

Extra dose of Dsn1p (Yir010wp) expressed from episomal plasmid in normal *Saccharomyces cerevisiae* diploid wild type has augmented growth rate and altered budding pattern, suggesting its growth stimulating property

Yiap, B.C.¹, C.M.V.L. Wong^{1,2}, Hirzun, M.Y.³, Raha, A.R.^{1,2 *}

¹Department of Biotechnology, Faculty of Food Science and Biotechnology,

²Fermentation Technology Unit, Enzyme & Microbial Technology Lab, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia.

³Sime Darby Technology Center, 2 Jalan Tandang, 46050, Petaling Jaya, Selangor, Malaysia.

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Abstract. *DSN1* is a spindle pole body component that functions in the chromosomal segregation process of yeast. The effect of additional *DSN1* gene dosage on the yeast cell has yet to be established. The work from this study indicated that an extra gene dosage of *DSN1* caused abnormalities in both cellular growth and budding pattern. The yeast cells carrying an additional copy of *DSN1* gene have an increased growth rate and they formed a highly retarded multiple bud complex. These phenomena suggest possible growth stimulating property of the gene product.

Keywords. Budding, *DSN1*, gene dosage sensitivity, growth stimulating.

INTRODUCTION

Dosage sensitivity implies that the effect of a gene and its product is dependant on the number of gene copy and hence its protein expression level (Balazs *et al.*, 2003). The basis for this phenomenon is known as the "Balance Hypothesis", whereby an imbalance in the concentration of the subunits of a protein-protein complex can be deleterious.

Homeostatic balance of the genes and gene products of a cell is crucial for maintaining its cellular physiology and morphology. Dsn1p is involved in the chromosomal segregation process of *Saccharomyces cerevisiae* because it is a component of the yeast spindle pole body (SPB). In yeast the spindle pole body represents the microtubule-organizing center (MTOC), which is usually represented by the centrosomes in most animal cells (Astrid, 2002).

In this work, we studied the effects of extra gene dosage of the newly characterized *DSN1*. We have looked into the changes of the yeast cell growth and budding pattern in relation to *DSN1*.

MATERIALS AND METHODS

Materials. All chemicals were obtained from Amresco Inc. while growth media were supplied by Duchefa and Scharlau. pUG23 and pUG34 GFP fusion expression vectors were gifts from Johannes H. Hegemann and co-workers.

The diploid SSC3 wild type host (*MAT a/α, ade2.1/ade2.1, leu2-111/leu2-111, his3-5/his3-5, trp1-1/trp1-1, ura3/ura3, can1-*

100/can1-100) was constructed from haploids SSC1 and SSC2 of both mating types with W303 origin.

Cloning strategy. The *YTR010W* gene was obtained by PCR amplification of its genomic copy from yeast with the primer pairs FWGFP/RWGFP being used for N-terminal cloning. PCR amplification using both primer pairs would give a product of about 1749 bp in size. The 5' region of the PCR product would have a *SmaI* restriction enzyme cutting site, whilst the *EcoRI* digestion site was situated at its 3' end. The products were cloned into yeast expression vector pUG34 (N-terminal GFP fusion). The vector has *CEN6/ARSH4* for replication in yeast and the *E.coli* origin of replication, ORI. Selection for yeast transformants was through growing on histidine minus dropout medium, whereas ampicillin resistance was the bacterial selective marker. The expression of the EGFP3 was governed by the *MET25* promoter and transcription ended by the *CYC1* terminator.

The vector was first digested with *SmaI*, purified and then subjected to a second digestion by *EcoRI* restriction endonuclease. Both the vectors and PCR products were repurified before being ligated.

Induction and suppression of GFP – YIR010Wp chimeric proteins. As the vectors contained the Met25 promoter, expression was suppressed by the addition of 1mM of

*Author for Correspondence.

Mailing address: Dept. of Biotechnology, Fac. of Food Sciences and Biotechnology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor. Tel: 03-8656 6421 / 8656 6423. Fax: 03-8656 7948 / 8942 3552. Email: adsade@yahoo.com

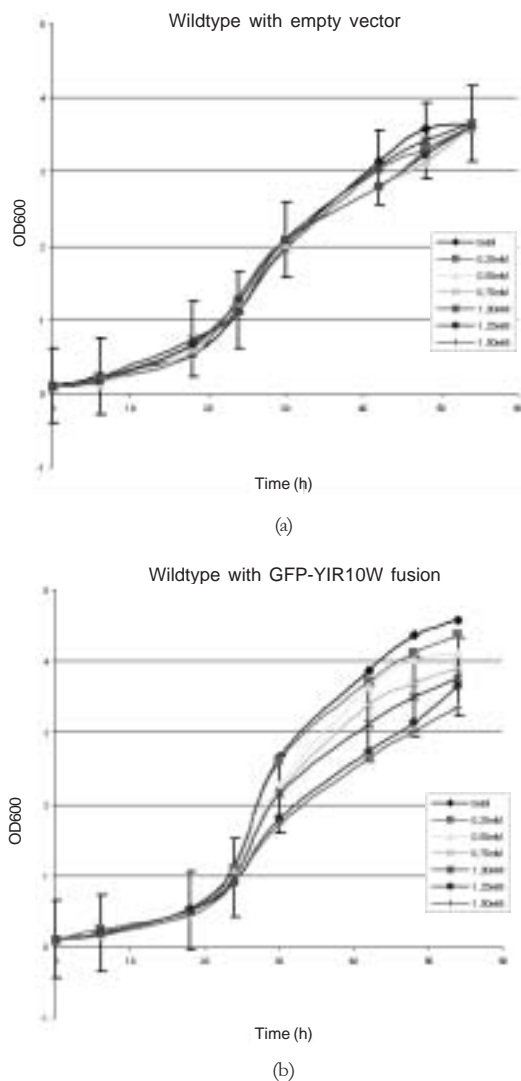


Figure 2a. Graph showing the OD_{600} reading of wildtype yeast cells with GFP empty vector [2 copies of *YIR010W* gene] over time at different concentrations of Methionine. **b** Graph showing the OD_{600} reading of wildtype yeast cells with pUG34 (N-Fus GFP-YIR010W) [3 copies of *YIR010W*] over time at different concentrations of Methionine

plasmid showed distinct disparity in growth performance at various methionine concentrations [Figure 2(b)]. The results also indicated significant growth differences between the wildtype with empty vector and wildtype with GFP – YIR010W fusion vector at 0mM to 0.75mM of methionine with decreasing difference as methionine concentration increased. Inherently, in growth condition with methionine concentration at 1.00 to 1.50 mM, the growth rates for both cell types was seen to be similar, without any significant differences.

Irregularity in budding pattern. One interesting finding when inspecting the bud morphology of the various cell types

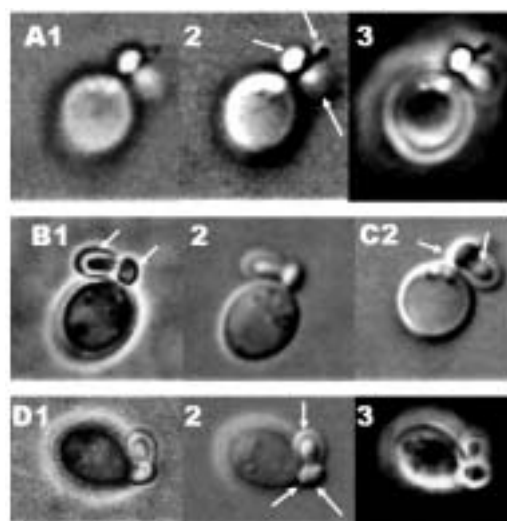


Figure 3 Upon induction of GFP-Yir010wp N-fusion hybrid protein in the diploid wildtype host, some of the cells produced peculiar structures of multiple consecutive buds (arrows) that failed to grow and eventually detached. All photo 1 were taken using normal visible light transmission microscopy, photo 2 were DIC (Normaski) and photo 3 were phase contrast pictures.

was the prevailing formation of peculiar budding structures in some of the mutants expressing the N-terminal GFP-Yir010wp. (Figure 3) shows that upon induction of GFP-Yir010wp N-fusion hybrid protein in diploid wild type host, some of the cells produced structures of multiple non-growing consecutive buds that eventually detached from the parent cell. We have proposed to name these consecutive buds that failed to grow as “Highly Retarded Multiple Buds Complex” or HRMBC. The structures could be separated from the mother cell by rough handling of the culture as shown in the (Figure 4). This has the appearance of a retarded small bud formed over the mother cell apex and another new bud elongated horizontally from the small bud. A third bud could be formed from the first retarded small bud as shown in panels A to D. A sheath-like structure was found to surround the second elongated bud as well. This structure was not seen in the wildtype host expressing C-fusion Yir010wp-GFP and was not detected in the single *YIR010W* disrupted mutant. Additionally, cells sprouting HRMBC structures constituted more than 67% of total cells observed (Data not shown).

On examining the property of the HRMBC under fluorescence microscopy, the multiple buds protruding from the mother cells (long full-lined arrows) were mostly brightly autofluorescing in golden yellow color but the mother cells (short full-lined arrows) appeared to be non-fluorescing (Figure 4 – 1 and 4 – 2). Results also showed that some of these structures were non-fluorescing (arrow head), while others might be partially autofluorescing and (dashed line arrow in Figure 4 – 3). Figure 4 – 3 showed that in several situations when the mother cell (diamond-head arrow) was highly

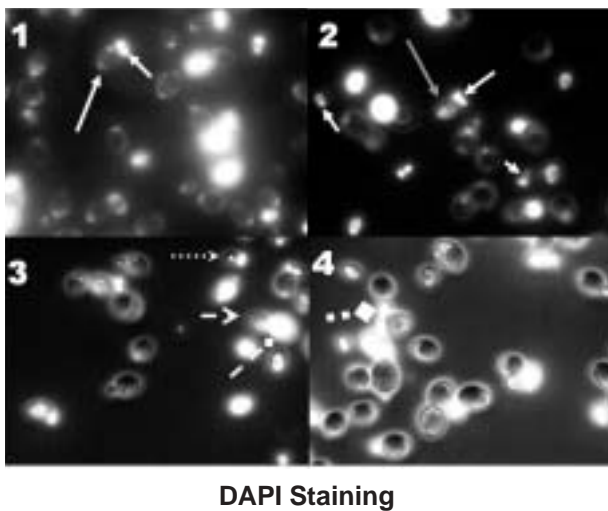


Figure 4. Autofluorescing characteristic of HRMBC which could be either attached to the mother cell or dislodged from it. Most of the structures were highly autofluorescing with bright golden yellowish color.

autofluorescing, its bud or daughter cell (blunt end arrow) was not. Figure 4 – 4 showed that the peculiar structure (dashed line Diamond Head arrow) could grow to a bigger size and could be almost of equal size, hence resembling a “Mickey Mouse-shaped” cell.

DISCUSSION

The reasons for the employment of pMET25 were that it has an inducible promoter and its expression was adequate but not too strong (Ulrich *et al.*, 1996). As the expression vectors were of the yeast centromeric type of plasmids, their cellular copy would be kept as low as their expression level. The inducibility of the promoter provided a better control over the level of expression of the fusion protein. This was important, as the fusion protein may be toxic to the host cell at an increased dosage. (David, 1991).

Methods to confirm successful cloning involved multiple steps such as PCR, RE digestion and sequencing, which were carried out to ascertain the correctness of the cloning process (Joseph *et al.*, 2001). Sequential verification steps were taken to confirm that the clones contained the correct gene and that the gene is devoid of any mutation. At the same time, proper gene alignment with the correct orientation will ensure that transcription and translation will be carried out efficiently. PCR amplification of the 1.8kb product from the vectors was to show the correct size of the insert. Additional digestion of the PCR product by different restriction endonucleases that were found in the *YTR010W* gene would support the identity of the insert.

The *YTR010W* gene displayed a dosage dependence characteristic when addition of extra copy (N-fus GFP-*YTR010W* only) into wildtype (with 2 copies of intact genomic *YTR010W*) cell boosted its growth rate. On the other hand, the mutated N-terminally fused GFP-Yir010wp chimeric protein might cause the faster growth rate seen in the wildtype yeast. Nevertheless, the reduction in heterologous fitness could not be applied to the N-fusion chimeric protein expressing wildtype host as it has a higher growth rate. An other explanation was necessary and perhaps the formation of the HRMBC structures were due to the increased growth rate. The chimeric recombinant protein could have stimulated and quickened the cell division cycle budding in the host. Yir010wp has an activation domain predicted at its C-terminal (protein domain prediction from SGD), so a GFP C-terminal fusion may prevent its functionality while the N-fusion GFP hybrid could be functional (John *et al.*, 2000). The advent of this phenomenon in the *YTR010W* single deletant might indicate the essentiality of Yir010wp for growth stimulation as the deletant has a lower gene dosage (one copy of gene in deletants versus three copies in wildtype expressing the chimeric proteins).

It is possible that the accumulation of either Yir010wp, because of its over-expression as in the case of additional production from the episomal plasmid, or the other protein due to Yir010wp under-expression as in the case of single deletion, could have prevented the formation of a more complicated complex. The imbalance of either one has diminished the final complex amount hence preventing its normal functionality and ultimately breaking down the cell division cycle mechanism (Bala' *zs et al.*, 2003). As this occurred, it could lead to premature cellular senescence through the presence of cells that were highly autofluoresced (Andrew *et al.*, 2001). The observation of HRMBC structures also indicate that perhaps GFP-Yir010wp complex could have given a false debilitating signal to the cell that affected its physiology.

The introduction of an additional copy of *YTR010W* gene into normal diploid wildtype yeast cells and the expression of the extra dose of functional Yir010wp have led to interesting observation of phenotypic changes. The mutant acquired a faster growth rate upon induction of expression. In addition, budding anomalies with the formation of peculiar budding structures (HRMBC) were also seen in the mutants. A high percentage of those structures were highly autofluorescing suggesting that premature cellular senescence could have taken place in them. As a whole, the results implied that Yir010wp has a growth stimulating property that could override cell division cycle check point mechanism and could eventually cause premature cell senescence.

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