ELUCIDATION OF A PERIBACTEROID MEMBRANE-BOUND bHLH TRANSCRIPTION FACTOR REQUIRED FOR LEGUME NITROGEN FIXATION

A THESIS SUBMITTED BY

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NAME	GENOTYPE	SOURCE
26972c	*MATa ura3 mep1-1 mep2-1*	Dubois & Grenson (1979)
26972c1	*MATa ura3 mep1-1Δ::KanMX4 mep2-1*	Kaiser etal., (unpublished)
26972c2	*MATa ura3 mep1-1 mep2-1 mep3Δ::KanMX4*	Kaiser etal., (unpublished)
31019b	*MATa ura3 mep1Δ mep2Δ::LEU2 mep3Δ :KanMX2*	Marini etal., (1997)
MLY115	*MATa/MATα ura3-52/ura3-52 leu2::hisG/leu2::hisG mep1::LEU2 /mep1::LEU2 mep2::LEU2/mep2::LEU2*	Lorenz & Heitmann (1998)

Table A1. Yeast strains used in the appendices. Note all yeast strains used are congenic with Σ1278b (Grenson etal., 1966).

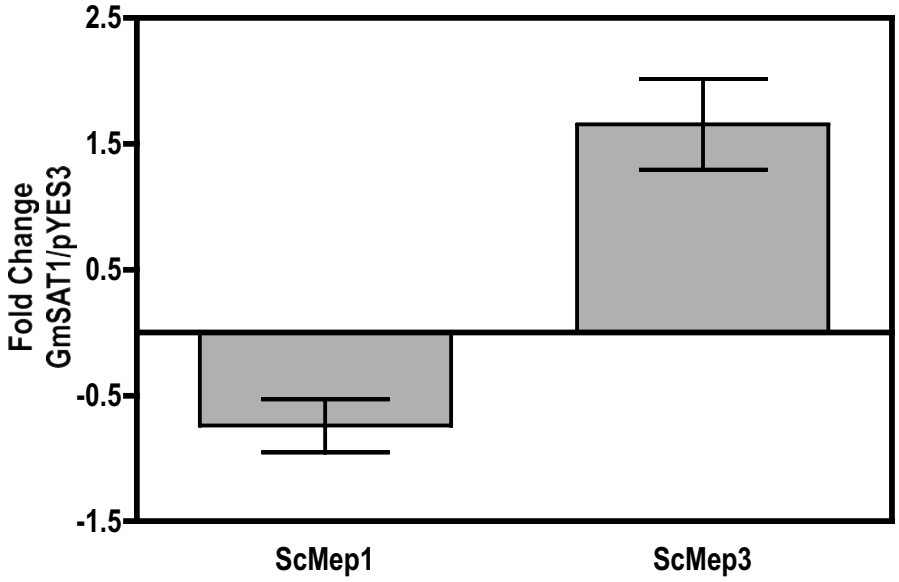

Figure A1. GmSAT1 expression increases Mep3 transcript abundance in 26972c. 26972c yeast harboring empty pYES3 vector (pYES3) or pYES3-GmSAT1 (GmSAT1) were grown to mid-log phase in minimal media supplemented with 2% (w/v) galactose and 0.1% (w/v) proline. Total RNA was extracted from equal volumes and OD_{600} readings of the yeast using the RNeasy kit (Qiagen). One µg of total RNA was reverse transcribed using the iScript cDNA synthesis kit (BioRad, Ca) according to the manufacturer's protocol. Transcript abundance was determined in each cDNA pool using a BioRad Icycler IQ (BioRad, Ca) for ScMep1, ScMep3 and ScTub1. cDNAs were amplified in 20 µl reaction volumes containing a 1X mix of IQ™ SYBR Green Supermix (BioRad, Ca), 200 nM of each primer and 1 µl of cDNA template (equivalent to 5 ng of total RNA). Mep1 and Mep3 gene expression were normalised against the alpha tubulin ScTub1 expression between each strain. Primer sequences: Mep1realtimeFW 5'-GCGATGCTCTTCTACGGTTGTC, Mep1realtimeRV 5'-CGTGTCCGCAGAGATAAGCAAC Mep3realtimeFW 5'-GGTGGTTGGTTGACGCATAAC, Mep3realtimeRV 5'-CGGCTTCCTCAGTGACTCTTAG, Tub1realtimeFW 5'-AGGAGGACGCGGCTAATAAT, Tub1realtimeRV 5'-AACCCATCACATTGGTCTGC. Results presented are averaged from three independent real time PCR runs and representative of two independent biological experiments. Thanks to Viviane Beucart for performing the real time PCRs.

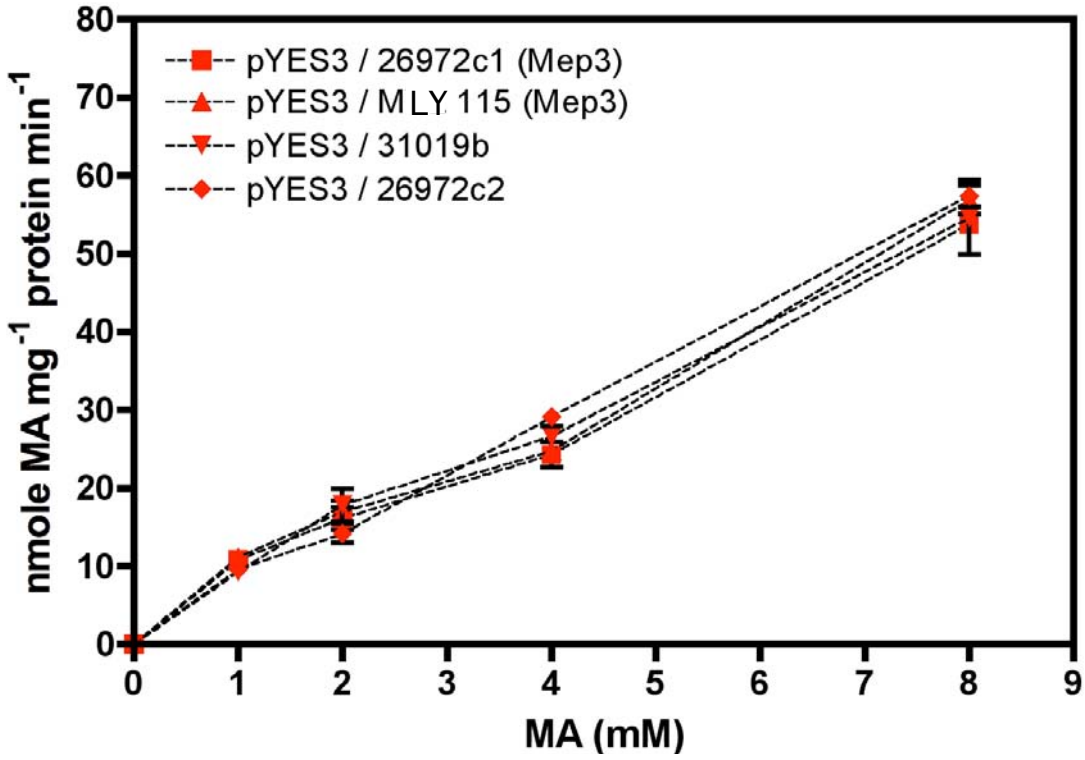

Figure A2. Mep3 does not contribute to MA uptake. ^{14}C-MA uptake experiments on yeast harbouring an empty pYES3 vector with either a functional Mep3 (26972c1 & MLY115), or devoid of any functional Meps (26972c2 & 31019b) were performed as described in section 2.4.5. Note that, similar to Marini etal., (1997), there was no enhanced MA accumulation in yeast with a functional Mep3 present compared with yeast devoid of all functional Meps. Uptake experiments performed by Dr. Brent Kaiser.

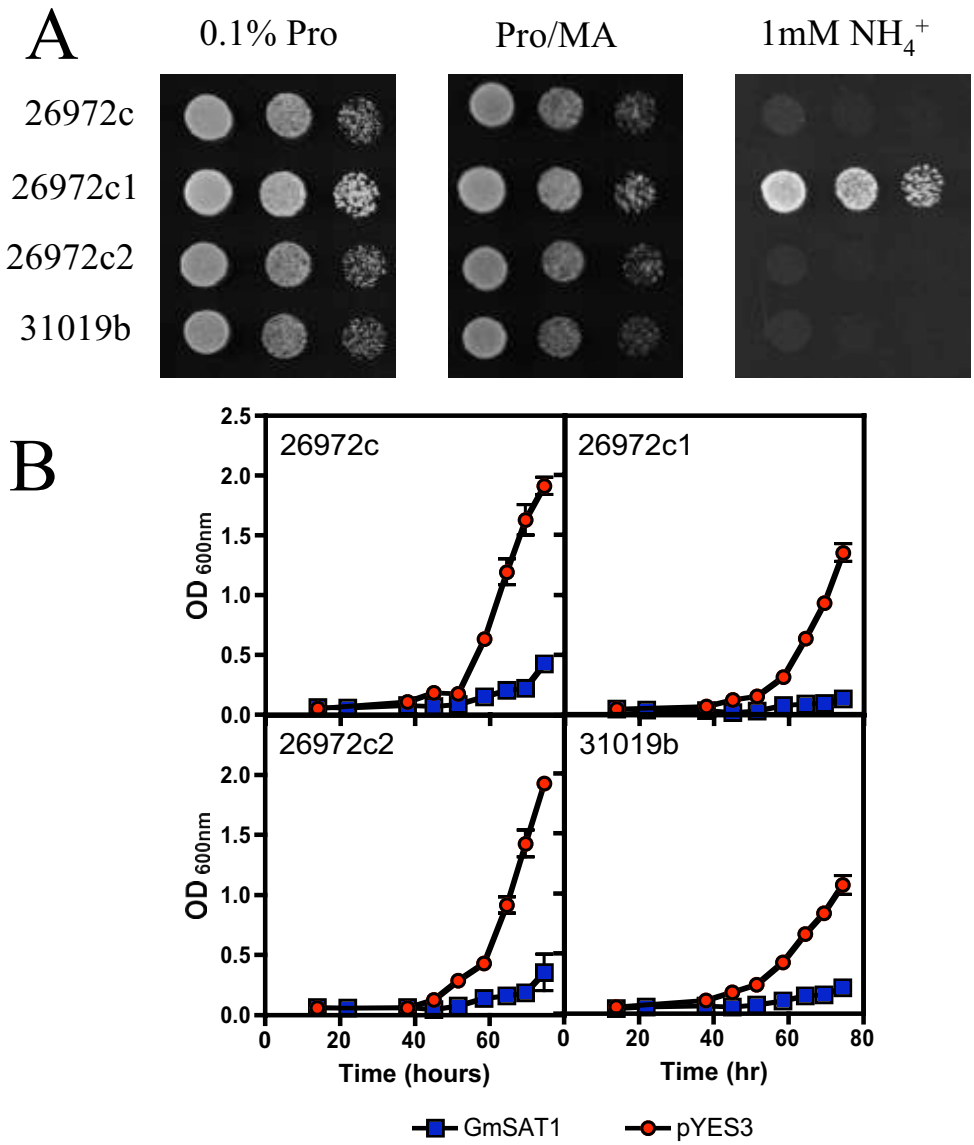

Figure A3. Mep3 transports sufficient ammonium to allow growth on 1mM ammonium media but does not cause toxicity when grown in 100 mM MA. **(A)** Yeast strains with a functional Mep3 (26972c1) or devoid of functional Meps (26972c, 26972c2 and 31019b) harboring an empty pYES3 vector were grown to mid-log phase in liquid YNB (w/o amino acids, Difco) plus 2% (w/v) glucose. Cells were washed twice in sterile dH_2O and a ten-fold dilution series spotted onto solid minimal media (Grenson, 1966) supplemented with 2% (w/v) galactose plus either 0.1% (w/v) L-proline (0.1% Pro), 0.1% (w/v) L-proline and 100 mM MA (Pro / MA) or 1 mM NH_4Cl (1mM NH_4^+). Only 26972c1 which has a functional Mep3 transporter was able to grow on 1mM NH_4^+ medium (right panel). 26972c1 also grows well on 100 mM MA (middle panel), which suggests Mep3 does not transport sufficient MA to induce toxicity at this concentration. **(B)** Liquid culture growth of 26972c, 26972c1, 26972c2 and 31019b in 100 mM MA media also demonstrate that Mep3 does not allow accumulation of MA, however GmSAT1 expression does. The described yeast strains (3 replicates of each) harboring either an empty pYES3 vector (pYES3) or pYES3-GmSAT1 (GmSAT1) were grown at 30ºC with 200 rpm agitation in 50mL cultures of minimal media supplemented with 0.1% (w/v) L-proline, 100 mM MA and 2% (w/v) galactose. 26972c1 yeast transformed with the empty pYES3 vector grow at a similar rate to the other yeast strains which lack a functional Mep3. Expression of GmSAT1 reduced the growth rate of all strains examined, implicating factor(s) other that Mep3 are responsible for MA toxicity associated with GmSAT1 expression. Thanks to Dr. Brent Kaiser for performing the growth curve study