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Original Article

Yeast as a model to study the roles of aging regulation path way Sip2-Sch9 in α-synuclein pathogenesis

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Abstract: A-synuclein is the main component of Lewy body which is main pathological presentation of Parkinson's disease. This study was aimed at observing the impact of Sip2 and Sch9 gene on α-synuclein (α-syn) toxicity, aggregation and reactive oxygen species (ROS) level in yeast. The pRS414-α-syn, pRS416-RFP and pRS416-α-syn-RFP plasmid were constructed, and were transformed into the YL36, and YL36+ΔSip2 or YL36+ΔSch9 yeast. Expression of α-syn was testified using western-blot. The spotting assay and growth curve were adopted to observe α-syn toxicity, the distribution of α-synwas observed with fluorescent microscope, and ROS level were detected with H2D-CFDA. The results show that yeast transformed into α-syn plasmid showed obviously growth retardation induced in galactose while no in glucose. The α-syn protein express on the cytomembrane induced in galactose for 4 h, and the aggregation was found from cytomembrane to cytoplasm for 8 h induction. A higher ROS level induced in galactose was detected than that uninduced in glucose. The yeast displayed obvious growth retardation when the gene for Sip2 is removed, while the yeast showed even better growthwhen the gene for Sch9 is removed. The expression and aggregation of α-syn, and ROS level were more obviously increased when the gene for Sip2 is removed. However, the expression of α-syn and ROS level was also increased when the gene for Sch9 is removed, but the aggregation was decreased. Our study showed that the ΔSch9 in yeast was protective for growth and aggregation formation while ΔSip2 in yeast could aggravate the growth retardation, expression and aggregation of α-syn, and the ROS level.

Keywords: α-synuclein, aggregation, ROS, yeast, Sip2, Sch9

Introduction

A-synuclein (α-syn) is the main component of Lewy body which is main pathological presentation of Parkinson's disease (PD), Research shows the pathological accumulation of α-syn in the brain is a typical neuropathological feature of PD [1-3]. And there is growing evidence that α-syn can spread from diseased to healthy cells or tissues, and then contributing to disease worsening [4-6]. However, the pathogenic mechanism of α-synas a role in neurodegenerative diseases is not completely understood. It can form oligomers and aggregation in concentration dependence pattern which result in toxicity to cells. In the formation of the aggregation, ROS plays an important part. With the function of protein quality control system, abnormal protein aggregation is not evident in health neuron, which can also neutralize excessive ROS in cell. However, some mutations, resulting in neurodegenerative diseases, mainly produce some proteins with evident aggregated trend and injure the function of the protein quality control system, or have the declined capacity for antioxidant defense [7-9].

In addition to that, the increased incidence of neurodegenerative diseases is related with the age, which can lead to abnormal protein aggregation and declined function of the protein quality control system, and elevated ROS level beyond capacity of antioxidant defense mechanisms [10, 11]. Therefore, researches exploring intrinsic factor to prolong life may be more attractive. For instance, the latest research has shown that Sip2 acetylation-Sch9 phosphorylation was an intrinsic aging defense pathway [12, 13].

These conclusions obtained mainly attribute to the studies in saccharomyces cerevisiae, which

Table 1. Primers and their sequences

Name	Cutting site	Sequence 5'→3'
416-RFP up	BglII	GAAGATCTATGGCCTCCTCCGAGGACG
416-RFP down	XboI	CCGCTCGAGTTAGGCGCCGGTGGAGTGG
416-syn-RFP up	XhaI	GCTCTAGAATGGATGTATTCATGAAAGG
416-syn-RFP down	BglII	GAAGATCTGGCTTCAGGTTCGTAGTCTTG
KanF1	NA	CCAGA ATACCCTCCT TGA
KanR1	NA	CTGAGCGAGCGAAATAC
ΔSip2 up	NA	AAGTATCTGAAATAATTGTGCTTTTGGAAGGGTACTGAGAGCAGTGAAGTCGGATCCCCGGGTTAATTAA
ΔSip2 down	NA	CCATAGCTGCCAAAAATATATACAATACGCCTTTAAAAAGTAGGAATCCCGAATTCGAGCTCGTTTAAAC
ΔSch9 up	NA	TAAGTCTGAGAATTATACTCGTATAAGCAAGAAATAAAGATACGAATATACGGATCCCCGGGTTAATTAA
ΔSch9 down	NA	AAAATAAAAAGAAAAGGAAAAGAAGGGAAGGGCAAGAGGAGCGATTGAGCGATGAATTCGAGCTCGTTT

is a practical model to study neurodegenerative diseases like protein aggregation, protein misfolding, oxidative stress and apoptosis, et al [14-16]. Features of diseases like Alzheimer and Parkinson's disease have been successfully modeled in yeast. The basic cellular machinery for neuronal function is conserved in yeast making it an attractive unicellular, genetically and biochemically tractable model organism to study pathways underlying neurodegeneration diseases. As the extension of cultivation in the stationary phase, the injury in cell is accumulated, except for that, the cell also display many features in aged mammal such as decline in protein synthesis, decreased metabolism, and changes of cell morphology. And the researches on yeast in stationary phase have indicated some genes regulating the age. Hence, the stationary phase yeast (aged yeast) is taken as an ideal model to clarify the interaction between genes in neurodegenerative disease research. In view of these, the purpose of this study is using yeast as model to explore the role of Sip2-Sch9 in a-syn toxicity.

Materials and methods

Plasmids and vector construction

The commercialized pRS416 (H4ARS, CEN3, Ampr, Ori, Ura) and pRS416 (H4ARS, CEN3, Ampr, Ori, trp) plasmids, which were cloned with the gene of gal1 promoter, c-myc tag, and cycle-terminator, were donated by the School of Life Sciences and Biomedical Center of Sun Yat-sen University. The RFP cDNA was isolated from pTol2HuC-mRFP cDNA via PCR amplification using the primers list in the **Table 1** that included, respectively, a BglII and XboI restriction site for cloning into the pRS416 plasmid. The α-syn cDNA was isolated from HtrA2 cDNA via PCR amplification using the primers list in the **Table 1** that included, respectively, the XhaI and BglII restriction site for cloning into the pRS416 plasmid. PCR kit Q5 and other enzymes were from NEB (#M0491L). PCR conditions were 98°C for 1 min, followed by 30 cycles of 98°C for 10 sec, and 65°C for 20 sec, and then 72°C for 30 sec. To confirm the fidelity of the PCR and cloning steps, all GFP and α-syn-GFP fusion constructs were sequenced.

Yeast strains and cultivation

Yeast strains used in this study were derived from YL36 (matα, ade2, ura3, his3, trp1, leu2, can100), which was donated by the School of Life Sciences and Biomedical Center of Sun Yat-sen University. Deletion of sip2 or sch9 was achieved by replacement of the gene with the PCR product containing a Kan gene which was amplificated using the pFa6a-kanMX6 as the template. Yeast cells were grown in non-selective medium (YPD) at 30°C and transformed as described. Selective synthetic complete medium (SCM) contained 2% glucose or 2% galactose and lacked the nutrient corresponding to the marker (tryptophan for pRS414 and uracil for pRS416). Yeast were first grown in SCM with glucose and lacking tryptophan/uracil overnight then shifted to SCM containing galactose for 12 hours to induce α-syn expression. After pre-incubation in galactose medium, yeast cells were shifted to SCM with glucose to shut off the promoter for 2 hours, and observed with a fluorescent microscope.

Spot assay and growth profile

For growth assays on solid medium, a serial dilution of yeasts was made in SCM medium ranging from an OD600 of 10^{-1} to 10^{-4}. Of these dilutions, 5 μl was spotted on SCM agar plates, which were then incubated at 30°C for at least

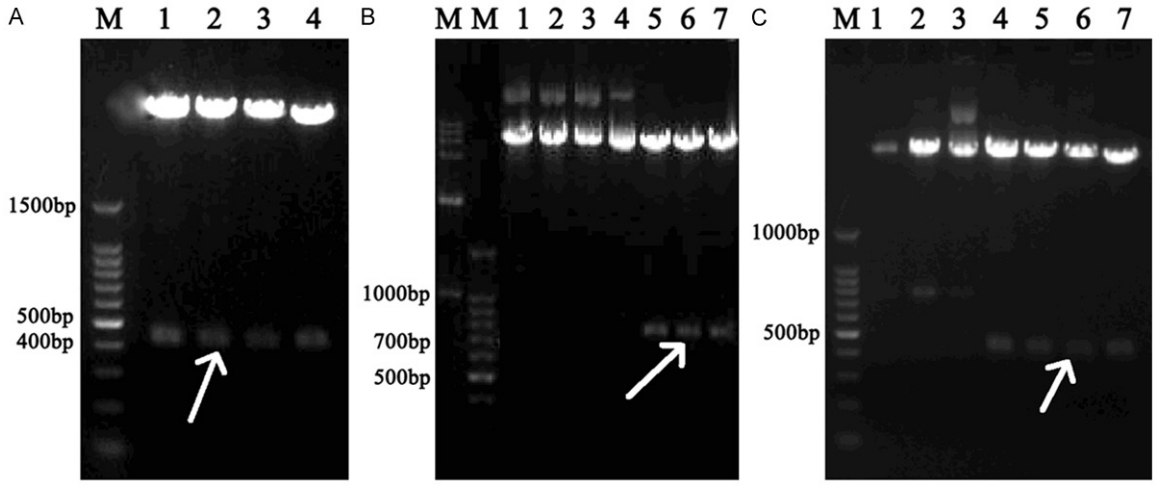

Figure 1. Electrophoresis of the PCR products was identified. A. The positive stripes within 400-500 bp shows that α-syn DNA was successfully amplified from pRS414-α-syn plasmid (arrow). B. The positive stripes within 700 bp shows that RFP DNA was successfully amplified from pRS416-RFP plasmid (arrow). C. The positive stripes within 400-500 bp shows that α-syn DNA was successfully amplified from pRS416-α-syn-RFP plasmid (arrow).

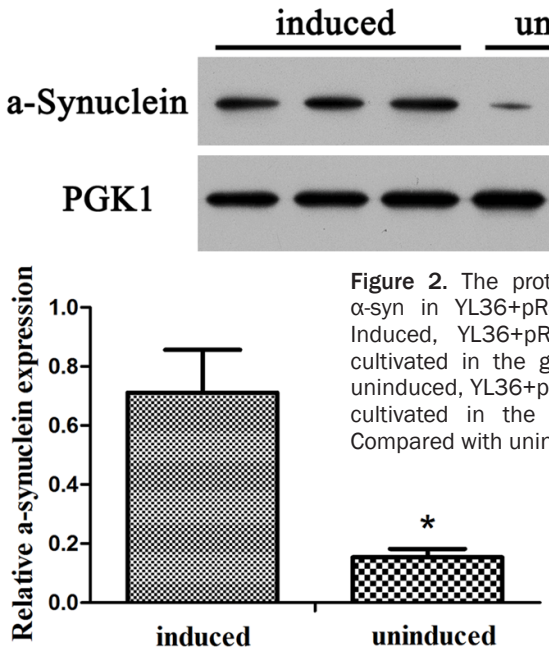

Figure 2. The protein expression of α-syn in YL36+pRS414-α-syn yeast. Induced, YL36+pRS414-α-syn yeast cultivated in the galactose medium; uninduced, YL36+pRS414-α-syn yeast cultivated in the glucose medium. Compared with uninduced, **P*<0.05.

48 h. At least three independent transformants were tested for the survival plating to rule out clonogenic variation of the effects. To determine survival upon a-syn expression, 5 ml yeast from overnight cultures inoculated in tubes on SCM was diluted to OD600 of 10^{-2}, and was grown at 30°C of 250 rpm for 28 h. The OD600 of yeast was tested every 4 h. The growth profiles were then established by measuring OD600 in a DU 800 Protein nucleic acid spectrophotometer (Beckman Coulter, California, USA). For all assays, every experiment was repeated three times. In order to test growth retardation caused by native α-Syn, the growth assay of the corresponding strains were compared to that of a control strain transformed with the empty vectors.

Measurement of intracellular ROS in yeast

For the ROS test, 10^7 yeasts and 10 ml YPD medium were mingled in 50 ml centrifuge tube cultivated for 16 h at 30°C and was centrifuged for 25 s at 13000 rpm. At the end of the specified treatment time, the yeast was collected into phosphate-buffered saline (PBS) with the final concentration of 10 μM H2DCFDA in Eppendorf tubes. Samples were read after incubated for 30 min in a Thermo Lab systems Fluoroskan Ascent microplate reader at 37°C.

Results

Construction and identification of pRS414-α-syn, pRS416-RFP and pRS416-α-syn-RFP plasmids

The pRS414-α-syn was successfully constructed. The positive stripes within 400-500 bp in 1-4 holes by PCR were observed in **Figure 1A**,

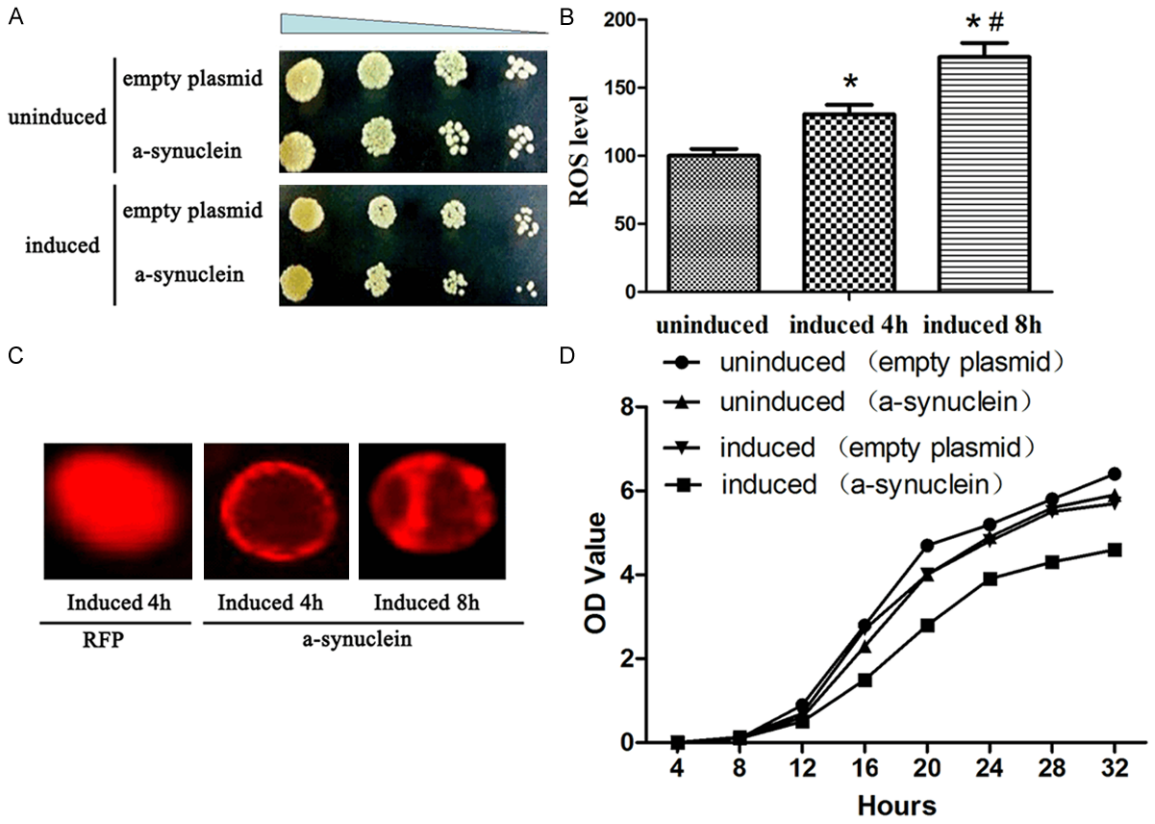

Figure 3. Effect of α-syn on physiological status of yeast. A. The growth retardation were observed in YL36+pRS414-α-syn yeast. B. The ROS level were detected in yeast. Compared with uninduced, **P*<0.05; compared with induced for 4 h, #*P*<0.05. C. The expression of α-syn and RFP in yeast. D. The growth profiles were then established by measuring OD600. Experiments were performed in triplicate.

which were in accordance with the stripe size of α-syn. The pRS416-RFP was successfully constructed. The positive strips approximately 700 bp in 1-7 holes by PCR were observed in **Figure 1B**, which were in accordance with the stripe size of RFP. The pRS416-α-syn-RFP was successfully constructed. The positive strips within 400-500 bp in 4-7 holes by PCR were observed in **Figure 1C**, which were in accordance with the stripe size of α-syn. The α-syn-RFP fusion constructs were sequenced to be with the right direction, no bass error and missing in genes.

Construction and identification of YL36+pRS414-α-syn yeast

YL36+PRS414-α-syn yeast was cultivated both in the glucose and galactose medium. The expression of α-syn cultivated in the galactose medium was significantly higher than in the glucose medium analyzed by western blot (**Figure 2**).

α-syn toxicity, aggregation and ROS level in yeast

When compared to the YL36+pRS414 yeast, the YL36+pRS414-α-syn yeast cultivated in the glucose and galactose medium were both observed with growth retardation. When compared to the yeast cultivated in the glucose medium, YL36+pRS414-α-syn yeast cultivated in the galacotose medium were observed with more growth retardation than the YL36+pRS414 yeast in the galacotose medium (**Figure 3A**).

When cultivated in glucose medium, the ROS level of YL36+pRS416-α-syn-RFP yeast was the lowest of three groups. While cultivated in galactose medium, the ROS level of YL36+pRS416-α-syn-RFP yeast was increased as the time from 4 h to 8 h (**Figure 3B**), there were significant differences between them (*P*< 0.05).

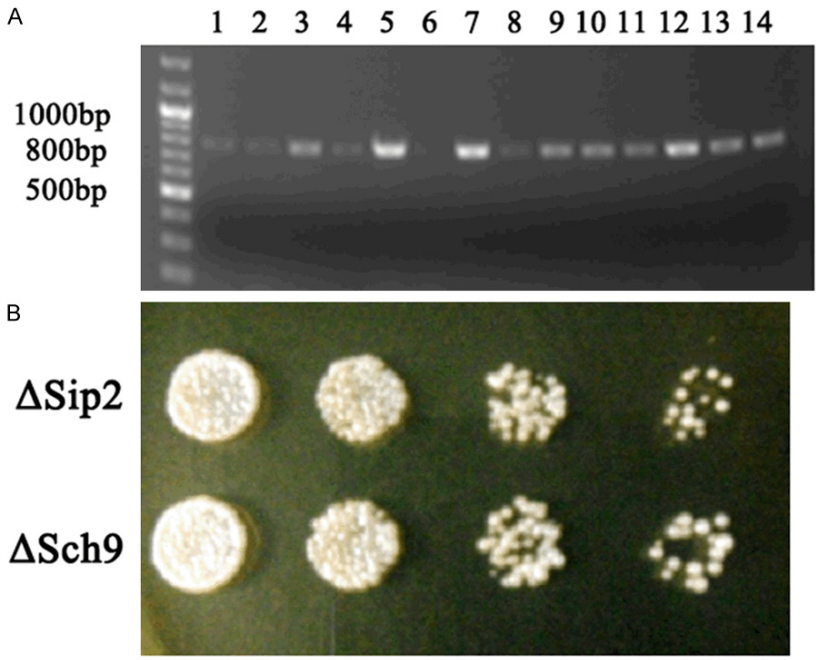

Figure 4. Construction and identification of YL36+ΔSip2 or YL36+ΔSch9 yeast. A. The YL36+ΔSip2 (1-5 holes) and YL36+ΔSch9 (8-14 holes) yeast were successfully constructed. The wild yeast without the positive stripe within 700-800 bp as the negative control (6 holes) and the Δ0mi yeast with the positive stripe as the positive control (7 holes). B. The YL36+ΔSip2 and YL36+ΔSch9 yeast grow in the G418 plate.

When cultured in the galactose medium for 4 h, the α-syn-RFP was emerged to be the small granular distributed in the cell membrane. When cultivated for 8 h, the α-syn-RFP increased and was emerged to be the large granular aggregations in both the cell membrane and cytoplasm. While the YL36+pRS416-RFP yeast induced in the galactose medium, the RFP was uniformly distributed in the cytoplasm (**Figure 3C**).

The value of OD600 was certified to increase as the time gone (0-32 h). And the growth curve was present the S shape. At the same time point, the OD600 value of YL36+pRS414-α-syn yeast was much lower than the YL36+pRS414 yeast. And when cultivated in the galactose medium, the OD600 value of YL36+pRS414-α-syn yeast was significantly higher in the glucose medium (**Figure 3D**).

Identification and toxicity of YL36+ΔSip2 or YL36+ΔSch9 yeast

The wild yeast without the positive stripe within 700-800 bp as the negative control and the Δ0mi yeast with the positive stripe as the positive control, there were positive stripes in 1-5 holes and in 8-14 holes which indicated that the YL36+ΔSip2 and YL36+ΔSch9 yeast were successfully constructed, respectively (**Figure 4A**). The YL36+ΔSip2 and YL36+ΔSch9 yeast grew well while the wide yeast could not grow in the G418 plate. There were no differences between them in growth after cultivating in YPD plate at 30°C for 3 days (**Figure 4B**).

Identification of YL36+ΔSip2+pRS414-α-syn and YL36+ΔSch9+pRS414-α-syn yeast

The ROS level in the YL36+ΔSip2+pRS416-α-syn-RFP yeast was higher than in the YL36+ΔSch9+pRS416-α-syn-RFP yeast and in the YL36+pRS416-α-syn-RFP yeast ($P<0.05$). And the ROS level in the YL36+ΔSch9+pRS416-α-syn-RFP yeast was higher than in the YL36+pRS416-α-syn-RFP yeast ($P<0.05$, **Figure 5A**, **5B**). When compared to cultivated in the glucose medium, the expression of α-syn was significantly higher in YL36+ΔSip2+pRS414-α-syn and YL36+ΔSch9+pRS414-α-syn yeast cultivated in galactose medium (**Figure 5C**, **5D**).

α-syn toxicity, aggregation and ROS level in ΔSip2 and ΔSch9 yeast

All these 6 groups (YL36+pRS416, YL36+pRS-416-α-syn-RFP, YL36+ΔSip2+pRS416, YL36+ΔSip2+pRS416-α-syn-RFP, YL36+ΔSch9+pRS-416, and YL36+ΔSch9+pRS416-α-syn-RFP) yeasts had no obvious differences in growth cultivated in glucose medium. But when cultivated in galactose medium, the YL36+ΔSip2+pRS416-α-syn-RFP yeast displayed an obviously growth retardation even when the OD600 value was 10-2 point. The growth of YL36+ΔSch9+pRS416-α-syn-RFP yeast was better than the YL36+pRS416-α-syn-RFP yeast, or even as good as the YL36+pRS416 yeast induced in the galactose medium (**Figure 6A**).

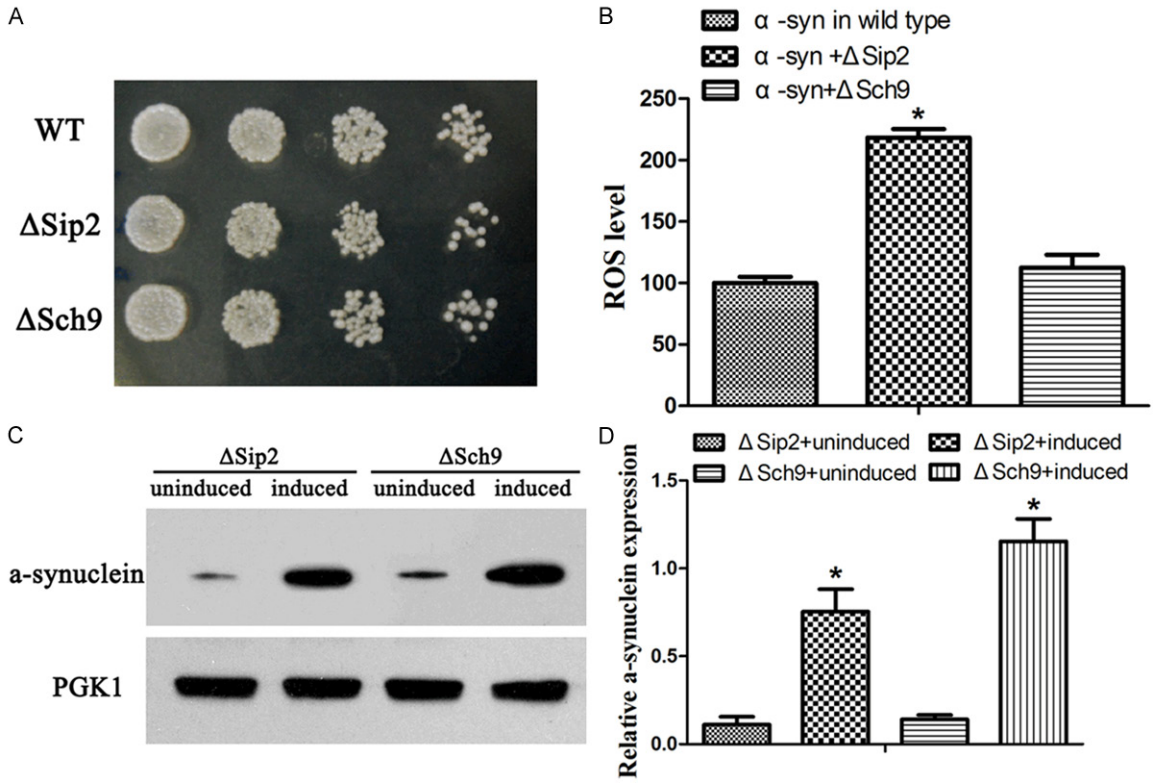

Figure 5. Construction and identification of YL36+ΔSip2+pRS414-α-syn and YL36+ΔSch9+pRS414-α-syn yeast. A, B. The ROS level were detected in yeast. WT, wild type yeast with pRS414-α-syn overexpression; ΔSip2, yeast with pRS414-α-syn overexpression and Sip2 knockout; ΔSch9, yeast with pRS414-α-syn overexpression and Sch9 knockout. Compared with WT, **P*<0.05. C, D. The protein expression of α-syn in yeast.compared with induced, **P*<0.05.

The α-syn expression of YL36+ΔSip2+pRS414-α-syn yeast and YL36+ΔSch9+pRS414-α-syn yeast were both higher than the pRS414-α-syn yeast (*P*<0.05), and the α-syn expression of YL36+ΔSip2+pRS414-α-syn yeast was even higher than the YL36+ΔSch9+pRS414-α-syn yeast (*P*<0.05, **Figure 6B**, **6C**).

When cultivated in galactose medium for 12 h, compared to the YL36+pRS416-α-syn-RFP yeast, the α-syn-RFP of YL36+ΔSip2+pRS416-α-syn-RFP yeast was increased to be the large granular aggregations in the cytoplasm, while the α-syn-RFP of YL36+ΔSch9+pRS416-α-syn-RFP yeast was decreased to be distributed only in the cytomembrane with light red, which was even less than the α-syn-RFP of YL36+pRS416-α-syn-RFP yeast (**Figure 6D**).

Discussion

The quantity of α-syn is the precondition for the formation of toxic. The wild type and A53T mutation α-syn were expressed in yeast through the way of secretion, the distribution of α-syn gathered on the cell membrane lead to the formation of inclusions [17-19]. The formation process of inclusion was started from the small nuclear formation in [20-22]. The study adopted the transformed pRS plasmid series with gal promoter, in which the α-syn could be induced in galactose while not in glucose. Yeast with α-syn expression induced in galactose had the growth retardation but the control had not the growth retardation, α-syn expression was increased as the extension of time in galactose medium by western blot, suggesting that α-syn caused growth retardation was positive correlated with the α-syn expression. Study clarified that α-syn level polyploidization were more likely to lead to the conclusion of the earlier onset of Parkinson's disease.

As to how increased α-syn generated the toxicity in cell, study indicated that it was the aggregation of α-syn which leads to the toxicity [23-25]. We successfully simulated the α-syn distribution from cell membrane to cytoplasm

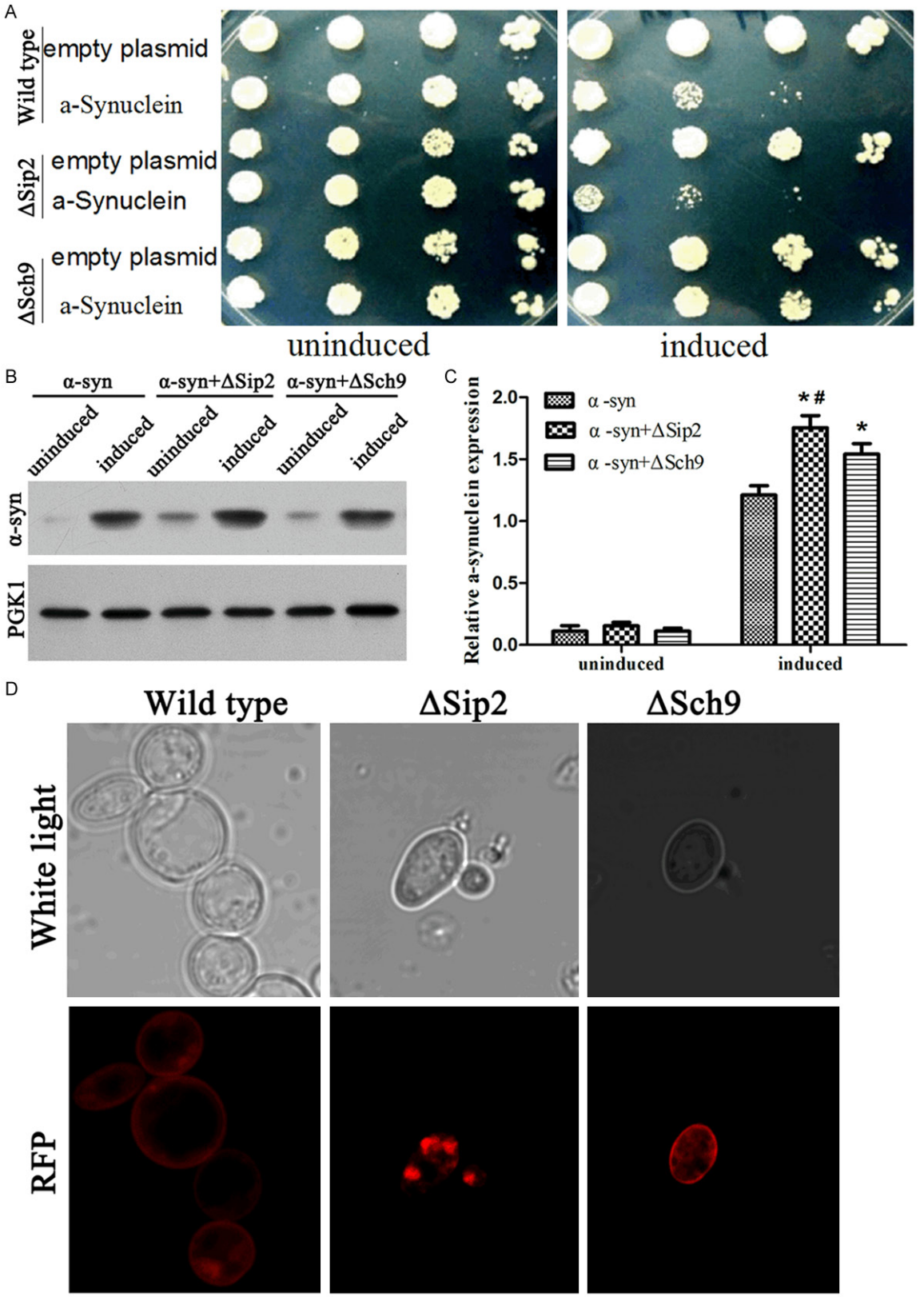

Figure 6. Effect of ΔSip2 and ΔSch9 on the α-syn toxicity, aggregation and ROS level in yeast. A. The growth retardation were observed in yeast. B, C. The protein expression of α-syn in yeast.Compared with α-syn, *P<0.05; compared with α-syn+ΔSch9, #P<0.05. D. The expression of α-syn and RFP in yeast.

forming inclusions, also detected the positive phase change with the content of ROS. Although α-syn was observed the concentration dependence of aggregation and toxicity to the cells, but the research was not specific the intermediate products lead to aggregation for the cytotoxicity. Although studies proposed that the early stage of oligomers formation had cytotoxic effect. The formation of intracytoplasmic inclusion was observed in yeast with wild type α-syn expression which was speculated that the formation of inclusion was a major factor for toxicity to die, while the rise of ROS level was both the stimulating factor and the product for formation of inclusions. When α-syn folding abnormally, the molecular chaperone regulatory proteins were the first to regroup which were degraded by the ubiquitin protein or CMA pathway. But when more α-syn folded abnormally to produce the aggregation, autophagy pathway was temporarily activated to keep the steady of proteins when these aggregation proteins were too much to be degraded by the pathway reminded above. With the further increased α-syn aggregation, the autophagy-lysosome pathway was damaged which lead to cytotoxicity, and the excessive α-syn diffused between cells that further aggravated the toxicity to cells.

The study was first aimed to construct the Sip2 and Sch9 single gene deletion which were certified to be harmless for the growth of yeast, then the yeast was transferred with α-syn to induce its expression. The two strains had a distinct difference on yeast growth, the α-syn toxicity in Sip2 deletion strain was more aggravated than the wild yeast while in Sch9 strain, the α-syn toxicity was relieved than the wild strain.

Sch9, Serine/threonine protein kinase, was found in transposon mutation screening for modulating the yeast age that the deletion of Sch9 gene lead to great life extension in yeast [26-28]. The level of Sch9 phosphorylation was found in aged yeast, and the upregulated phosphorylation level of Sch9 by TOR in TOR-Sch9 pathway could decline the life in yeast. This study was designed to reduce the phosphorylation level by deleting the Sch9 gene, which turned out that both α-syn toxicity and aggregation could be relieved. In conclusion, it was speculated that the decline of Sch9 phosphorylation level had the function against the α-syn toxicity that was the possible mechanism of life extension, and at the same time to state that toxicity protein was an important factor of age.

Though sch9 deletion yeast had more α-syn expression and increased ROS level than the wild group, there was no obvious α-syn aggregation, which could be explained that the overexpressed α-syn was not enough to form the aggregation (or might be the upregulated degradation of α-syn). The TOR pathway involved in autophagy, but Sch9 as the substrate of TOR, the deletion of Sch9 was speculated to increase the activated function of autophagy to clear the α-syn in cell that the light red protein was distributed in the cell membrane as observed in this study. However, there was a need to detect the autophagy in the study to certify the protected function of phosphorylation deficiency in yeast.

Sch9 is TOR the substrate, the TOR pathway involved in cell autophagy [29], speculated that the lack of Sch9 can increase the activation of autophagy to clear the alpha syn, results in a decrease of alpha syn in the yeast cell, so the distribution of the membrane as the observed pink ring light circle. So you need under the lack of alpha syn expression of Sch9 detect the changes of autophagy further clear Sch9 phosphorylation of lack of protection.

Sip2, a subunit of Snf1 of AMPK, was a moderator of Snf1. When Sip2 acetylation increased, the interaction between Sip2 and Snf1 was strengthened that downregulated the activity of Snf1 kinase, then declined phosphorylation of downstream Sch9 was participated in the age regulation in yeast, which proved the function of upstream Sip2 acetylation in the α-syn toxicity. The α-syn toxicity and aggregation were obvious increased in yeast with reduced acetylation level by deleting the Sip2 gene. And the toxicity resistant function was verificated in Sip2 in our study.

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Disclosure of conflict of interest

None.

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References

[1] Al-Hilaly YK, Biasetti L, Blakeman BJ, Pollack SJ, Zibaee S, Abdul-Sada A, Thorpe JR, Xue WF, Serpell LC. The involvement of dityrosine cross-linking in α-synuclein assembly and deposition in Lewy Bodies in Parkinson's disease. Sci Rep 2016; 6: 39171.

[2] Lulla A, Barnhill L, Bitan G, Ivanova MI, Nguyen B, O'Donnell K, Stahl MC, Yamashiro C, Klärner FG, Schrader T, Sagasti A, Bronstein JM. Neurotoxicity of the Parkinson disease-associated pesticideziram is synuclein-dependent in zebrafish embryos. Environ Health Perspect 2016; 124: 1766-1775.

[3] Hegarty SV, Sullivan AM, O'Keeffe GW. The Epigenome as a therapeutic target for Parkinson's disease. Neural Regen Res 2016; 11: 1735-1738.

[4] Breid S, Bernis ME, Babila JT, Garza MC, Wille H, Tamgüney G. Neuroinvasion of α-synuclein-prionoids after intraperitoneal and intraglossal inoculation. J Virol 2016; 90: 9182-93.

[5] Flagmeier P, Meisl G, Vendruscolo M, Knowles TP, Dobson CM, Buell AK, Galvagnion C. Mutations associated with familial Parkinson's disease alter the initiation and amplification steps of α-synuclein aggregation. Proc Natl Acad Sci U S A 2016; 113: 10328-33.

[6] Luo HT, Zhang JP, Miao F. Effects of pramipexole treatment on the α-synuclein content in serum exosomes of Parkinson's disease patients. Exp Ther Med 2016; 12: 1373-1376.

[7] Bonow RO, Leon MB, Doshi D, Moat N. Management strategies and future challenges for aortic valve disease. Lancet 2016; 387:1312-23.

[8] Halliwell B. Proteasomal dysfunction: a common feature of neurodegenerative diseases? Implications for the environmental origins of neurodegeneration. Antioxid Redox Signal 2006; 8: 2007-19.

[9] Halliwell B. Oxidative stress and neurodegeneration: where are we now? Jneurochem 2006; 97: 1634-58.

[10] Paschen W, Mengesdorf T. Endoplasmic reticulum stress response and neurodegeneration. Cell Calcium 2005; 38: 409-15.

[11] Hol EM, Scheper W. Protein quality control in neurodegeneration: walking the tight rope between health and disease. Jmol Neurosci 2008; 34: 23-33.

[12] Koutsilieri E, Scheller C, Grünblatt E, Nara K, Li J, Riederer P. Free radicals in Parkinson's disease. Jneurol 2002; 249 Suppl 2: II1-5.

[13] Lu JY, Lin YY, Sheu JC, Wu JT, Lee FJ, Chen Y, Lin MI, Chiang FT, Tai TY, Berger SL, Zhao Y, Tsai KS, Zhu H, Chuang LM, Boeke JD. Acetylation of yeast AMPK controls intrinsic aging independently of caloric restriction. Cell 2011; 146: 969-79.

[14] Mulak A, Bonaz B. Brain-gut-microbiota axis in Parkinson's disease. World J Gastroenterol 2015; 21: 10609-20.

[15] Cebollero E, Reggiori F. Regulation of autophagy in yeast Saccharomyces cerevisiae. Biochim Biophys Acta 2009; 1793: 1413-21.

[16] Haynes CM, Caldwell S, Cooper AA. An HRD/DER-independent ER quality control mechanism involves Rsp5p-dependent ubiquitination and ER-Golgi transport. J Cell Biol 2002; 158: 91-101.

[17] Roy A, Rangasamy SB, Kundu M, Pahan K. BPOZ-2 gene delivery ameliorates Alpha-Synucleinopathy in A53T transgenic mouse model of Parkinson's disease. Sci Rep 2016; 6: 22067.

[18] Yamasaki T, Fujinaga M, Kawamura K, Furutsuka K, Nengaki N, Shimoda Y, Shiomi S, Takei M, Hashimoto H, Yui J, Wakizaka H, Hatori A, Xie L, Kumata K, Zhang MR. Dynamic changes in striatal mGluR1 but not mGluR5 during pathological progression of Parkinson's disease in human Alpha-Synuclein A53T transgenic rats: a multi-PET imaging study. J Neurosci 2016; 36: 375-84.

[19] Deusser J, Schmidt S, Ettle B, Plötz S, Huber S, Müller CP, Masliah E, Winkler J, Kohl Z. Serotonergic dysfunction in the A53T alpha-synuclein mouse model of Parkinson's disease. J Neurochem 2015; 135: 589-97.

[20] Temple MD, Perrone GG, Dawes IW. Complex cellular responses to reactive oxygen species. Trends Cell Biol 2005; 15: 319-26.

[21] Dixon C, Mathias N, Zweig RM, Davis DA, Gross DS. Alpha-synuclein targets the plasma membrane via the secretory pathway and induces toxicity in yeast. Genetics 2005; 170: 47-59.

[22] Zabrocki P, Pellens K, Vanhelmont T, Vandebroek T, Griffioen G, Wera S, Van Leuven F, Winderickx J. Characterization of alpha-synuclein aggregation and synergistic toxicity with protein tau in yeast. FEBS J 2005; 272: 1386-400.

[23] Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Rochet JC, McCaffery JM, Barlowe C, Lindquist

S. The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis. Proc Natl Acad Sci U S A 2008; 105: 145-50.
[24] Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, Patel S, Oehler A, Lowe JK, Kravitz SN, Geschwind DH, Glidden DV, Halliday GM, Middleton LT, Gentleman SM, Grinberg LT, Giles K. Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc Natl Acad Sci U S A 2015; 112: E5308-17.
[25] Stefanis L. alpha-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2: a009399.
[26] Sinclair D, Mills K, Guarente L. Aging in Saccharomyces cerevisiae. Annu Rev Microbiol 1998; 52: 533-60.
[27] Longo VD. Mutations in signal transduction proteins increase stress resistance and longevity in yeast, nematodes, fruit flies, and mammalian neuronal cells. Neurobiol Aging 1999; 20: 479-86.
[28] Longo VD, Fabrizio P. Regulation of longevity and stress resistance: a molecular strategy conserved from yeast to humans? Cell Mol Life Sci 2002; 59: 903-8.
[29] Chen Q, Thorpe J, Ding Q, El-Amouri IS, Keller JN. Proteasome synthesis and assembly are required for survival during stationary phase. Free Radic Biol Med 2004; 37: 859-68.