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The Influence of ALS-associated MATR3 Toxicity on Cell Size in the Yeast Model

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Background

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease[1]. The pathology of ALS is described as the progressive degeneration of motor neurons that initially leads to atrophy of the voluntary muscles followed by the involuntary muscles[1,2]. Ultimately, the cause of death is pulmonary distress due to loss of function of the diaphragm[2]. Life expectancy after diagnosis is usually one year, and there are currently no cures or effective treatments for this fatal disease[3].

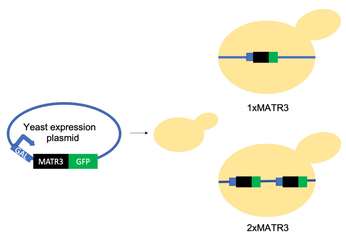
Approximately 90% of all ALS cases are sporadic meaning that the disease is developed randomly, while around 10% of the cases are familial meaning that the disease is passed down within a family[3,4]. Over 30 years, many genes have been identified and linked to the development of familial ALS. One of these genes is MATR3 which codes for Matrin-3, a nuclear matrix protein that binds to DNA and RNA with various roles in the nucleus[5]. Matrin-3 is normally found in the nucleus; however, when the gene is mutated, Matrin-3 is depleted from the nucleus and accumulates in clusters in the cytoplasm[6]. Matrin-3 associated toxicity is hypothesized to be either due to the loss of function of the protein in the nucleus or a gain of toxicity function in the cytoplasm leading to neuronal cell death. Furthermore, with increasing MATR3 toxicity in yeast, an increase in cell size was observed.

Objective

Confirm the increase in cell size when MATR3 is expressed and determine whether the increase in cell size is associated to MATR3 toxicity.

Experimental Design

1. Express human MATR3 in yeast
2. Quantify an increase in cell size with MATR3 expression
3. Identify genetic suppressors of MATR3 toxicity
4. Determine whether suppressors have a direct effect on Matrin-3
5. Determine whether suppressors revert cell size to wild-type



MATR3 is toxic to yeast

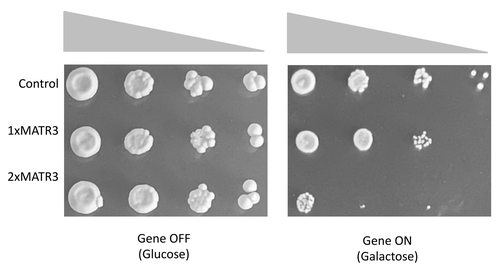


Figure 1.
Spotting assay of control, one copy of MATR3, and two copies of MATR3 strains onto plates containing glucose (gene off) and galactose (gene on). Pictures taken after 3 days of growth.

MATR3 increases cell size

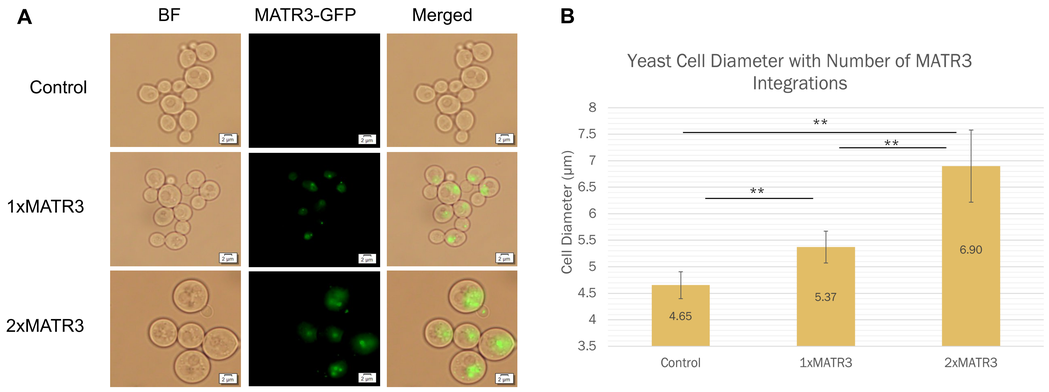


Figure 2.
(A) Brightfield and fluorescence microscopy of control (n=1124), one copy of MATR3 tagged with Green Fluorescent Protein (GFP) (n=1135), and two copies of MATR3 tagged with GFP (n=1190) after 6 hour induction in galactose. Merged images show Matrin-3 protein presence within the cell. Scale= 2µm.
(B) Quantification of cell sizes via CellSens software after 6 hour induction in galactose. **p<0.01.

Suppressors rescue MATR3 toxicity

A

Entry Name	Protein Name
ZC11A	Zinc finger CCCH domain-containing protein 11A
RPGF4	Rap guanine nucleotide exchange factor 4
H12	Histone 1.2
SRTD1	SERTA domain-containing protein 1
HXC4	Homeobox protein box-C4
VTA1	Vacuolar protein sorting-associated protein VTA1 homolog
CCNC	Cyclin C
ANXA2	Annexin II
TFAP4	Transcription factor AP-4
SRTD4	SERTA domain-containing protein 4
PLD1	Phospholipase D1
VSX2	Visual system homeobox 2
PDE4D	cAMP-specific 3',5'-cyclic phosphodiesterase 4D
RPB7	DNA-directed RNA polymerase II subunit RPB7
RASA3	Pas GTPase-activating protein 3
SRTD2	SERTA domain-containing protein 2

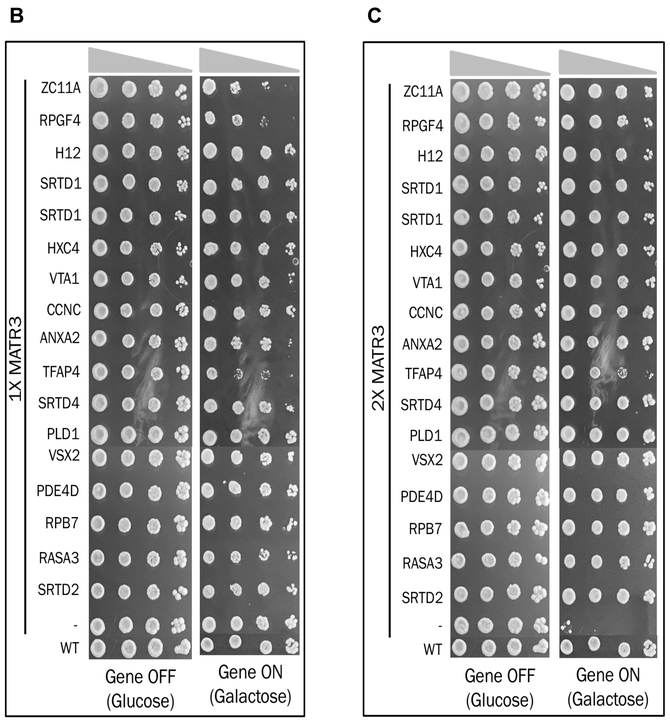


Figure 3.
(A) Chart of known human suppressor genes with protein names. **(B)** Spotting assay of one copy of MATR3 (-), wild-type (WT), and one copy of MATR3 with suppressor genes on plates containing glucose (gene off) and galactose (gene on). Pictures taken after three days of growth. **(C)** Spotting assay of two copies of MATR3 (-), WT, and two copies of MATR3 with suppressor genes on plates containing glucose (gene off) and galactose (gene on). Pictures taken after three days of growth.

Suppressors have a direct effect on Matrin-3

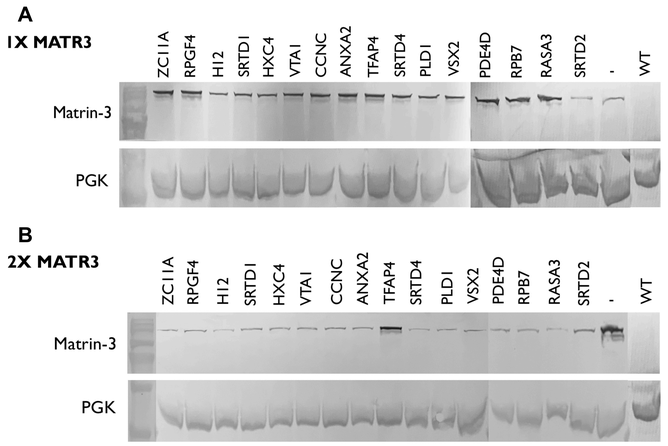


Figure 4.
(A) Western blot for one copy of MATR3 (-), wild-type (WT), and one copy of MATR3 with suppressor genes after 6 hour induction in galactose. PGK1 served as the loading control. **(B)** Western blot for two copies of MATR3 (-), WT, and two copies of MATR3 with suppressor genes after 6 hour induction in galactose. PGK1 served as the loading control. The images are representative of two independent trials.

Conclusions

- MATR3 is toxic in yeast
- Cell size increases when MATR3 is expressed
- Suppressors rescue toxicity with two copies of MATR3, but does not rescue toxicity with one copy of MATR3
- Suppressor have a direct reduction of Matrin-3 with two copies of MATR3, but does not have a reduction of Matrin-3 with one copy of MATR3

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Acknowledgements

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