP/Rh family translocate NH_3 or whether some translocate NH_4^+.

McDonald and Ward 2016

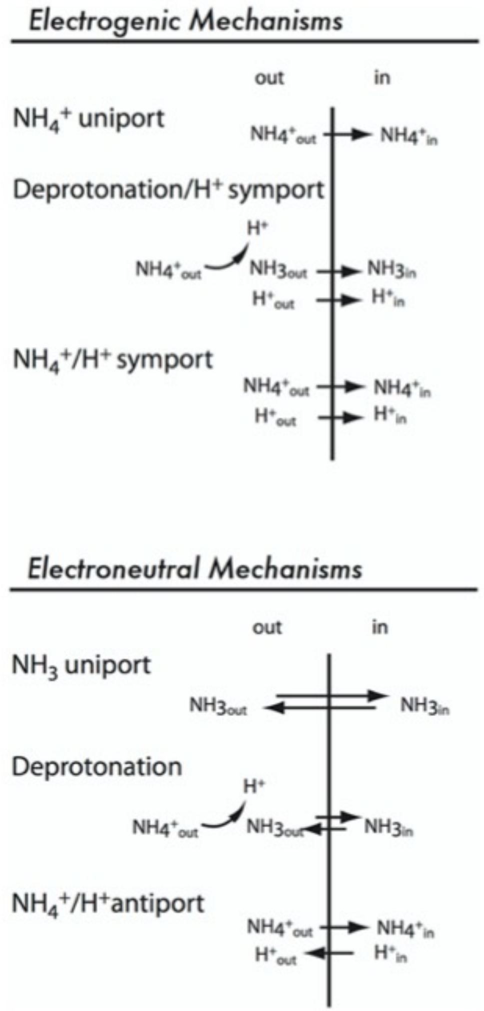


FIGURE 5 | Possible electroneutral and electrogenic mechanisms for AMT/MEP/Rh proteins.

When there is too much NH_4^+: NH_4^+ toxicity is universal

… and in microorganisms (e.g., bacteria, yeast)

How do yeasts cope with ammonium toxicity?

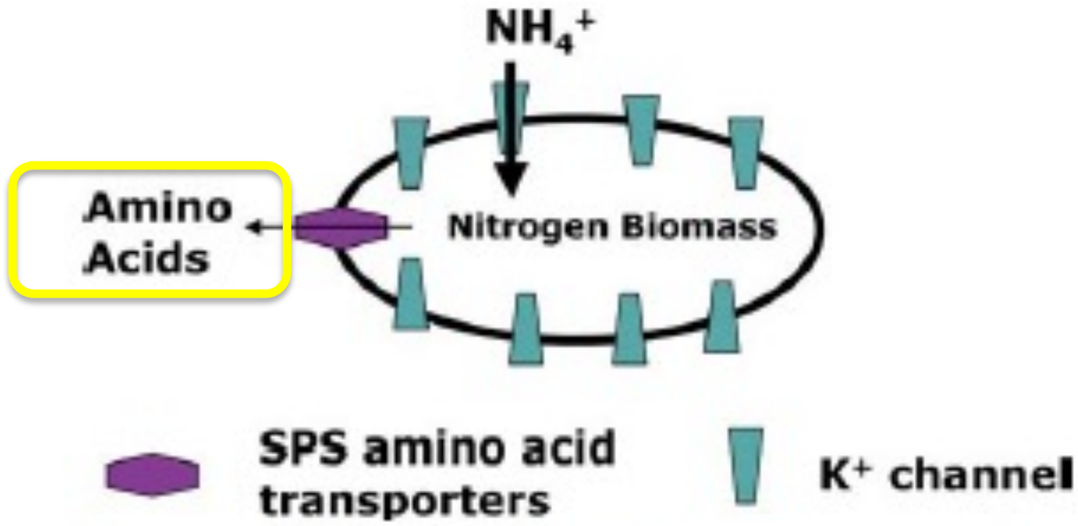
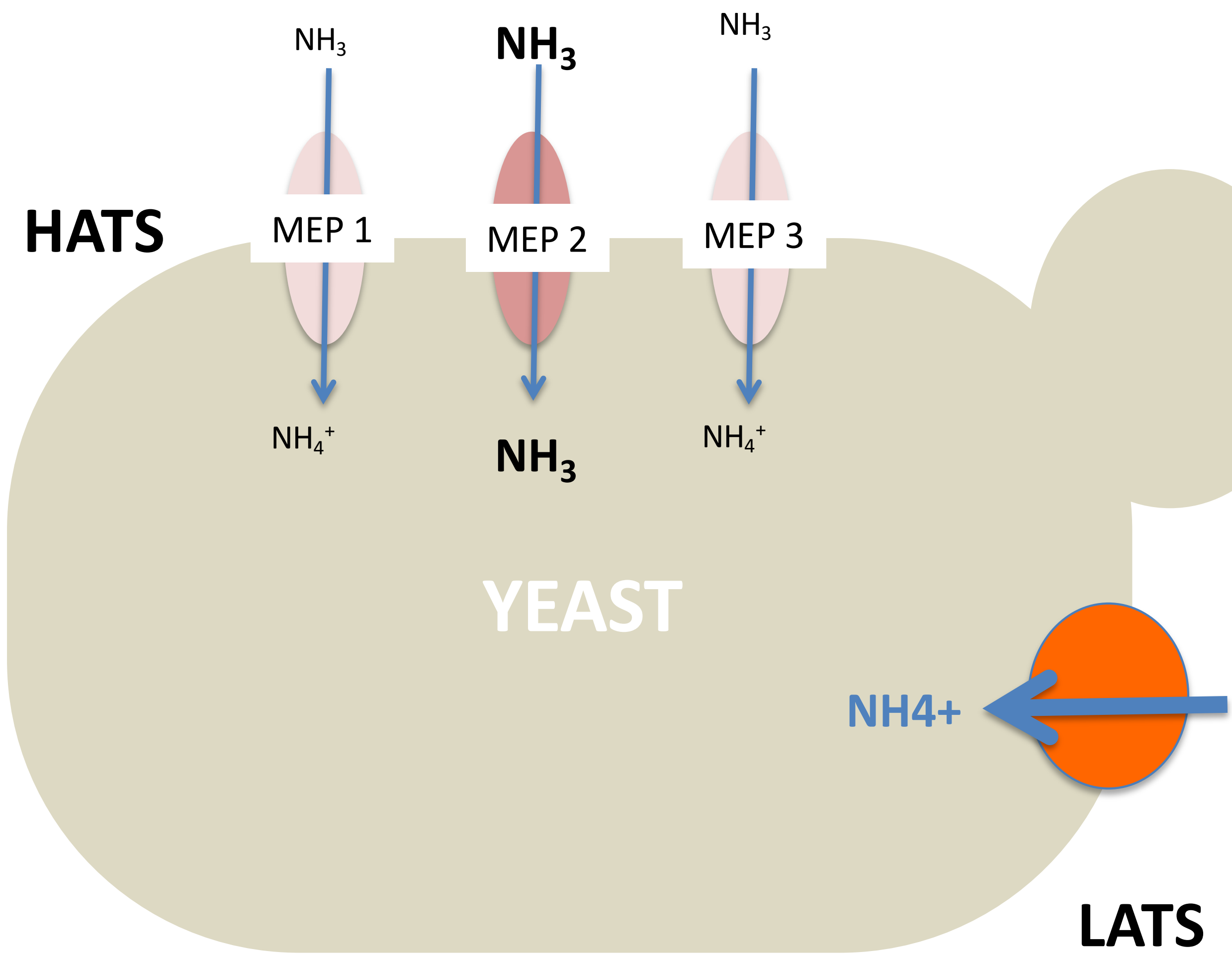


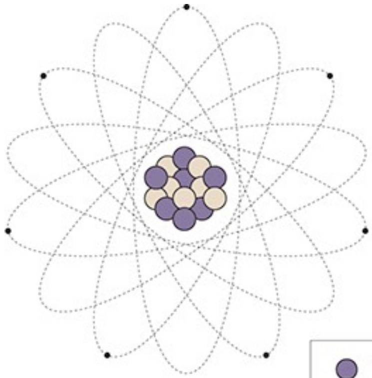
Figure 10. Model for Ammonium Toxicity and Detoxification in Yeast
We believe the results of this paper support the following model. If ammonium is present in high concentrations in the environment, then ammonium ions can enter the cell unregulated via potassium channels. Although most of the ammonium can be taken up into new biomass (if excess carbon and other nutrients are available), the unregulated flux creates an excess of internal ammonium that becomes toxic. To reduce internal ammonium levels, amino acids are excreted (most likely through the SPS amino acid transporters). The nitrogen affixed to amino acids will not be taken up through the potassium channels and is thus detoxified with respect to the cell.
DOI: 10.1371/journal.pbio.0040351.g010

Ammonium transporters in yeast (only MEP for HATS)

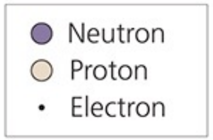


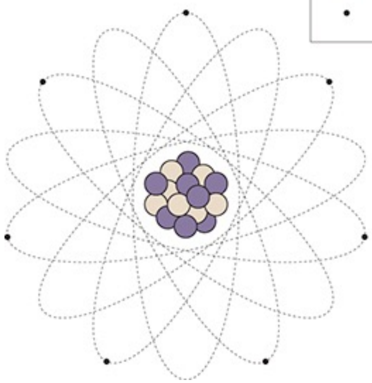
MEP 2 - Repressed at $[NH_4^+] > 5$ mM

N stable isotopes



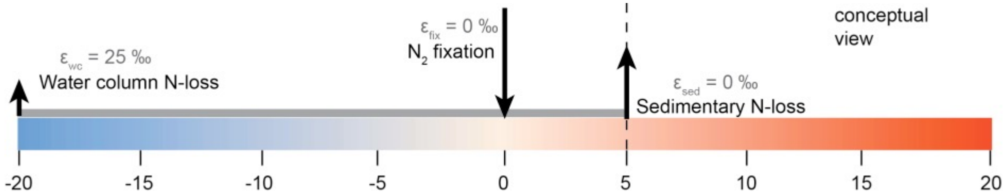
^{14}N = Nitrogen 14
- Stable
- Seven neutrons and seven protons
- Atomic number = 7
- Mass number = 14.00307
- Makes up 99.63% of Earth's total nitrogen

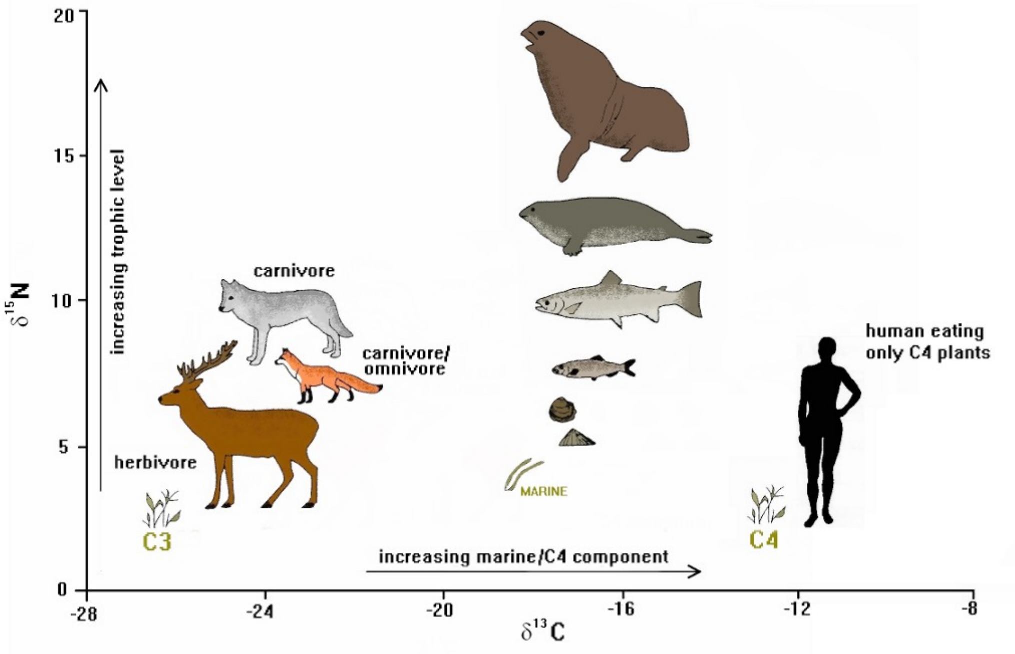




^{15}N = Nitrogen 15
- Stable
- Eight neutrons and seven protons
- Atomic number = 7
- Mass number = 15.0001
- Makes up 0.37% of Earth's total nitrogen

NH_4^+ containing ^{14}N are more easily deprotonated than those containing ^{15}N, leading to an isotopic fractionatior linked to an isotope effect, at 30°C, of 1.044 between NH_4^+ and NH_3





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RESEARCH PAPER

Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K^+-sensitive and -insensitive components of NH_4^+ transport

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Received 8 August 2007; Revised 8 November 2007; Accepted 12 November 2007

Evidences of K^+-dependent component of ammonium toxicity
(when K^+ is a limiting nutrient) in:

PLANTS

YEAST

Plant, Cell & Environment
Plant, Cell and Environment (2010) **33**, 23–34 doi: 10.1111/j.1365-3040.2009.02046.x

Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72)

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OPEN ACCESS Freely available online PLoS BIOLOGY

Ammonium Toxicity and Potassium Limitation in Yeast

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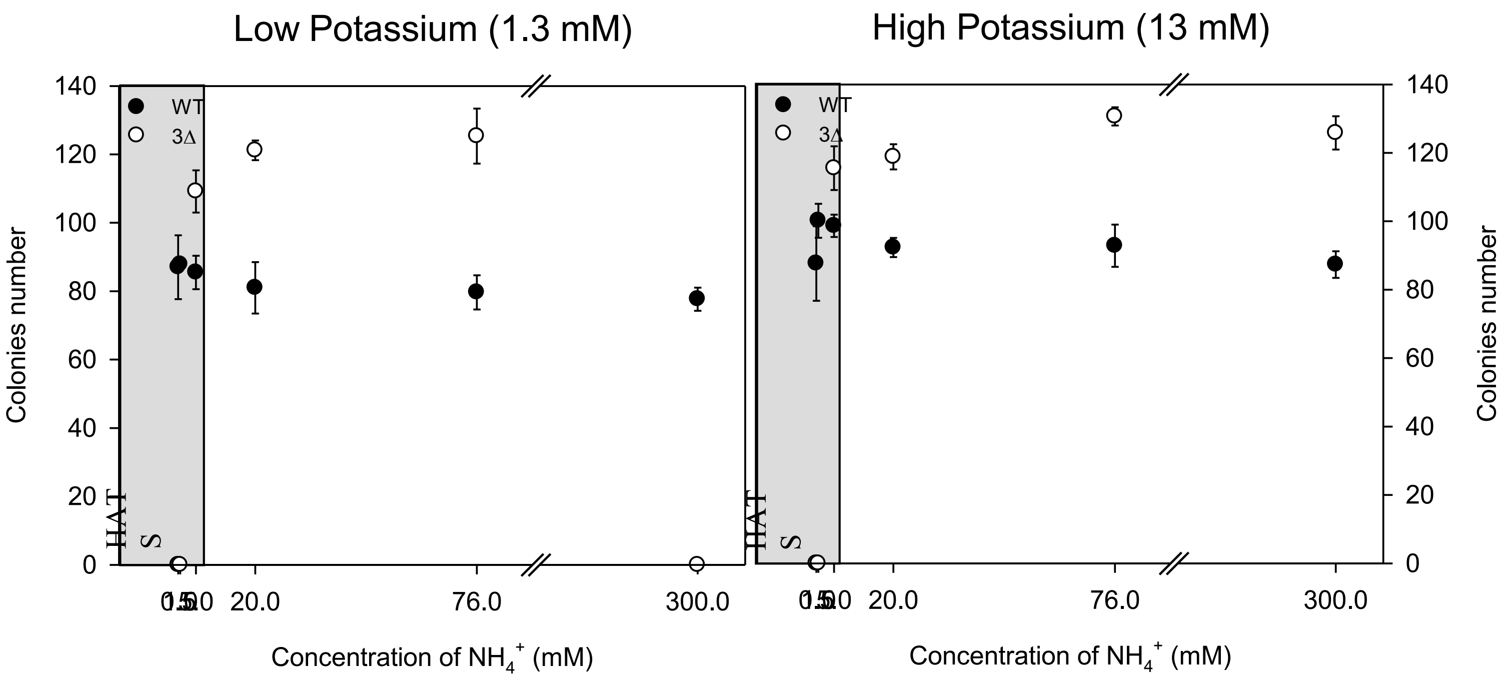
Experimental design using yeast

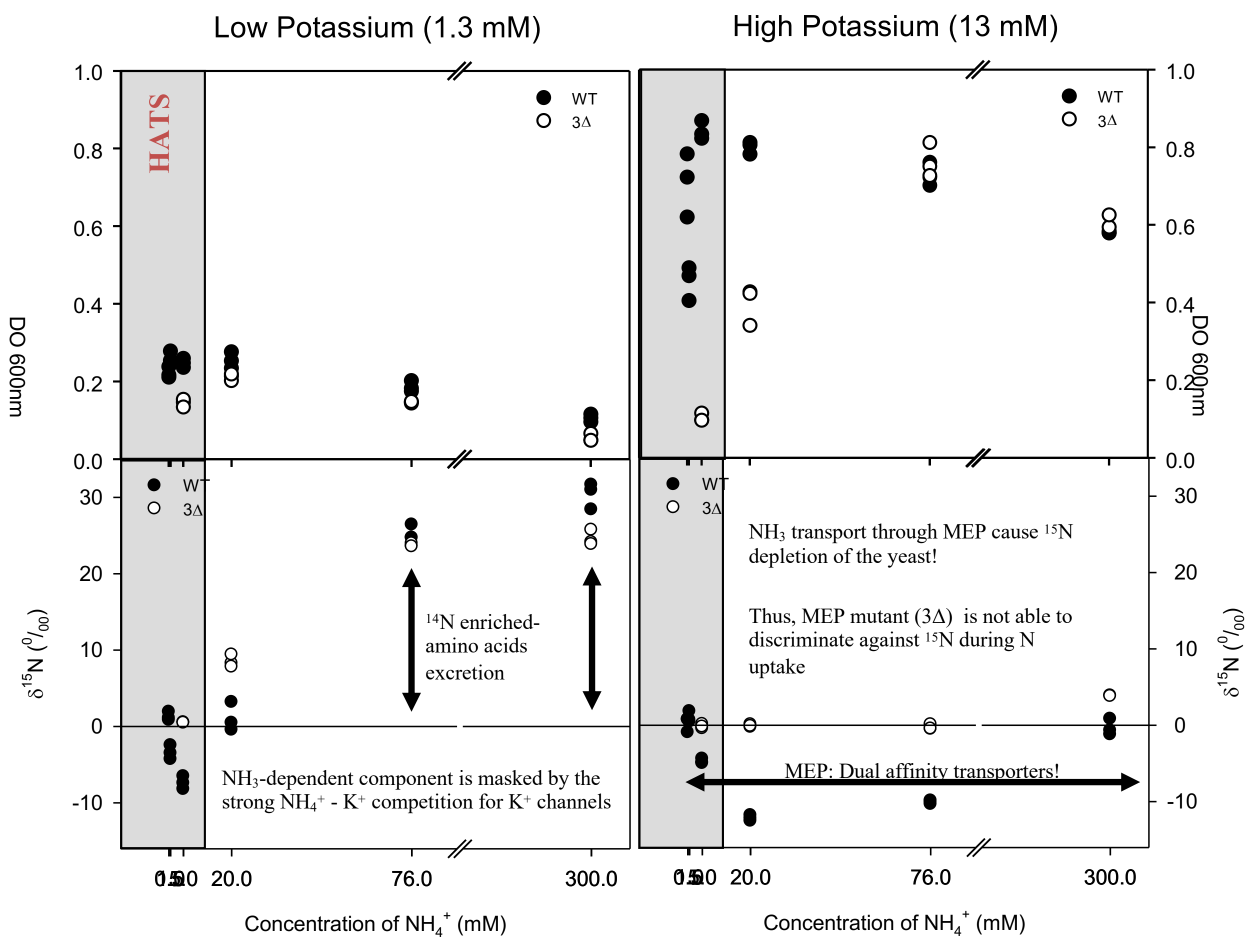
- **Yeast strains**: *Wild types* (Soil *S.cerevisiae* and Σ1278b), *3ΔMEP* mutants (31019b and 31019b+PFL38_empty vector – *no MEP; only LATS is working*)
- 2 $\mathbf{K^+}$ levels (1.3 and 13 mM)
- **Increasing** $\mathbf{NH_4^+}$ concentrations (0.5, 1, 5, 20, 76 and 300 mM)
- Relationship between uptake of NH_4^+/NH_3 and $\boldsymbol{\delta^{15}N}$ of organisms



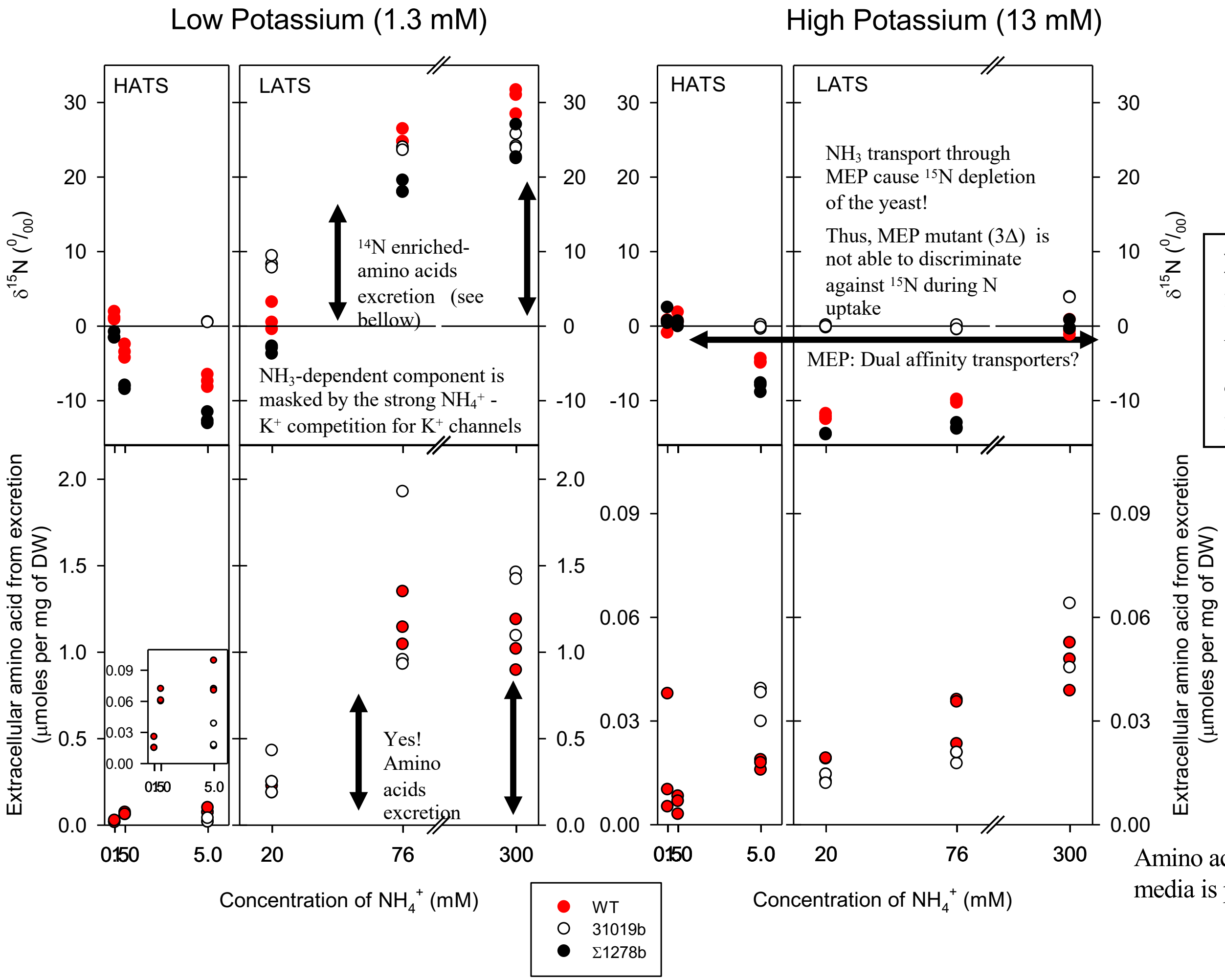
Number of yeast colonies indicates the lethal treatments.

Growth

^{15}N discrimination



Valores de δ^{15}N de las fuentes de N: $(NH_4)_2SO_4$=0.044; Uracil=-3.1



δ15N of N source $(NH_4)_2SO_4$=0.04 4

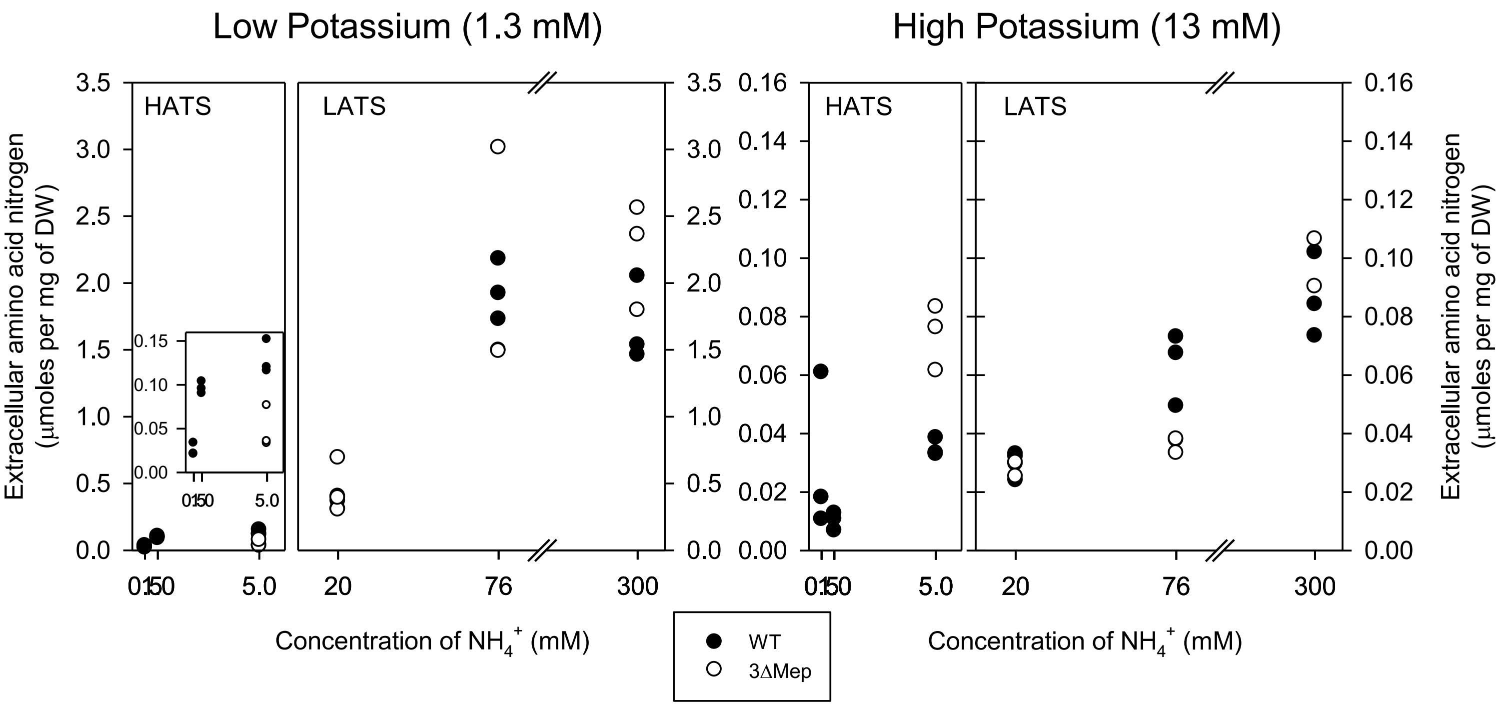
Σ1278b = wild type for 31019b

WT: is a wild *S. cerevisiae* isolated from the soil

Amino acids detection in Σ1278b media is pending (in process)

Now, it would be interesting to know which is the MEP transporter that works under LATS conditions? Could it work at 76mM? at 100mM? and even at 200mM? Interesting strains to test:

31019b+Mep1
31019b+Mep2
31019b+Mep3

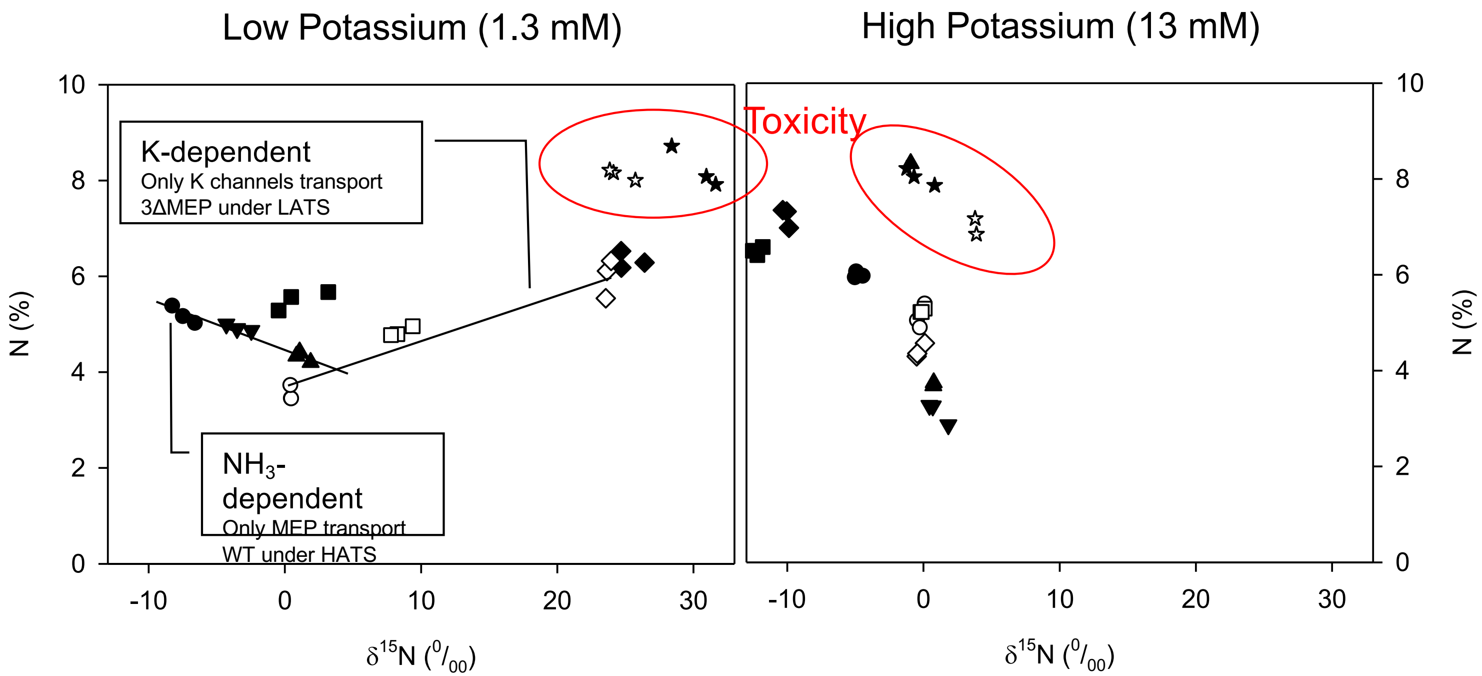


There is ^{15}N discrimination during amino acids excretion which means that most excreted amino acids contain ^{14}N.

Therefore, yeast cells coping with ammonium toxicity become enriched in the heavier isotope!

The 2 components of NH_4^+/NH_3 transport

(K dependent and K independent)

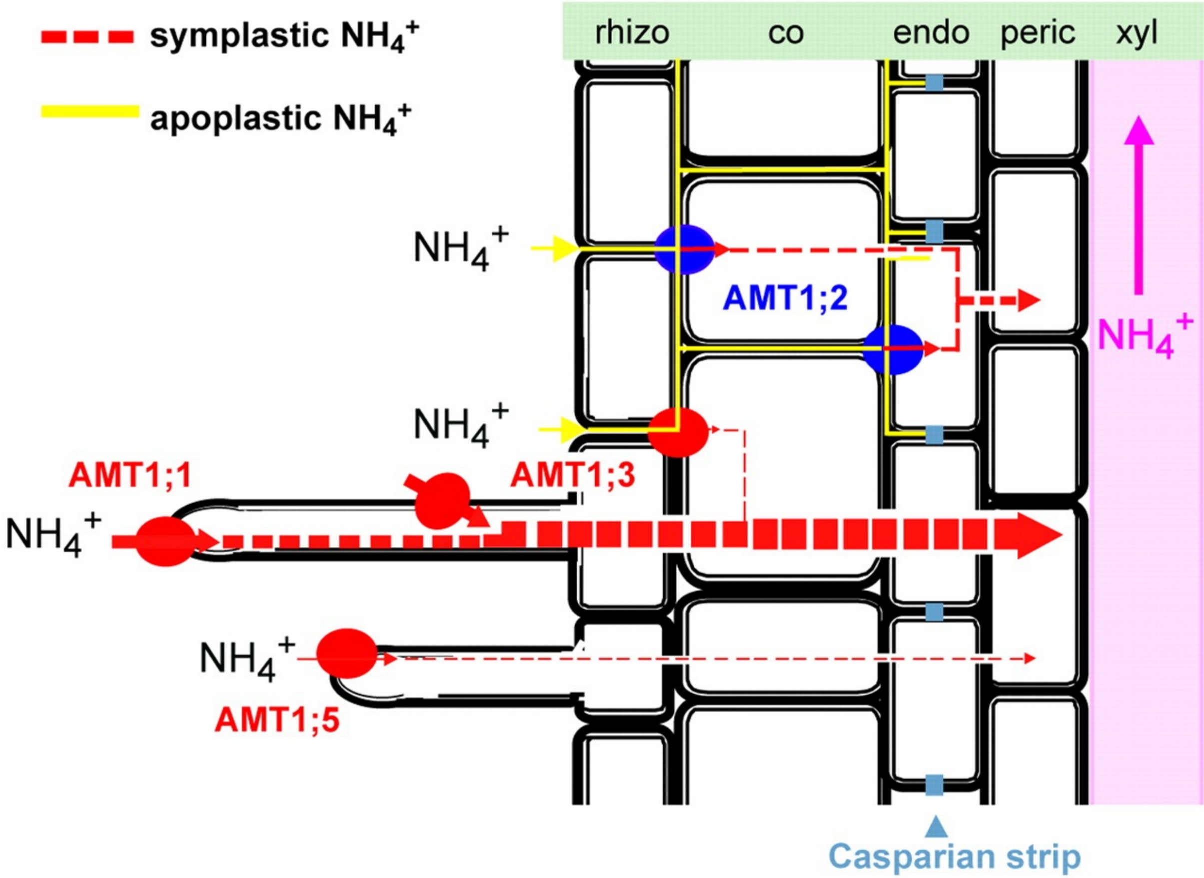


The natural N abundance is correlated to N percentage.

However, this relationship is “positive” or “negative” depending on the main NH_4^+/NH_3 transport mechanism which is working: K^+-dependent (no discriminatory) or NH_3-dependent (discriminatory)

What about in other kingdoms?

Example:
Ammonium
transporters
in plants
Very
complex to
study *in vivo*



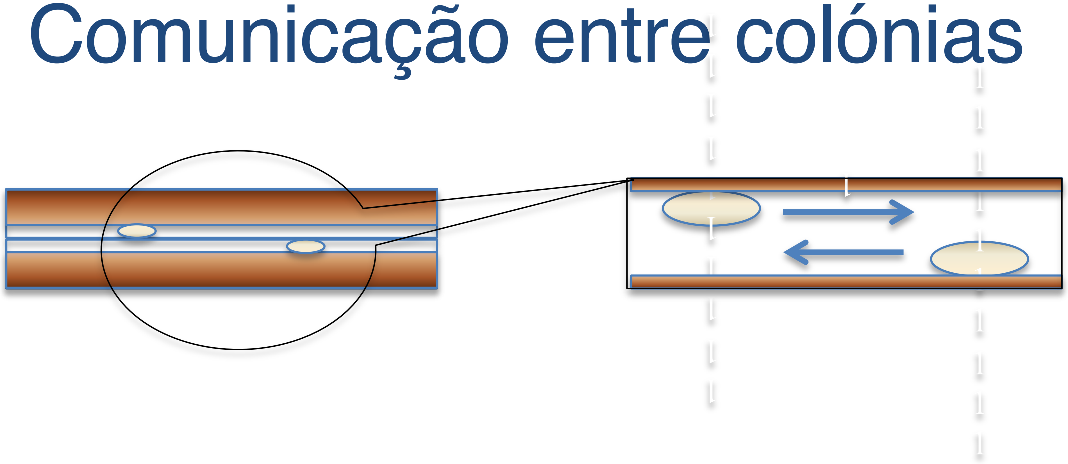
Using heterologous expression in yeast cells it is possible to study ammonium transporters

see Ariz et al 2018

Diferentes concentrações de amónio e de potássio

		$[NH_4^+]$ (mM)					
WT	$[K^+]$ (mM)	1		76		300	
	1,3						
	13						
ΔMEP		1		76		300	
	1,3						
	13						
MEP2		1		76		300	
	1,3						
	13						

Comunicação entre colónias



- WT
- ΔMep
- Mep2

Amónio como indutor da resistência a antifúngico