# *Need-based activation of ammonium uptake in Escherichia coli*

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# *Introduction*

- The efficient sequestration of nutrients is vital for the growth and survival of microorganisms
- Study the delicate management of ammonium ($NH_4^+/NH_3$) sequestration by E. coli cells using microfluidic chemostats
- A delicate control of ammonium sequestration strategy

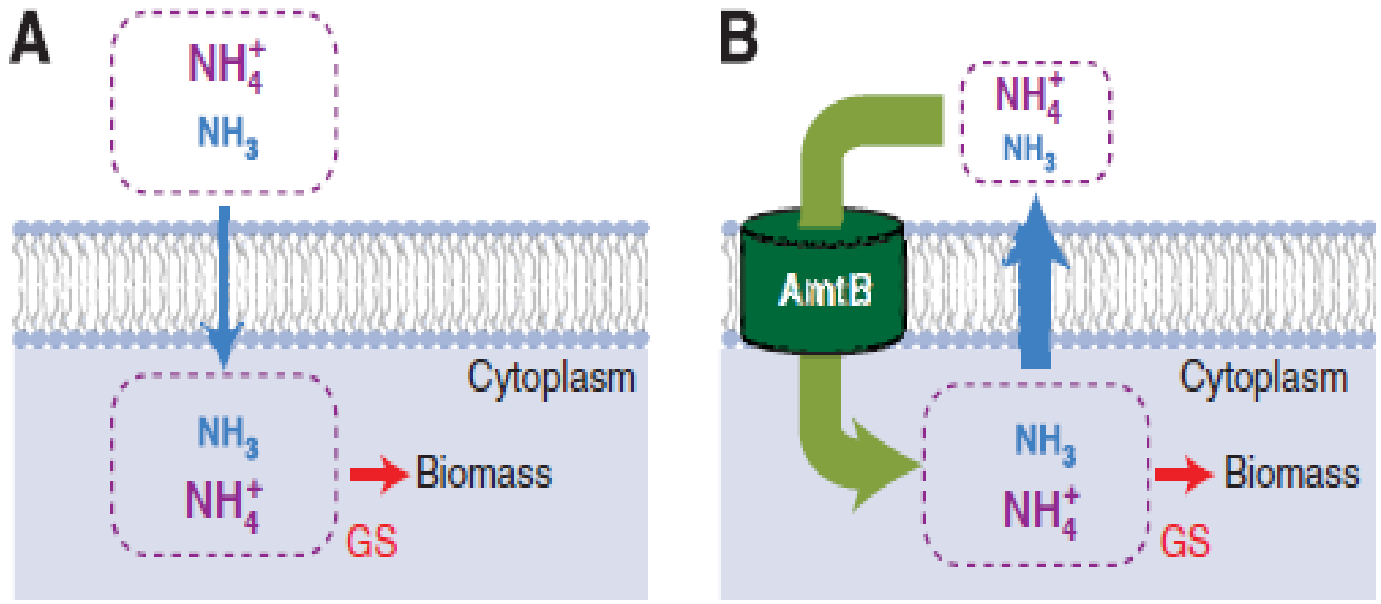
# *Results*

- 1.AmtB is necessary to maintain rapid cell growth at low ambient ammonium concentrations
- 2.GS and AmtB expression is upregulated at low ammonium concentrations
- 3.Deducing the internal ammonium concentration of ΔamtB strain

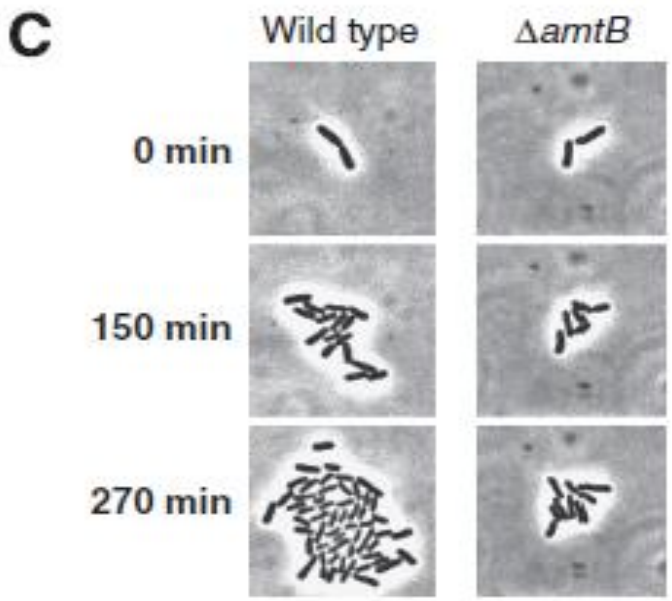
# *Results*

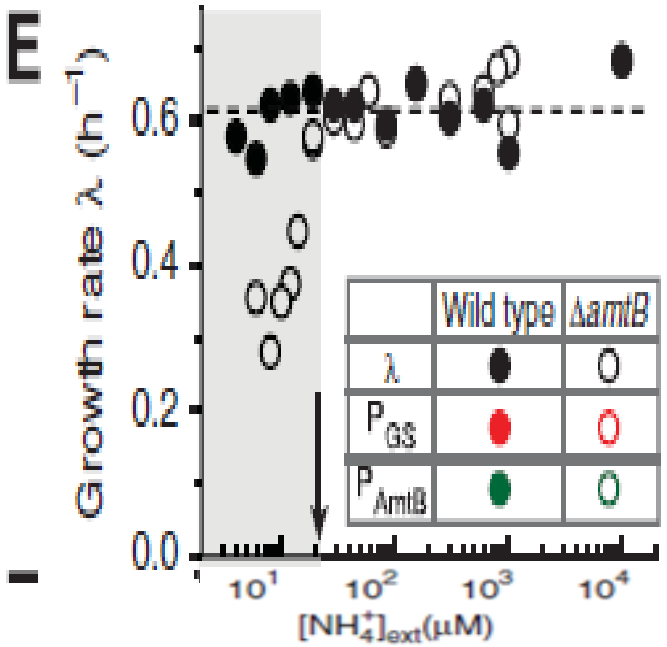
- 4.Deducing the GS activity
- 5.Deducing the AmtB activity
- 6.The activity of AmtB is delicately controlled
- 7.The delicate control of AmtB activity is coordinated with cellular nitrogen demand

# 1.AmtB is necessary to maintain rapid cell growth at low ambient ammonium concentrations




- At high ambient ammonium concentrations
- At low ambient ammonium concentrations

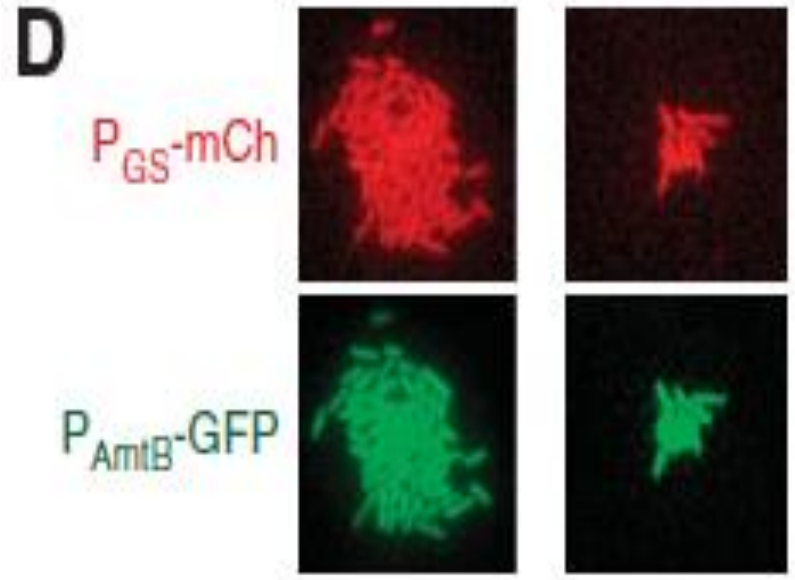



ΔamtB strains growing more slowly than the wild type at 12 μMof NH4+

The wild-type strain maintained its growth rate; the ΔamtB strain grew more slowly below ~20 μM

# 2.GS and AmtB expression is upregulated at low ammonium concentrations

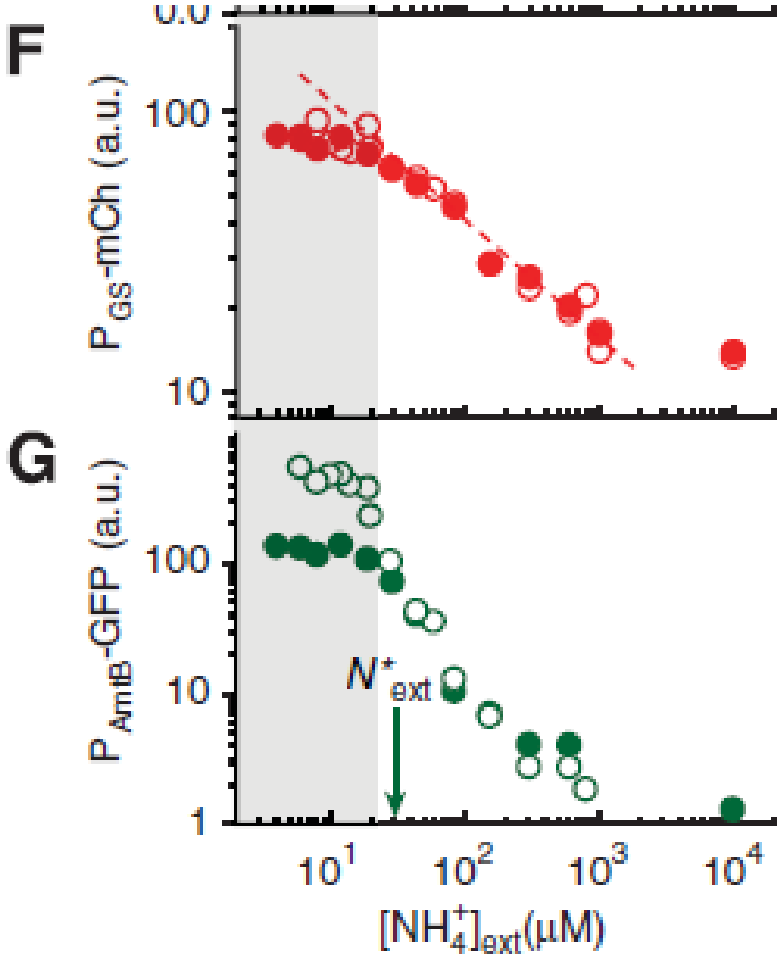



GS reported using the mCherry fluorescence protein

AmtB promoter green reported by green fluorescence protein (GFP)

The wild-type strain

The ΔamtB strain




Cell growth is maintained by elevating the GS expression level

Above $N^*$ext≈30μM (green arrow) indistinguishable; Below this higher plateau

# 3.Deducing the internal ammonium concentration of ΔamtB strain

$$J_{diffusion} = \kappa_{ext}[NH_4^+]_{ext} - \kappa_{int}[NH_4^+]_{int} \qquad (1)$$

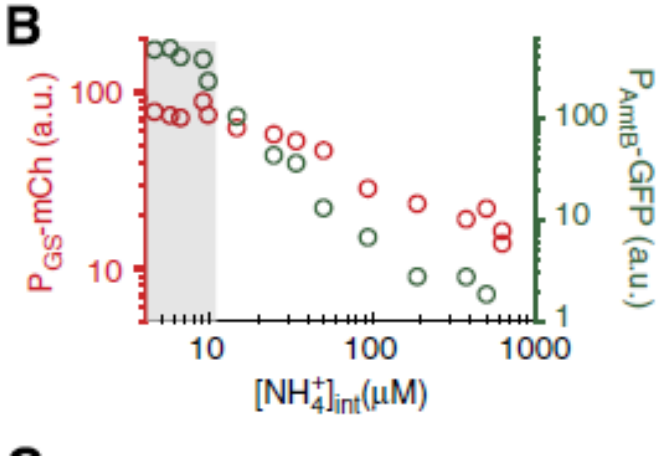
$J_{diffusion}$:the diffusive flux;$k_{ext}$ and $k_{int}$ :proportionality constants given by the NH3 permeability, cell geometry, and extra- and intra-cellular pH;

$$J_{biomass} = \lambda \times n_0. \qquad (2)$$

$J_{biomass}$:nitrogen assimilation,$n_0$:nitrogen content of biomass ,$\lambda$: the measured growth rate

$J_{diffusion}$=$J_{biomass}$

# 4.Deducing the GS activity



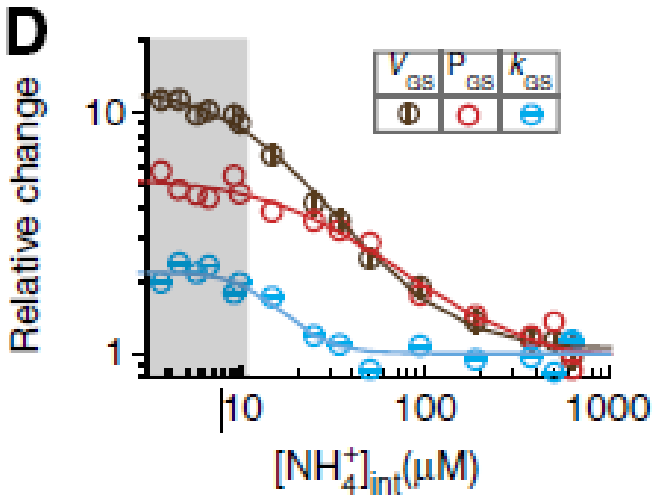

revealing a simple dependence of GS promoter activity on the internal NH4 +concentration

GS activity can be altered by glutamine via adenylylation and by the end products of glutamine metabolism via allosteric inhibition

$$J_{GS} = V_{GS} \times \frac{[NH_4^+]_{int}}{[NH_4^+]_{int} + K_{GS}} \quad (3)$$

$$J_{biomass} = \lambda \times n_0. \quad (2)$$

$V_{GS}$:$V_{max}$ $K_{GS}$:Michaelis constant $K_{GS}$~100 μM for NH4+



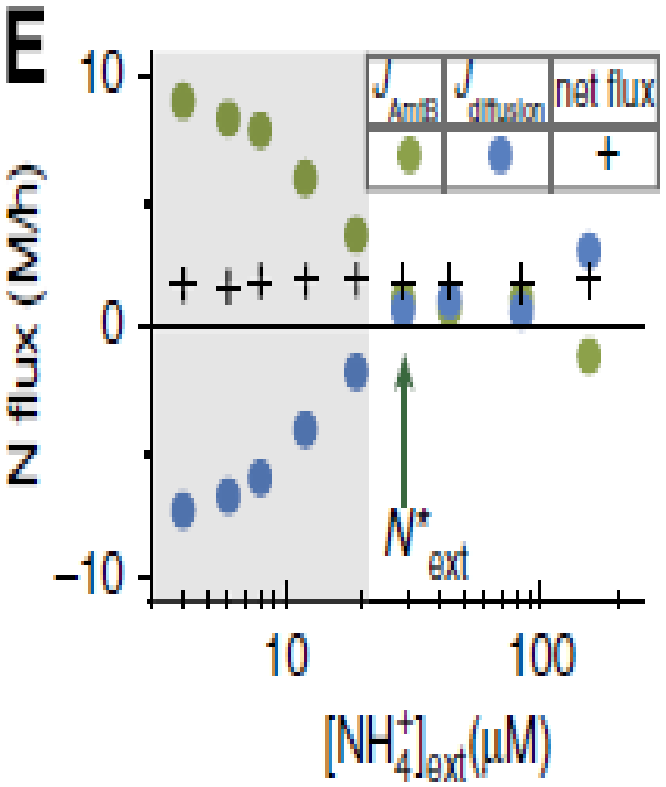

The ratio of $V_{GS}$（[NH4+]int）and $P_{GS}$（[NH4+]int）gives the relative changes in the specific activity of GS, $k_{GS}$

GS is approximately halfadenylylated in nitrogen-replete conditions and completely unadenylylated in nitrogen-limited conditions

# 5.Deducing the AmtB activity

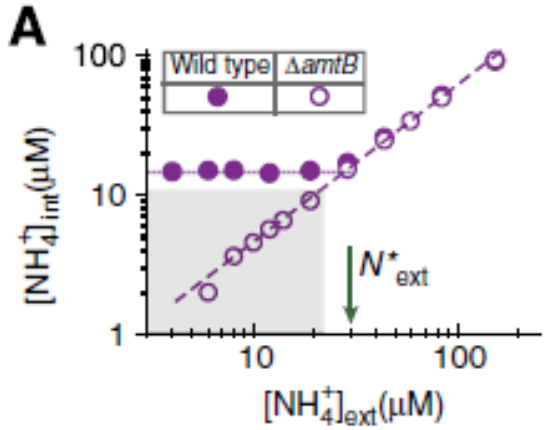
- AmtB expression  is upregulated below ~1mM of external NH4+, its activity  is turned on only below $N^*_{ext}$.
- AmtB activity is inhibited by the regulatory protein  GlnK
- Determine the internal NH4+ concentration of wild type first

$$J_{\mathrm{AmtB}} + J_{\mathrm{diffusion}} = J_{\mathrm{biomass}}, \qquad (4)$$

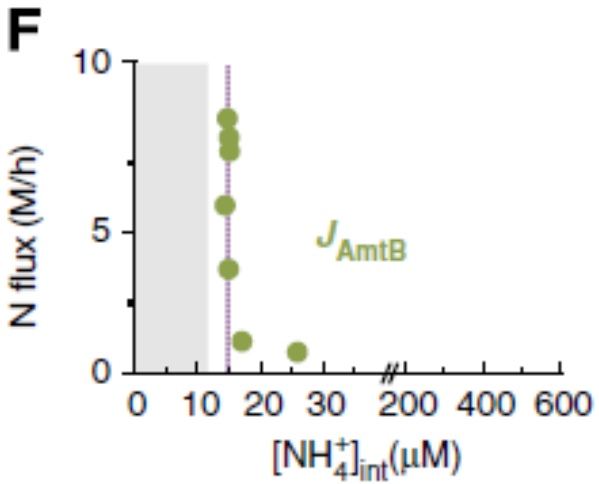


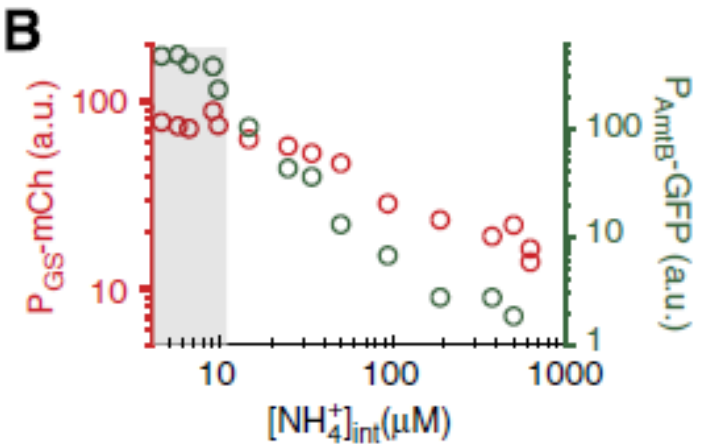

the netammonium uptake flux, given by the sum of $J_{diffusion}$ and$J_{AmtB}$, as the crosses.

# 6.The activity of AmtB is delicately controlled

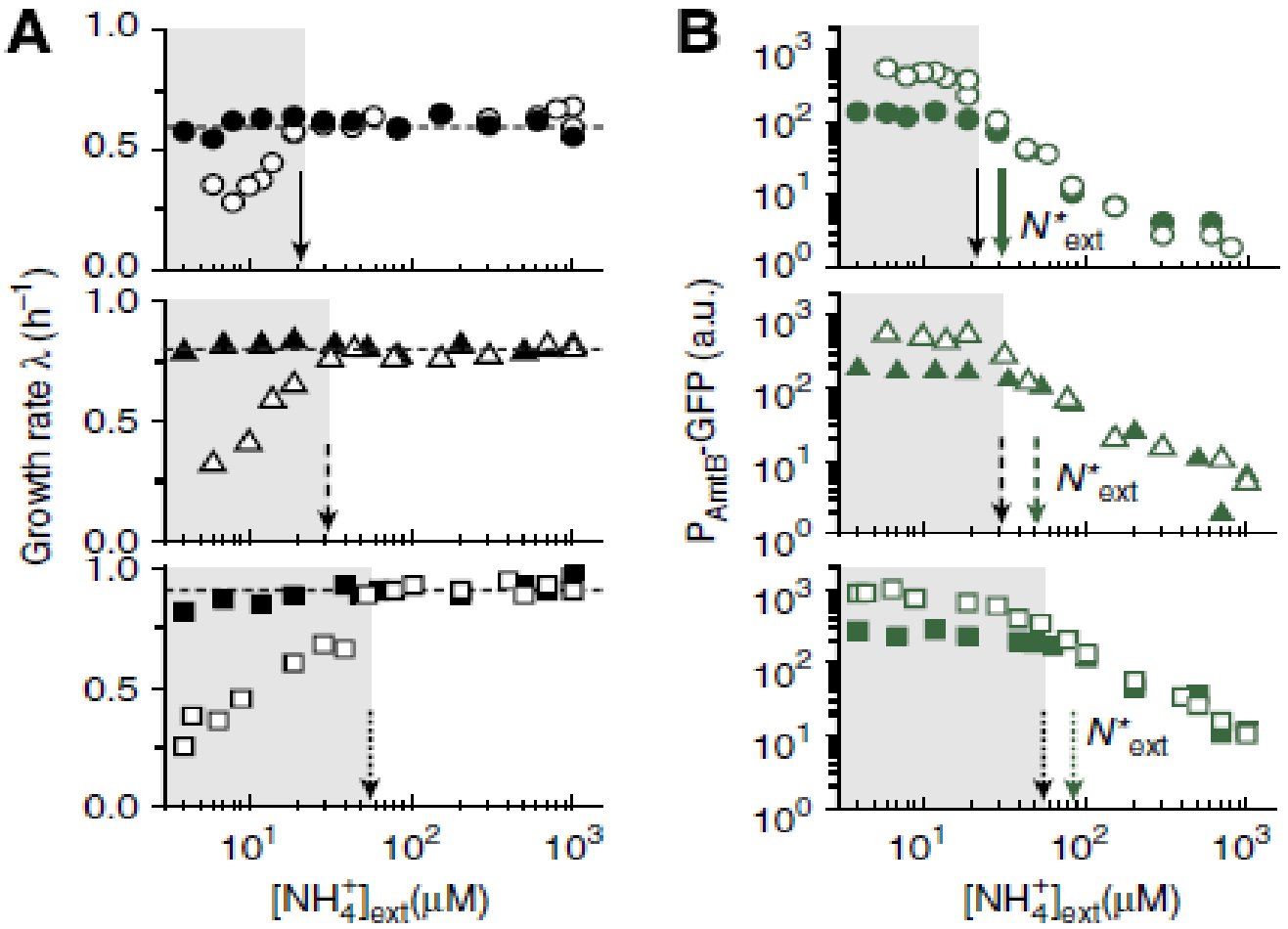


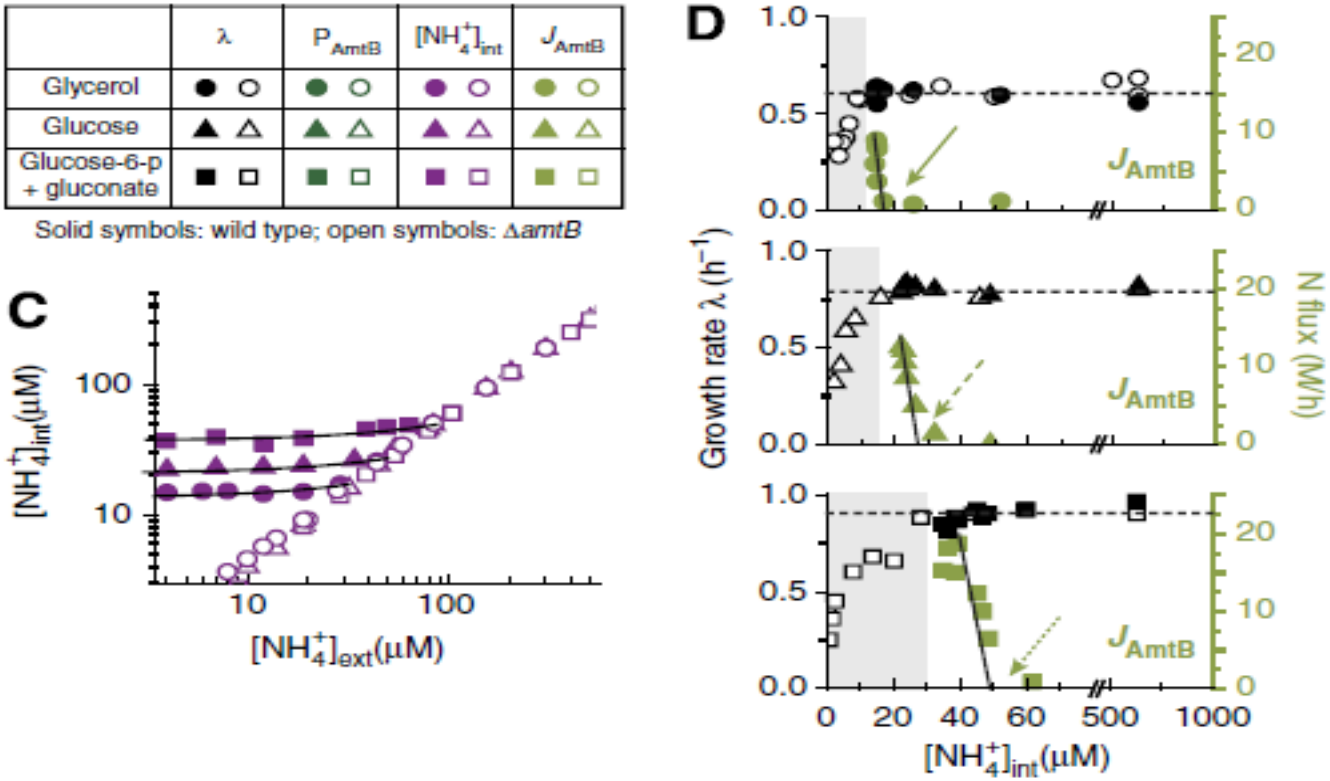

Below $N^*_{ext}$,(30μM)wild type maintained its internal NH4+ concentration at an approximately constant value (~15 μM), referred to as the ‘maintenance concentration’



# 7.The delicate control of AmtB activity is coordinated with cellular nitrogen demand





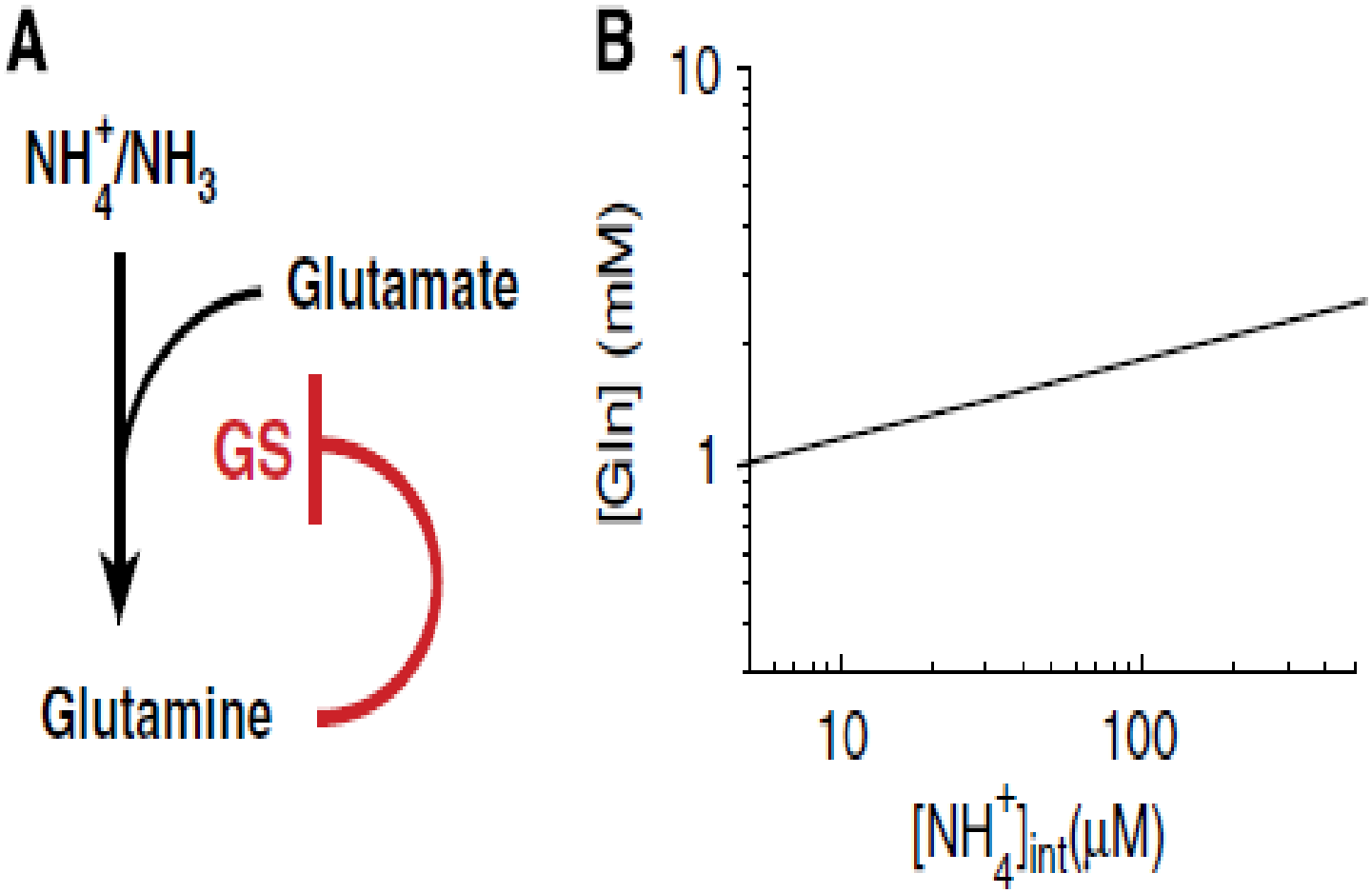

The onset of ammonium transport and the maintenance level of the internal NH4+ is not preset to a fixed value, but is instead determined dynamically, such that ammonium transport by AmtB is only employed as necessary to maintain cell growth.

# Discussion

- Glutamine is unlikely to be a signal controlling AmtB activity:

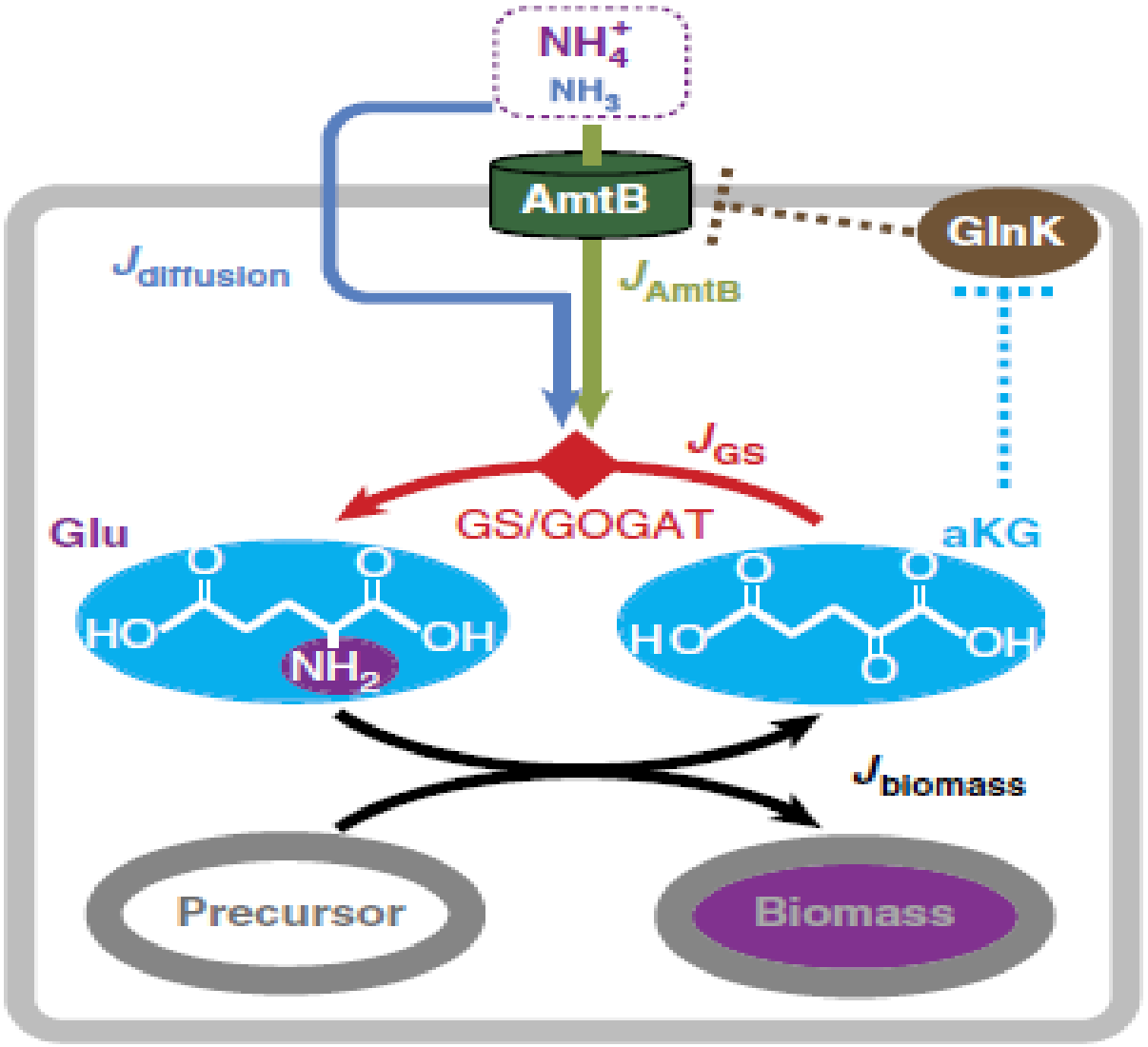
  (i)The weak dependence of the glutamine pool on the internal $NH_4$ concentration,

  (ii)the steady maintenance of the internal $NH_4$ concentration when AmtB activity abruptly increases


A
$NH_4^+/NH_3$
Glutamate
GS
Glutamine
B
10
1
[Gln] (mM)
10
100
$[NH_4^+]_{int}(\mu M)$

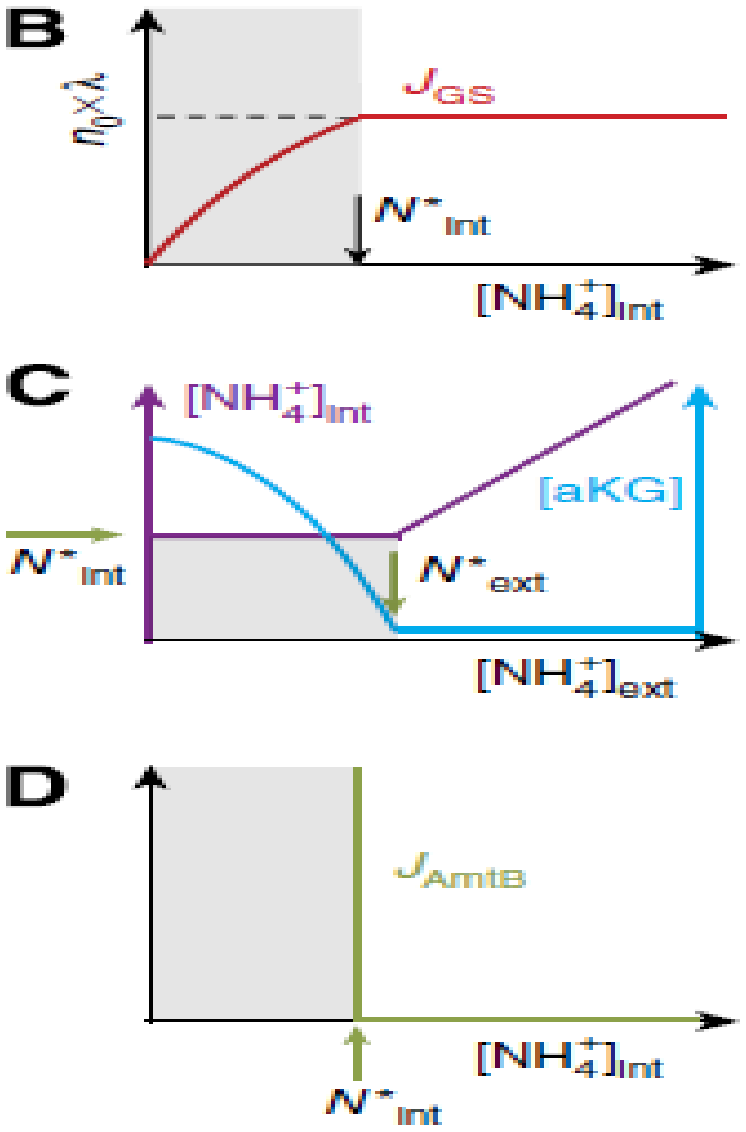
# Discussion

- AmtB is regulated by a-ketogluta rate via an integral feedback control



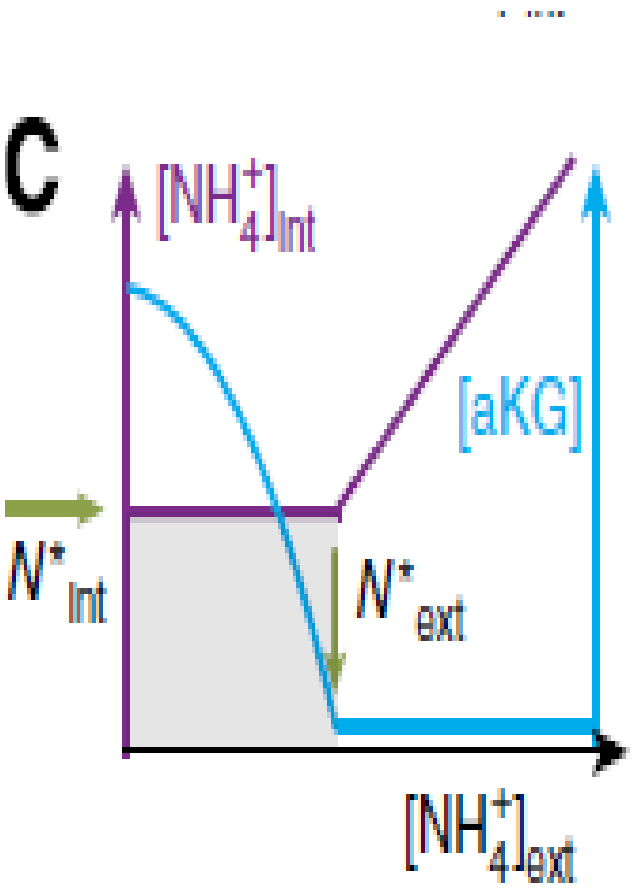
# Discussion

- The robust maintenance of internal ammonium concentration and the abrupt onset of ammonium transport through AmtB



# Discussion

- Tight coordination of GS and AmtB activities
- Perspective



Thanks for your attention!