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Gene Dosage Variation of YIR010W (DSN1) in *Saccharomyces cerevisiae* Caused Growth and Nuclear Anomalies

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Abstract: DSN1 is a SPB component that functions in yeast chromosome segregation process. It has been shown to be of haploinsufficient but its nature of gene dosage sensitivity was yet to be established. This study indicated that gene dosage variation of it has caused abnormalities in both cellular growth and nuclear division. Alteration of cell size and compromised budding index were part of the phenotypic manifestations. Changes in gene copy number also led to nuclear division and separation errors such as aneuploidy and nuclear disjunction. The results of this study provide direct evidence of the function of DSN1 in chromosomal segregation process. Gene dosage sensitivity of the gene also implied that its gene product would be forming complex with another protein.

Key words: Aneuploidy, chromosomal segregation, DSN1 (YOR010W), haploinsufficient

INTRODUCTION

Cellular physiology and morphology of a cell are greatly relied on the homeostatic balance of its genes and gene products[1]. This is especially so for proteins that form complex structure with others whereby the correct stoichiometry of all the components of the complex must be maintained[2,3]. This formation of complex was predicted by the balance hypothesis[4]. Haploinsufficiency was a manifestation of this phenomenon as shown by disruption of a copy of a particular gene in a diploid host[5].

DSN1 was implicated earlier as a component of yeast spindle pole body (SPB) that showed haploinsufficiency[6]. This was demonstrated by the low spore viability, which appeared to be part of the phenotypes, as detected through tetrad analysis. Sensitivity of SPB related genes to gene dosage was confirmed by the aneuploidy and polyploidy phenotypes in host with altered gene dosage[7]. In the budding yeast *Saccharomyces cerevisiae,* the response by corrective mechanism requires a number of gene products; damages in these products terminate cell cycle response to faults in nuclear division, leading to lethal division when such events occur[8].

The aim of this study was conducted to find out the effects of altered gene dosage of the newly characterized DSN1 and to determine the changes of the yeast cell growth and nuclear division in mutants with various numbers of DSN1 gene.

MATERIALS AND METHODS

The diploid SSC3 wild type host (MAT a/α, ade2.1/ade2.1, leu2-111/leu2-111, his 3-5/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. Diploid YIR010W single disruption mutant was constructed using PCR-based homologous recombination techniques as previously described[9,10].

All chemicals were obtained from Amresco Inc. while growing media were purchased from Duchefa and Scharlau. pUG23 and pUG34 GFP fusion expression vectors were gifts from Johannes H. Hegemann.

Cloning of YIR010W into GFP fusion expression vectors and yeast transformation: The YIR010W gene was amplified from genomic template through PCR reaction by using complementary primers having SmaI (Forward) and EcoRI (Reverse) restriction endonuclease sites. The PCR

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product was double digested by the two enzymes and ligated into GFP fusion expression vectors. pUG23 conferred C-terminal fusion of GFP to YIR010W whilst N-terminal fusion was done in pUG34. The recombinant vectors were transformed into the appropriate yeast hosts to construct hosts with assorted YIR010W gene copy numbers[11].

Induction and suppression of Yir010wp-GFP chimeric proteins: As the vectors contained Met 25 promoter, suppression of expression was achieved by addition of 1 mM of methionine into growth medium as previously described. Removal of methionine from growth medium led to derepression, hence induction of expression of the hybrid proteins.

Growth performance assay: All samples were grown in suppressive media prior to the beginning of the experiment. The experimental period was counted from the starting of removal of methionine from media. Samples were grown in synthetic dropout media lacking histidine for the selection of the plasmids. Hundred μl of each sample cultures were mixed with 900 μl of ultra pure water before their OD600 reading were recorded. A graph of OD600 reading versus time was plotted accordingly with a standard error bar protruding from line of the wild type control cell without any vectors.

Sampling and scoring for cell size and budding index: Random sampling of various cell sizes and budding stages of yeast hosts were carried out. More than 100 yeast cells were measured and an average cell size was calculated. Around 500 cells were scored for their budding stages, from no budding cell to cell with equal size bud.

Nuclear staining and viewing: The nuclei of the various hosts were stained with DAPI and view under a Leica Epi-fluorescent microscope. The filter cube A was utilized to produce the appropriate emission and excitation wavelength fluorescent light. Imaging was done by a CCD camera mounted on the microscope.

RESULTS

Cell size and budding index: Table 1 showed the average cell size of different host from both wild types and disruptants of YIR010W. The wildtype carrying the empty GFP vector was obviously having similar size as that of the wildtype without it. At the same time, wildtypes with the recombinant fusion GFP-YIR010W vector were also of equal size when the protein expression was suppressed. On the other hand, expressions of the recombinant hybrid proteins have rescued the diploid single disruptant of YIR010W by showing almost equivalent cell size to that of the wildtype control. As with the wildtype, the cell sizes of both the deletants with or without the GFP empty vector were more or less alike.

Typically, the budding indices for those rescued deletants and recombinant plasmid carrying wild type hosts (that did not expressing the chimeric proteins) were approximately comparable with those wild type host with or without the GFP vectors. Deletion of YIR010W gene in yeast cell led to the plunging of the budding index to about 20%. However, the expression of GFP in the deletant did not have any implication on its budding index. Nonetheless, expression of N-fusion GFP-Yir010wp in diploid wildtype host was the source of its diminished budding index to that seen in the diploid single disruptant. On the other hand, the production of Yir010wp-GFP C-terminal fusion protein did not affected its budding index that badly but just halving it to around 40%.

Nuclear division and appearances: In the previous study, YIR010W disruptant was suspected to display the occurrence of aneuploidy due to the lowered spore viability. This hypothesis was confirmed with the visualizations of multiple nuclei in single cell of budding or, worse still, non-budding. Figure 1 presented nuclear anomalies shown by either single disruptant of YIR010W or wildtype host carrying and expressing C-fusion or N-fusion Yir010wp-GFP chimeric protein. Figure 1c–e showed binucleated yeast cells, whereby Fig. 1a and b showed unbud cells with double nuclei that were starting to divide or failed to separate. Figure 1f showed a big bud cell with a nucleus in its daughter cell but two nuclei were visible in the mother cell. The nucleus in the daughter cell (and one of the nucleus in the mother cell as well) seem to be smaller that might due to unequal division of nucleus caused by retention of part of the daughter nucleus in the mother cell. Figure 1g showed a small bud cell having one of its two nuclei started to divide. Figure 1h showed unbud yeast cell with three nuclei, the upper nucleus appeared normal in size but the lower two were smaller in size as compared to the upper one and among themselves.

The error in nuclear division originated from assorted gene dosages was further strengthen by the viewing of translocation of undivided or divided nucleus in daughter bud that not supposedly to have at its stage of cell cycle. Figure 2 showed the normal nuclear division of yeast cell, whereby the nucleus in cell having small bud would reside to the apex opposite the bud, medium sided bud cell has its nucleus moved to the bud proximity, larger bud would

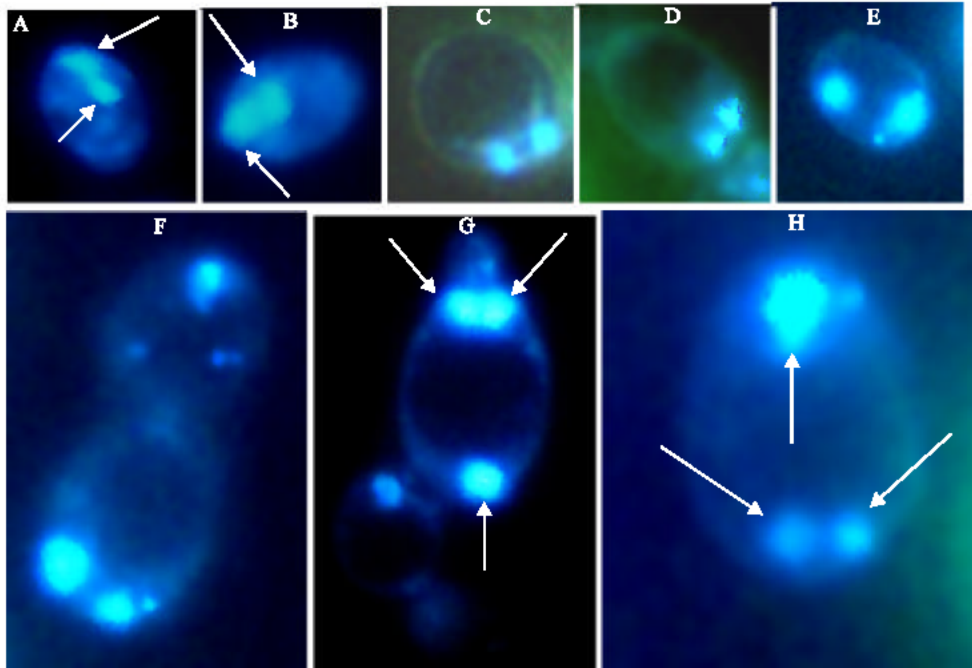

Fig. 1a-h: Aneuploidy or multiple nuclei shown by mutants carrying various copy number (1 or 3 in a diploid wild type host) or YIR010W gene

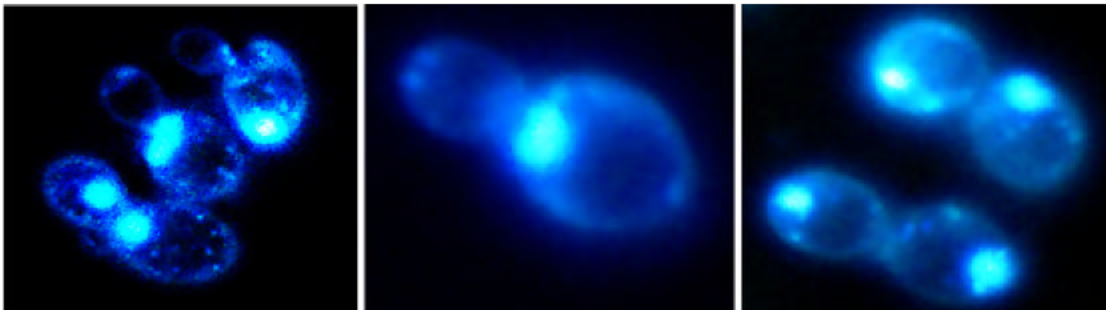

Fig. 2: Showing wildtype control yeast cells with normal cell division cycle

showed a newly divided nucleus went into the daughter cell and finally when both the daughter bud/ cell and mother cell were of equal size, the two nuclei would reside to the two apex away from each other. Figure 3 showed another nuclear anomaly, whereby photo Fig. 3a-c each showing the failing to divide double nuclei were residing in the daughter cell while the mother cell was deprived of nucleus. Figure 3d showed nuclei residing to apex of both the daughter cell and that of mother cell in a medium-sized budding yeast cell, a position only in cell-bud of equal size could have. Figure 3d also showed the nucleus in the proximity of a small bud when it should have been in the distal apex of the cell. Figure 3e-g showed nuclear retention in big bud mother cell.

Those errors (unequal division of nucleus and retention of nucleus) mentioned above, could have been the basis for the following observation. Figure 4a implicated the appearance of a diffused nucleus seem in some of the diploid host cell with copy number of YIR010W gene other than two. Additionally, most of them also have sprinkle of DAPI stainable particles diffused over the whole cell but particularly concentrated near the nucleus (Fig. 4b). Some of the nuclei appeared to be smaller than normal and were not roundly shape but irregular in shape (for instance, elongated).

Growth performance assay: The results of growth performance of yeast cells with varying number of

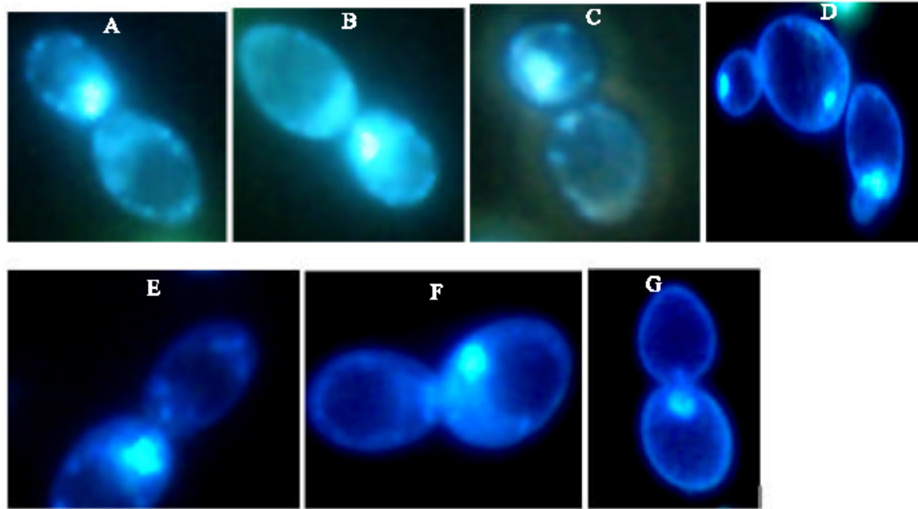

Fig. 3a-d: Showing migration of whole or part of the nucleus into daughter cell's bud and (e-g) showed retention of whole nucleus in mother cells, these took place in the mutants

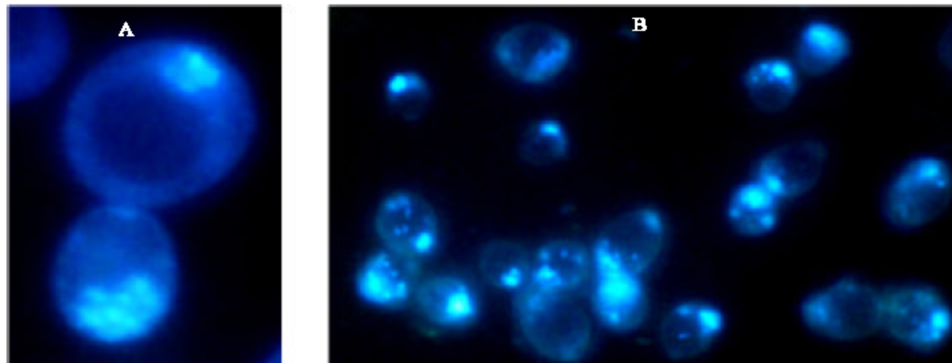

Fig. 4a and b: Showing mutants of various copy numbers of YIR010W gene displaying the diffused and fragmented nuclei characteristics

Table 1: The average cell size and budding index of different host from both wild types and disruptants of YIR010W

Cell type	Total size	Number of cell measured	Average size (um)	Bud	Unbud	Total	Budding index %
WtEmp	535.6	103	5.2	410	120	530	77.3
WtGFP	561.3	110	5.1	393	107	500	78.6
Wt23W	429.5	116	3.7	230	360	590	39.0
Wt23W-Sup	578.2	109	5.3	451	129	580	77.8
Wt34W	393.9	123	3.2	100	432	532	19.0
Wt34W-Sup	576.9	111	5.2	400	120	520	76.9
DEmp	410.0	107	3.8	108	405	513	21.0
D23W	535.3	101	5.3	401	107	508	78.9
D34W	588.2	113	5.2	378	107	485	78.0
DGFP	444.3	120	3.7	90	408	498	18.0

YIR010W gene over time at non-suppressive condition (0 mM methionine) (Fig. 5). From the result, wildtype hosts without any vector and that with empty GFP vector (both having 2 copies of intact genomic YIR010W gene) were having similar growth rate. Disruption of single copy of the gene caused reduction in growth rate. The same was observed for the disruptant carrying just the empty GFP vector.

Single disruptant expressing the fusion protein was able to restore its wildtype phenotype when it has identical growing performance as its wildtype yeast cell. On the other hand, both C-fus and N-fus chimeric protein

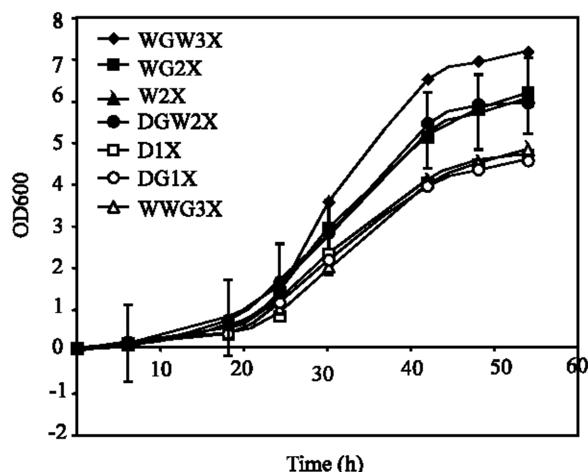

Fig. 5: Showing the OD600 reading of yeast cells with different copies of YIR010W gene over time at 0 mM of methionine

WGW3X → Wildtype with pUG34 (N-Fus GFP-YIR010W) [3 copies of YIR010W]

WG2X → Wildtype with empty GFP vectors [2 copies of YIR010W]

W2X → Wildtype without any vector [2 copies of YIR010W]

DGW2X → YIR010W Single Deletant with pUG34 (N-Fus GFP-YIR010W) [2 copies of YIR010W]

D1X → YIR010W Single Deletant without any vector [1 copies of YIR010W]

DG1X → YIR010W Single Deletant with empty GFP vectors [1 copies of YIR010W]

WWG3X → Wildtype with pUG23 (C-Fus YIR010W-GFP) [3 copies of YIR010W]

of GFP-YIR010W were expressed in wildtype host having intact double copies of YIR010W gene. Expression of N-fus GFP-YIR010Wp led to increment of growth rate while C-fus YIR010W-GFPp caused the host growth performance to tumble to that of single disruptant.

DISCUSSION

Cell size and budding index: As expected, the cell sizes and budding indices of the YIR010W mutants were lesser than their wildtype control counterparts as being predicted by the previous results showing reduction in heterologous fitness (the balance hypothesis)[4]. Nevertheless, the reduction in heterologous fitness could not be applied to the N-fusion chimeric protein expressing wildtype host as it has faster growth rate. The chimeric recombinant protein could have stimulated and fastening cell division cycle (in general) and/or (specifically on) budding in the host. Yir010wp has an activation domain predicted at its C-terminal (protein domains prediction from SGD), so C-fusion of GFP to it prevent its functionality while N-fusion GFP hybrid could work perfectly fine. The yet to be found of this phenomenon in the YIR010W single deletant might indirectly hint on the essentiality of Yir010wp for the growth and/or cell division stimulation as the deletant has lower gene dosage (one copy of gene in deletants versus three copies in wildtype expressing the chimeric proteins).

Nuclear division and appearances: The defective cell division cycle mechanism in mutants with differed gene copy numbers of YIR010W was further elaborated by the inaccuracy in nuclear division and positioning. Aneuploidy (through nuclear disjunction) and sheared nuclear elements (through nuclear retention) found in all types of mutants may account for the dimerization capability of Yir010wp with another protein[7]. It is possible that the accumulation of either Yir010wp (because of its over-expression as in the case of additional production of it 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[3]. 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.

Growth performance assay: The YIR010W gene displayed a dosage dependence characteristic when addition of extra copy of it (N-fus GFP-Yir010wp only) into wildtype (with 2 copies of intact genomic YIR010W) cell boosted its growth rate, whilst the deletion of a copy of YIR010W in diploid yeast cell has the consequence of reduction in its growth rate. However the presence of C-fus Yir010wp in the wildtype host led to similar plunging in its growth rate as that of the single disruptant of its gene. The logical explanation to this surprising finding could be that the fusion of GFP C-terminally to it could have been disrupting the normal function of it by altering its structure. According to SGD prediction, there is a Leucine zipper at its Carboxy-terminal for the purpose of dimerization. Therefore, the carboxy fusion of GFP onto the Yir010wp could have prevented dimerization hence rendering it non-functional. These non-functional aggregates of fusion protein might be toxic to the cell leading to the observed slow growth phenotype[2]. This prediction could only be ascertained when structural studies were done on those proteins.

Conversely, the C-terminally fused Yir010wp-GFP hybrid could be just perfectly fine, it is its increased dosage that has the detrimental effect on cell growth, in accordance to the balance hypothesis. Whilst the faster growth rate seen in the yeast might be caused by the mutated N-terminally fused GFP-Yir010wp chimeric

protein. In order to distinguish the real culprit, expression of addition native Yir010wp in wildtype yeast cell must be done by eliminating the GFP fusion from it.

As a conclusion, alterations in the gene copy numbers of the YIR010W gene have profound effects on its cellular physiology and morphology detected through growth performance assay and microscopic analysis respectively. DSN1P would have formed dimer with other protein due to its gene dosage sensitivity.

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