Chemical chaperone - sorbitol corrects cohesion and translational defects in the Roberts mutant

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Abstract-

Cohesin protein complex plays a very important role in chromosome segregation transcription, DNA replication and chromosome condensation. Mutations in cohesin proteins give rise to a disease called Cohesinopathies. The major cause of cohesinopathies is due to defects associated with gene expression that give rise to developmental disorders. In this study we have used the yeast (*Saccharomyces cerevisiae*) strain to mimic the cohesinopathy disorder Roberts syndrome with mutations (*eco1W216G*) homologous to that of humans (*esco2*). We have used sorbitol, as a chemical chaperone, to check, if it can restore the chromosome segregation, gene expression misregulation and the translational defects. We have also checked levels of autophagy in the Roberts mutant and found that in samples treated with sorbitol the autophagy defects were corrected.

Keywords-Cohesinopathies, Roberts syndrome, Sorbitol, Chromosome segregation, Translation

Article Highlights-

- **In presence of sorbitol temperature sensitivity of the Roberts mutants is rescued**
- **Chromosome cohesion defect is reduced in presence of sorbitol**
- **Translational defects are minimized in presence of sorbitol**
- **Autophagy enhances in presence of sorbitol**

1.Introduction-

Cohesin proteins are composed of a group of proteins that bind to the sister chromatids and maintains cohesion between chromatids along the entire length of chromosome [1].These remain attached till metaphase and till proper separation of sister chromatids occurs at anaphase[1]. In addition to the cohesin complex there are associated proteins which also plays an important role to provide stability and maintain genomic integrity [2] such as eco1, pds5 etc. These cohesin proteins are cell cycle dependent. Mutations in these proteins lead to

conditions referred to as Cohesinopathies [2] .In addition, to chromosome segregation there are also defects at transcriptional level which cause developmental disorders[2,3].In this study we are working on a rare autosomal genetic disorder called Roberts syndrome(RS) caused due to mutations in eco1 (human esco2) proteins[4]. RS is caused due to mutation in the eco1 gene(human homologue esco2) [5]. It is important to understand the effects caused due to these mutations. Different models such as yeast, mice, zebrafish and *Drosophila* are used to study the pathophysiology of RS [6]. For our work we have used haploid yeast model to make mutations in eco1, analogous to the human mutation. In this study we are working on the yeast model system which mimics the disease model of human Roberts syndrome caused due to point mutation *eco1W216G* [5].

The RS mutant is a stress response mutant lethal at 37°C temperature and is associated with defects in protein translation [3,7]. From our earlier published work, we know that cohesin proteins are involved in regulation of translation where it influences the enhancers and promoters during transcription.[3,8]. Roberts' mutant is a stress response mutant [7]with defects in translation [3]. Our study aims in finding answers to check if the chemical chaperone sorbitol, can alter the elevated expression of the stress response genes in the mutant well as shows changes in chromosome segregation and translation.

Due to translational defects, there is an accumulation of misfolded proteins[9]. These misfolded proteins tend to aggregate in the endoplasmic reticulum (ER) [10] generating cellular stress [11]. To rescue the cells from stress there is either shut down of transcription or activation of various pathways like unfolded protein response(UPR), stress regulation, CWI pathway [12]. Due to changes in expression levels of these collective proteins, newly synthesized misfolded proteins, are minimally produced, which gives more time for degradation pathways to dispose off unwanted residues[12,13]. Moreover, changes in gene expression of protective sugars and enzymes is reported, which helps the cells to survive stressed conditions [13]. Similar to neurodegenerative diseases[14], it is hypothesized that in the *eco1* mutant, there is a built up of ER stress which causes accumulation of unwanted proteins. Under these conditions, it tends to increase the expression of chaperones[15], to degrade abnormal and toxic proteins which might correct the misfolding. This helps , to rescue the cells from the stressed situation [16].

These misfolded proteins are corrected with the help of molecular chaperones [17]; but due to continuous production of misfolded proteins it increases the load on the chaperone machinery hence researchers use external chaperones which assist the misfolded proteins to fold properly

and regain its function. [18-20]. .Use of cellular chaperones are known for correcting misfolded proteins and ultimately reduce the severity which is observed in case of many of the neurodegenerative diseases[21]. It has been reported that the chemical chaperones are used in treatment of various neurodegenerative diseases like Huntington, Amyotrophic lateral sclerosis, Alzheimer and Parkinsons disease[21,22].

There are a large number of studies which use low molecular weight compounds such as glycerol, TMAO[23] and various amino acid derivatives. These chemical chaperones can reduce and reverse aggregation of misfolded proteins [24]. Also they are well known to reduce ER stress [25]. Hence chemical chaperones can be considered as potential alternative to biological chaperones. The exact mechanism of action of these chemical chaperone is not clear but probably it functions by preventing nonproductive interactions as well as by altering endogenous activities of chaperones. This stabilizes misfolded proteins and ultimately reduces protein aggregation [24].

The chemical chaperones are either polyhydric alcohol and sugars (like Trehalose, Sorbitol, Glycerol, Arabitol and Myo-inositol), amino acids derivatives (Proline ,Glycine, Alanine, Taurine) or Methyl ammonium derivatives (Trimethelamine N-Oxide i.e.TMAO, Glycreo-Phosporyl choline). Hence depending on the mechanism of action for providing stability, these osmolytes are classified as compatible and counteracting osmolytes. The first type of osmolytes are compatible type of osmolytes which includes aminoacids and polyols like sorbitol. These donot have much effect on the functional properties of the protein and provide stability under native conditions. The second class is the counteracting class, which causes modification in function of proteins and helps to provide stability and inactivates the protein. These includes methyl ammonium derivatives [26, 27].

It was observed that those proteins, that are expressed in presence of osmolytes are more stable than those which are expressed in absence of the osmolytes under denaturing conditions. This happens as these osmolytes can enhance protein folding and the stability of the protein [26].

During protein folding, osmolytes help in stabilizing proteins by interacting with the peptide backbone of protein. This interaction allows protein domains to gain its preferred hydration [26]. Compounds like sorbitol tend to avoid direct contact with polypeptides. Hence, at higher concentrations, we can observe increased hydration around polypeptides because proteins pack themselves more tightly and decrease their surface area.This phenomenon of packing of polypeptides in presence of osmolytes increases stability of the proteins.[26,27]. It has also

been reported that the presence of these polyols and glycine, betaine are best osmoprotectants and they preferentially exclude water molecule and increase thermal stability of proteins [28].

Sorbitol (glucitol) is a sugar alcohol/polyol and comes under the compatible type of osmolytes. It is widely used as a stabilizing agent and in food and pharmaceutical industries[28]. It remains stable at all temperatures and is a chemically inert molecule which dose not have much effect on the function of proteins [29]. It helps to provide stability to proteins and maintain their native conformation[30]. In this study we have used sorbitol as a chemical chaperone and various assays to check if it can rescue the diseased condition in the Roberts' mutant. It has been reported that it can pass through the blood brain barrier, has a short half life in serum, hence will not accumulate in blood. Sorbitol is easily metabolized by the brain. In brief, it can be used as an effective osmotherapeutic drug in optimal doses[31]. Taking into view, the following characteristics of sorbitol, we have used it as chemical chaperone which can rescue the diseased condition in yeast. It has been reported that due to hyperosmolarity, in presence of sorbitol, there is a changes in the transcription and translational processes, cell cycle progression and retention of various osmolytes[32]. The results obtained showed that it possibly acts as a rescuer of the phenotypic manifestation of the diseased condition.

2.Materials and methods-

2.1. Serial dilution assay-

Wild type and *eco1*W216*G* strains were grown in YPD Broth medium for overnight. Next day O.D.(Optical Density)was adjusted up to 0.2 then culture was diluted serially and spotted on YPD plates with different concentrations of sorbitol(0.5M,1M,1.5M,2M) and incubated for 48hrs. for each biological triplicates. After 48 hrs results were checked for growth pattern and further analyzed[33].

2.2. Growth Assay-

Wild type and *eco1*W216*G* cultures of yeasts were grown in YPD broth till its growth reaches mid-log phase. Then cultures were grown in a 96 well plate containing YPD added with sorbitol medium and YPD medium as control. This was performed in three biological replicates. Experiment was performed for 48 hours at 30 with continuous shaking in the Epoch2 BioTek Spectrophotometer and O.D. was recorded after every 30 min. The data was acquired and graph was plotted using GraphPad software[34].

2.3. RTPCR-

Cultures of Wild type and *eco1W*216*G* were grown in YPD with and without sorbitol in a shaking incubator overnight. The RNA was isolated using Ambion RNA extraction kit method. The RNA was treated with Turbo DNAse. The RNA (2ug) was then quantitated using nanodrop. From RNA cDNA was made using dNTP mix and Random 6 mers and Takara cDNA kit. RT PCR was performed using cDNA (5-fold dilution) ,18S and 25S forward and reverse primers Here we have used actin gene as housekeeping gene. Gene expression values were plotted using graph pad software[3].

2.4. Microscopy-

Wild type and *eco1W*216*G* strains of yeast were grown overnight in YPD medium. After 24 hours, O.D. was adjusted till 0.2 and A 10 µl of culture was placed on cellulose coated slide (to reduce mobility of cells) and were observed under Nikon Ti Eclipse microscope with GFP laser using 60x and 100x objectives. All the images were acquired at 5X zoom using 100X plan objective with 1.49 numerical aperture, using the manufacturer's software. FIJI software was used to process Images. The cells showing one spot and Two spot were counted and number of cells from 100 different fields were calculated calculated.Graphs were prepared using GraphPad 9 software[3].

2.5. Western blot-

Cultures of wild type and *eco1W*216*G* cells of yeast grown in presence and absence of Sorbitol with YPD as basic growth medium were grown to an OD of approximately 0.8. Then cells were pelleted down by centrifugation and washed. Cells were lysed with lysis buffer using lysing matrix containing glass beads and lysis buffer (Tris10 mM(pH 7.4), NaCl, 1 mM EDTA100 mM, EGTA1 mM, NaF 1 mM, $Na_4P_2O_7$20 mM, Na3VO42 mM, SDS 0.1%, sodium deoxycholate 0.5%, Triton-X 1001%, glycerol10%, and PMSF1 mM, protease inhibitor cocktail (Sigma)).Then cell lysis was done using bead beater followed by centrifugation .The supernatant was collected and quantified using bradford method. The Equal amounts of protein were loaded for every sample. Protein samples were separated by SDS gel electrophoresis using 12% SDS-polyacrylamide followed by its transfer on nitrocellulose filters. After transfer the blots were probed with primary antibody phospho-specific eIF2a (Ser-51) (Cell Signaling). This is followed by secondary antibody (anti rabbit).The bands were was visualized by using developer(Thermo)and Amersham imager 600(GE Healthcare Life Sciences)[3, 35].

Similar procedure for SDS and transfer was followed to check autophagy levels. The detection was done using ATG8(Thermo Fisher Scientific) as primary antibody [36]and anti Rabbit antibody as secondary antibody bands were visualized using developer(Thermo Fisher Scientific) and Amersham imager 600(GE Healthcare Life Sciences).

2.6. Polysome profiling-

For polysome analysis 200 ml of yeast culture was grown up to an OD 0.8(at 600).Cells were pelleted down washed with PBS and treated with 20 mg/ml of cycloheximide for 10 mins on ice. After 10 min cells were subjected for centrifugation. The obtained cell pellets were added in lysing matrix tubes containing glass beads and lysis buffer (Tris-HCl10 mM pH 7.5, NaCl100 mM, $MgCl_2$30 mM, cycloheximide100 ug/ml, heparin 0.2 mg/ml in DEPC). This is followed by cell lysis using cells bead beater(MP Biomedicals) centrifuged and 500µl of supernatant was loaded on top of an 11 ml 7–47% sucrose gradient prepared in High Salt Resolving Buffer (Tris-Cl 15 mM pH 7.4, NH_4Cl 140 mM and MgOAc-4H_2O 7.8 mM) Tubes were then subjected for ultracentrifugation at 27,000 rpm for 2.5 h in swing bucket SW41 rotor . After centrifugation the gradients were fractionated and OD was monitored Absorbance at 254 nm using a Bio comp fractionator station. Peaks were analyzed after measuring Polysome to monosome ratio[37,38].

3. Results-

3.1. Temperature sensitivity of mutant strains get rescued in presence of sorbitol-

The wild type and mutant cells were checked for a period of 48 hrs in presence of different concentrations of sorbitol (0.5M, 1M, 1.5M, 2M) by spot dilution assay. Due to mutation and translational defects, these cells show moderate growth at 30°C , slow growth at 33°C **(Supplementary Fig 1)** and lethal at 37°C. After 48 hrs we observed that YPD plates that have been incubated at 37°C showed no growth at 0.5M and 1M where as at 1.5M and 2M sorbitol the growth is revived

We observed that plates with 2M sorbitol, growth is revived but there was a slight reduction in the growth of wild type cells at 37°C. Thus, we have selected 1.5M sorbitol concentration for further assays. After primary screening, we can hypothesize that sorbitol acts as a chemical chaperone suppressor and our mutant is showing growth rescue at 37°C in presence of sorbitol.

To further consolidate the above results we have analyzed the growth patterns of wild type and Roberts mutant cells at 37°C using growth curve assay, where wild type and mutant cells were

grown in YPD medium in absence and presence of 1.5M sorbitol. We monitored the growth by following the absorbance at 600nm at intervals of 30 min for 24 hrs. This data was analysed using GraphPad Prism 9. We observed rapid growth with very short lag phase in wild type and mutant cells inoculated in YPD in absence of sorbitol.

On the other hand, the cells inoculated at 1.5M took a longer lag phase but showed better growth after log phase, indicating the rescue of the mutant cells in presence of 1.5M sorbitol.

3.2. One Spot Two Spot assay to check if sorbitol changes chromosome cohesion defects

The wild type and mutant strain that have been used for checking chromosome cohesion assay, consists of an array of lac repressor site integrated into the chromosome IV R arm with LacI - GFP system[5]. To check if addition of sorbitol has any effect on chromosome cohesion we have used wild type and *eco1* mutant, treated with nocodazole for arresting at G2/M phase, and checked for presence of one or two spots. Two distinct spots at one locus indicates precocious separation of sister chromatids prior to anaphase[5]. Around 100 cells were checked and the data was analysed using GraphPad Prism 9. Our data suggests, that in untreated mutant samples, GFP spots were more than two times of those present in wild type cells, where as after treatment with 1.5M sorbitol the number of two spots reduced.

3.3. Effect of sorbitol on stress response genes

Sorbitol acts as an ER and hyperosmotic stress inducer [39]. To check whether there are changes in expression levels of stress response genes der1, ero1 and kar1 we have carried out RT- PCR in presence of 1.5M and 2M sorbitol concentration. The relative transcription level of the targets genes was determined by relating the Ct values of the sample with reference to the reference gene (ACT1) **Fig3(a), (Supplementary fig2).**

Our data showed that in comparison to untreated samples, there is little or no change in gene expression levels of wild type. In mutant cells there is a decrease in the expression of der1 and kar1 genes when treated with sorbitol.

To find out profile changes of gene expression in response to presence of sorbitol we performed RNA sequence analysis. In the gene expression study, the data showed that overall 541 genes are upregulated and 291 genes are downregulated where as 5243 genes are neutrally regulated. There is an upregulation of many uncharacterized proteins along with other genes.

To identify gene expression that was affected we compared upregulated and downregulated genes in the group **(Fig3 (b))**

The gene ontology data suggest that amongst upregulated genes 48.60%genes are involved in biological processes, 59.70% genes are cellular component genes, 40.30% genes are involved in molecular function, 38.90%and 80.60% are PTM and other genes respectively whereas 72% are cellular component genes, 44% of each gene is involved in biological processes and molecular function, 56.00% and 84% PTM and other genes respectively are downregulated **(Fig3(c)).**

3.4. Does sorbitol correct impaired protein translation in cohesinopathy mutant strains?

Phosphorylation of eiF2α is considered as an indicator of translational initiation inhibition and it is conserved during the process of evolution. Due to eif2α phosphorylation in the ternary complex, there is an inhibition of exchange from GDP to GTP hence translation gets stalled (3). Here we have used western blot analysis to measure levels of phosphorylation of eif2α protein **(Fig4a)** and total eif2α **(Fig4b)**. Analysis of western blot image shows that reduced levels of phosphorylation of mutants **(Fig 4c),** which is corrected in samples treated with sorbitol. We have used eif2α as the loading control to depict equal loading of samples in each well.

3.5. Changes in actively translating ribosome fraction in presence of sorbitol

Polysome profiling is a technique that detects association of mRNA with that of ribosomes as well as gives an idea about overall translation taking place in the cells [38]. This polysome profile is used to detect which step of translation is getting affected [37] i.e. if there is a defect in translation initiation polysome to monosome ratio decreases where as if there is a translation elongation defect the polysome to monosome ratio gets increases.

In this case we have observed that peaks for 80S is more pronounced in wild type than that of mutants. Also it is already known that the polysome profile changes during stressed conditions. We have performed polysome profiling of wild type and mutant cells treated with 1.5M sorbitol and compared with the polysome profiles of untreated wild type and mutant samples. After calculating polysome to monosome ratio we have observed that the polysome to monosome ratio of mutants treated with sorbitol is similar to the values of wild types, which is indicates that there is a changes in gene expression pattern after addition of sorbitol which might be helping in rescuing our mutant cells.

3.6. Sorbitol might play a role in regulating autophagy

Autophagy is a recycling process of the cell which helps the cell to survive under stressed condition During this cell breakdown and reuse various proteins and macromolecules. In this case presence of increased levels of misfolded proteins are harmful for cells hence cells use mechanism of autophagy where cells eliminate and reuse for cell.

Here we hypothesize that use of chemical chaperone sorbitol may affect levels of autophagy in mutant strains. Our data suggests that there is an increase in the levels of autophagy in the mutant strain treated with sorbitol than the untreated cells where as in wild type the levels are almost similar **(Fig6a)**. We have used GAPDH **(Fig6b)** as loading control and data analysis showed that there is equal showed that there is equal loading of each sample.

To summarize the data for spot dilution assay and growth curve assay suggests that growth of Roberts mutant at 37°in presence of 1.5M sorbitol cells shows rescue of mutant cells **(Fig1a, 1b).** In one spot two spot assay also we could find reduction in two spots after treatment with sorbitol. Results for RNA sequencing and RT PCR showed reduced levels of stress response gene. To analyze the effect of sorbitol on translation we have performed western blot assay determining eif2α levels and polysome profile where we found that in sorbitol treated mutants the levels of phosphorylation of eif2α were reduced by 75% **(Fig4a,b,c).** The polysome to monosome ratio returns to almost wild type levels **(Fig5)**.To analyze changes in autophagy levels we have used western blot analysis and determined changes in levels of ATG8-GFP **(Fig 6a,b,c)** and found that the ATG8 levels are increased in mutants treated with sorbitol.

4. Discussion

The chaperones that are naturally present in cells play a very important role in the cells by helping proteins to fold and maintain its native conformation. It also helps to remove misfolded proteins by the activated UPR systems[27]. Sometimes there is an excess amount of misfolded proteins which increases the load on the chaperones as well as on the protein degradation machinery. As a result it creates ER stress[21]. To reduce ER stress we can substitute with different chemical chaperones that can be added externally. Work on sorbitol by other groups suggests that, it increases solubility of cellular aggregates and the ER to golgi transport [40] .It also allows proper folding of proteins. Studies have been reported where sorbitol and trehalose

reduce fibrillation in proteins [41. Bioinformatics analysis on interaction of amyloid fibrils with sorbitol suggests changes in peptide conformation, kinetics and physical characteristics of the protein[41,42]. Hence we have selected sorbitol as the chemical chaperone.

In this work we have used spot dilution and growth curve assay to check the effect of sorbitol on the growth patterns of wild type and *eco1W*216*G* strains at permissive and nonpermissive temperature. Interestingly, screening these temperature sensitive mutants in presence of sorbitol, we found that the our mutant strains rescues temperature sensitivity at 37°C in presence of sorbitol **(Fig.1a,b)**. Here we have checked the growth phenotype of the *eco1* mutant at different concentration of sorbitol using spot dilution and growth curve assays upto 2M concentration. At this concentration, it shows better rescue of mutants, but it is observed that growth of wild type cells is also affected to a certain extent. Hence we have selected 1.5M sorbitol concentration for further studies.

Similar rescue in temperature sensitivity have been observed by research groups working on the cohesin mutant *mcd1-1* mutant [1] treated with sorbitol. and suggested that this rescue of temperature sensitive phenotypes could be due to correction of cohesion defects [1]. To check if addition of sorbitol helps in rescuing cohesion defects we have performed one spot two spot chromosome cohesion assay [3]. The data obtained from this assay, shows that the number of two spots is getting reduced in samples that are treated with 1.5M sorbitol (**Fig 2**). Since presence of two spots indicate premature (precocious) separation of sister chromatids, we can conclude that addition of sorbitol rescues the cohesion defects in the *eco1W*216*G* mutants.

Every cell responds to environmental stress by altering the expression levels of certain proteins that are required to adapt the cells to stressed situation, as well as for the survival of the cell[43]. Usually this alteration in protein expression is done by changes in expression levels of mRNA and by altering attachment of mRNA with that of ribosomes[44]. In some cases during stressed situations, it was found that various degradation pathways like proteosome and autophagy are upregulated whereas pathways that controls protein synthesis are downregulated[12]. We have subjected our wild type and mutant cells to different concentrations of sorbitol and checked the effect for the presence of hyperosmotic pressure on wild type and mutant cells with respect to growth, transcription and translation and its effect on autophagy .

Protein synthesis is controlled at various steps [45]. At translational initiation step, phosphorylation of translation initiation factor is one of the key controlling steps, since phosphorylation of eif2α reduces protein synthesis [46]. Thus it is considered to be an indicator of translational inhibition which is evolutionary conserved [3]. Our observations on the results of the western blot suggests that in presence of 1.5M sorbitol, the levels of phosphorylation in the cells decreases in the mutant when treated with sorbitol compared to the control strains. In presence of sorbitol eif2α phosphorylation is inhibited. Data from **Fig4c** shows phosphorylation levels were elevated by 75%.

Autophagy is the pathway known for degradation and reuse of proteins playing important roles during various cellular processes such as nutrient starvation and misfolded protein accumulation [18-20]. It has been observed that during hyperosmotic stress, genes for LC3(Autophagosomal ortholog of yeast Atg8) [47] gets induced and triggers autophagy related protein (ATG8) production to clear protein aggregates and rescues the cells from a stressed situation[48]. We wanted to check if there is an induction of autophagy level, in presence of sorbitol. At present ATG8 is the most widely used organelle marker to determine autophagy[49].

In contrast to the results of eif2α phosporylation ,the autophagy levels of ATG8 in the mutants treated with sorbitol increases. In a separate study in nutrient starved yeasts, we find that phosphorylation of eif2α is elevated, where as autophagy detected from expression levels of ATG8 are reduced [50,51]. This data supports our findings. In the *eco1* mutant we find eif2α phosphorylation increases and autophagy decreases as evidenced from levels of ATG8-GFP. In cells treated with sorbitol, we find that the levels of eiF2α phosphorylation is reduced and autophagy is restored to wild type conditions. The increase in levels of autophagy is an indication of the presence of excess ER stress or external osmotic pressure that is built up in the cells [52].

Like trehalose, there is a possibility that presence of sorbitol might play a chaperone like function to clear off the aggregates present in the cell which are possibly interfering with the autophagy in the cells [53].

Cells under favorable conditions can grow optimally but when they are under stress , some stress response genes get upregulated [54]. In this study we have checked the osmotic stress of sorbitol, to see if there are any changes in the expression of the stress response genes by using RNA sequencing. We have also tried validating the levels of the stress response gene in

presence of sorbitol by RT PCR. The results indicate that the levels of kar1, an ER stress response gene, in the mutant is reduced in presence of sorbitol, compared to heightened levels of kar1 alone in the eco1 mutant.

The *eco1* mutant generates ER stress and in response to this stress there is a change in the translation pattern [35]. It was important to check the polysome profile of these cells. It was already reported that cells treated with 1.5M sorbitol show considerable changes in their polysome profile than the control (38). Polysome profiles in the mutant is different in comparison to that of the wild type. Polysome to monosome ratio decreases in the absence of sorbitol in the mutant, but when treated with 1.5M sorbitol tends to be similar to that of the wild type.

To correlate the activity between eiF2α phosphorylation and autophagy, we found increased levels of eiF2α phosphorylation [35] and a decrease in the autophagy level as evidenced from expression levels of ATG-8-GFP [55]. When these mutants, were treated with sorbitol, we found a decrease in the phosphorylation levels of eiF2α and ATG8-GFP levels were enhanced [56]. The polysome profile reverted to wild type conditions in presence of sorbitol. Summarizing these results, we can conclude, that the use of sorbitol will rescue the cell from the stressed condition and global protein translation together with chromosome cohesion defects.

5. Conclusions-

From data presented here we conclude that ER stress plays role in chromosomal abnormalities of cell [57] and data for one spot two spot assay suggests that these chromosomal abnormalities are reduced on addition of sorbitol. Also results of RT PCR using stress response genes kar1 showed that ER stress is increased in mutants but on addition of sorbitol, ER stress is reduced. The eif2α levels are reduced and polysome to monosome ratio is corrected which suggests that translational initiation defect tend to be corrected in presence of sorbitol.The aggregates that are present in the cells get cleared from the cell during the process of mitosis and cytokinesis through autophagy to maintain cellular proteostasis [58]. However, *eco1W*216*G* mutant having defects in the cell division process where the cells stall during G2/M phase [3], can be one of the reasons behind protein aggregation and increase of ER stress in the mutant cells. Reduced levels of autophagy in mutant cells causes protein built up in cells but addition of sorbitol causes increase in levels of autophagy. This indicates that addition of sorbitol might be helpful for autophagy [59]. We

hypothesize that sorbitol acts as chemical chaperone which might interact hydrophobically with protein molecule and help them fold towards its native structure. This stabilizes proteins and prevents from formation of aggregates.

Data Availability Statement-

Strains are available on request.The authors affirm that all data necessary for conclusions of article are present in this article, figure and supplementary material.

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Both authors share equal contribution

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Conflict of Interest-

The authors declare no conflict of interest

Abbreviations Used-

Wildtype-WT

Roberts syndrome-RS

Endoplasmic reticulum -ER

Unfolded protein response-UPR

Cell wall integrity-CWI

eIf2α-Eukaryotic initiation factor 2 α

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Fig.1(a)

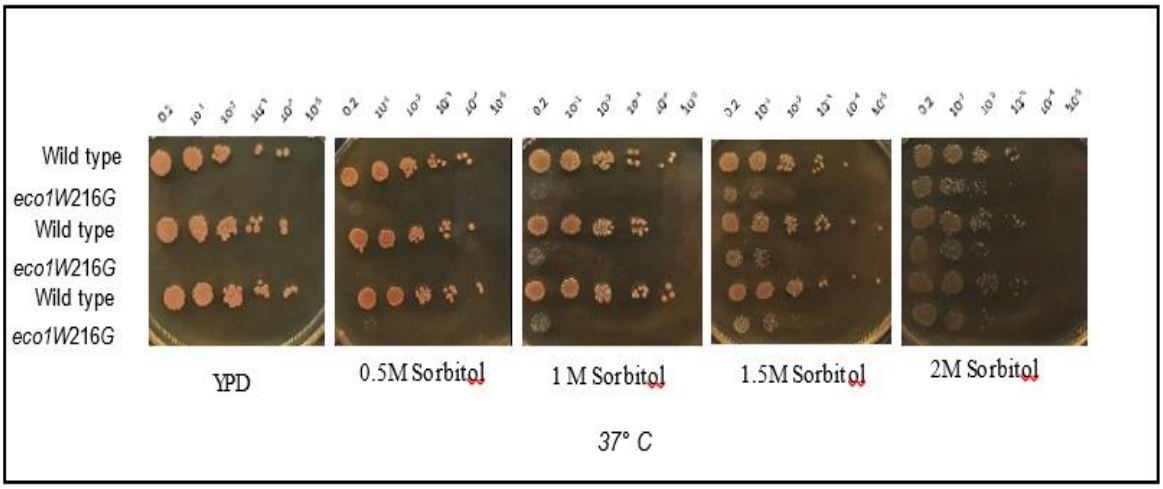

Wild type
eco1W216G
Wild type
eco1W216G
Wild type
eco1W216G
YPD
0.5M Sorbitol
1 M Sorbitol
1.5M Sorbitol
2M Sorbitol
37° C

Fig.1(b)

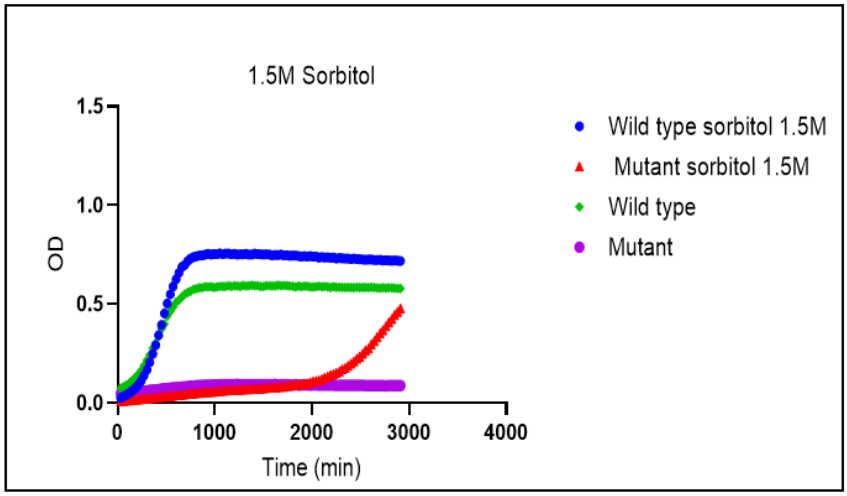

1.5M Sorbitol
OD
1.5
1.0
0.5
0.0
0
1000
2000
3000
4000
Time (min)
Wild type sorbitol 1.5M
Mutant sorbitol 1.5M
Wild type
Mutant

Fig.2

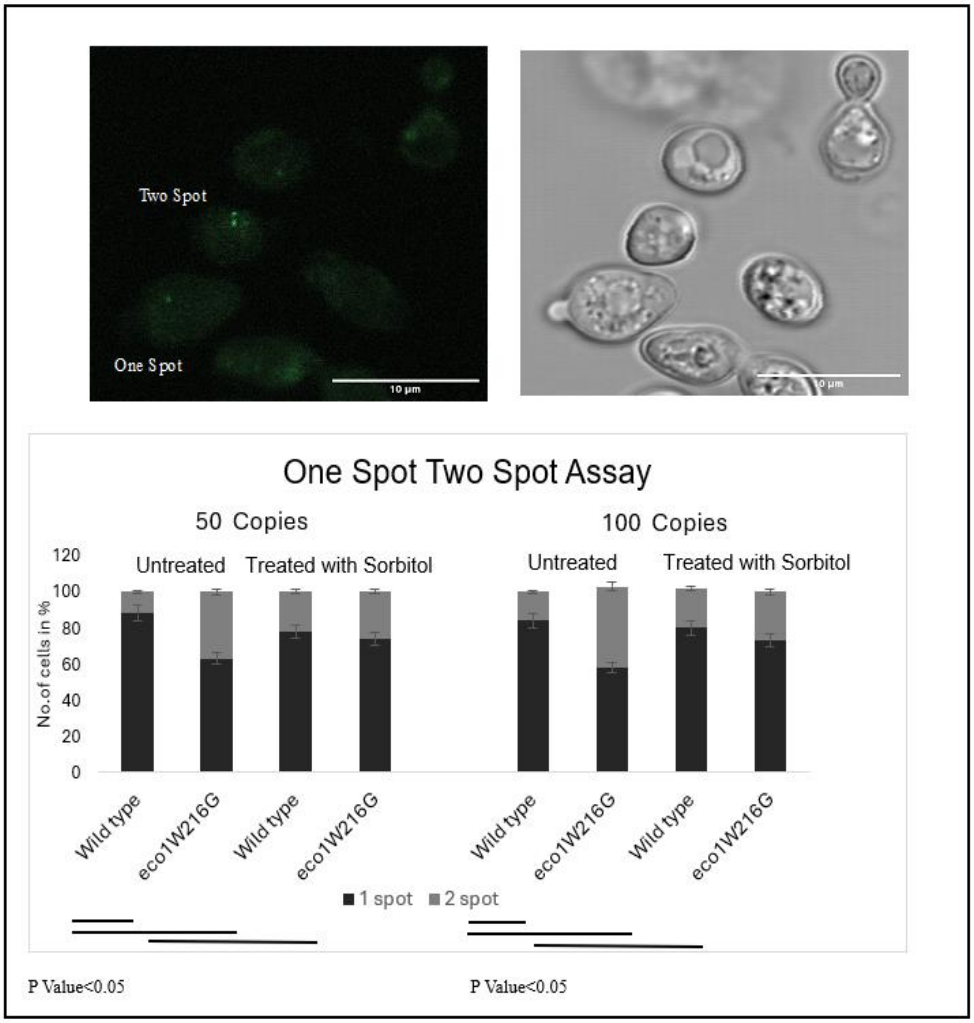

Two Spot
One Spot
10 µm
10 µm
One Spot Two Spot Assay
50 Copies
100 Copies
Untreated
Treated with Sorbitol
Untreated
Treated with Sorbitol
No.of cells in %
120
100
80
60
40
20
0
Wild type
eco1W216G
Wild type
eco1W216G
Wild type
eco1W216G
Wild type
eco1W216G
1 spot
2 spot
P Value<0.05
P Value<0.05

Fig.3(a)

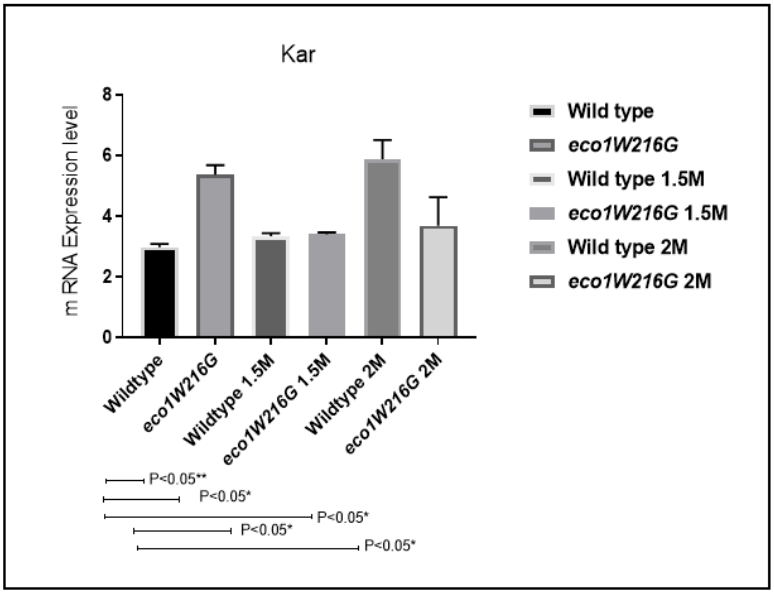
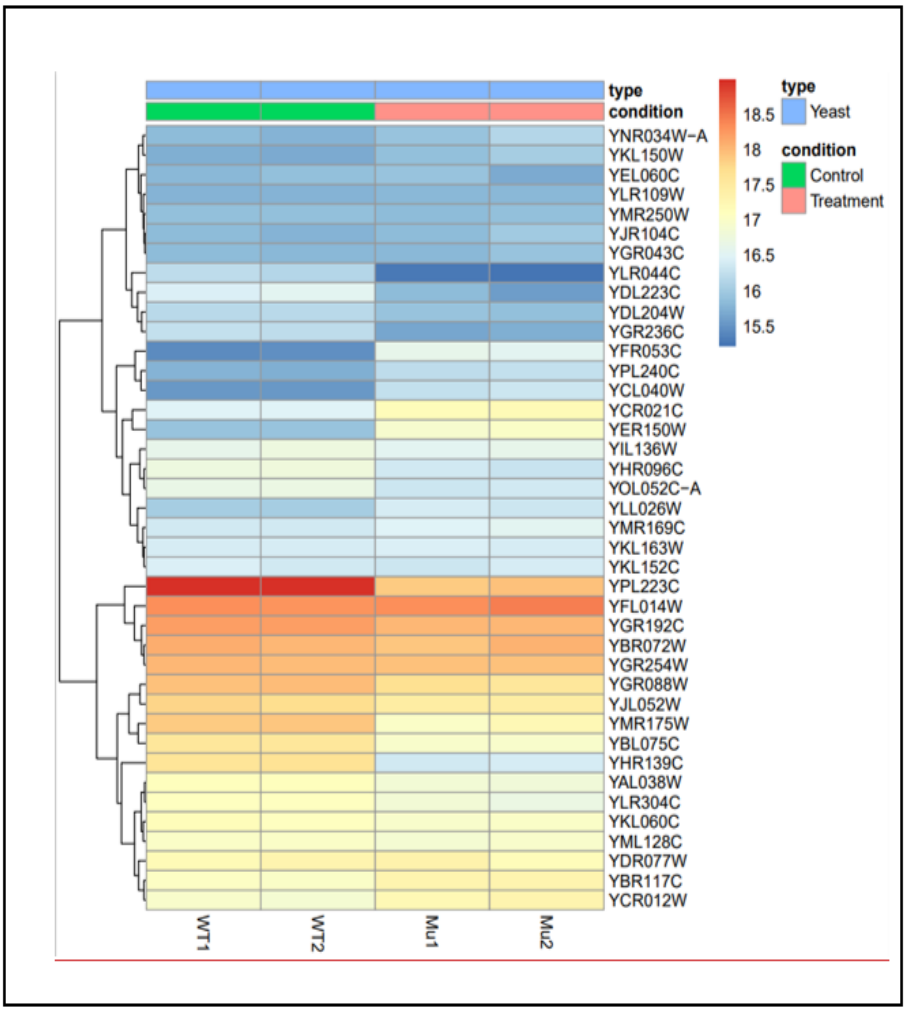

Kar
m RNA Expression level
8
6
4
2
0
Wildtype
eco1W216G
Wildtype 1.5M
eco1W216G 1.5M
Wildtype 2M
eco1W216G 2M
Wild type
eco1W216G
Wild type 1.5M
eco1W216G 1.5M
Wild type 2M
eco1W216G 2M
P<0.05**
P<0.05*
P<0.05*
P<0.05*
P<0.05*

Fig3(b)


type
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YDL204W
YGR236C
YFR053C
YPL240C
YCL040W
YCR021C
YER150W
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YHR096C
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YLL026W
YMR169C
YKL163W
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YCR012W
WT1
WT2
Mu1
Mu2
18.5
18
17.5
17
16.5
16
15.5
type
Yeast
condition
Control
Treatment

Fig3(c)

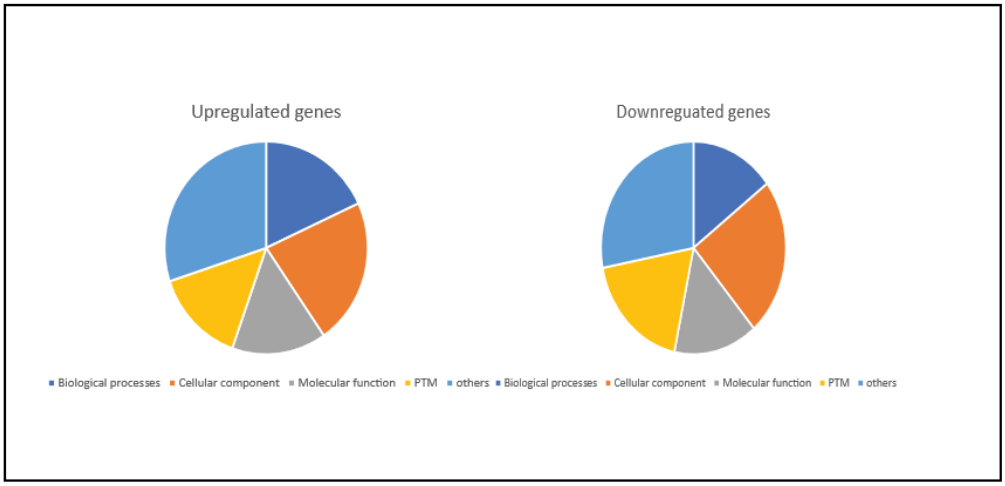
Upregulated genes
Downreguated genes
Biological processes
Cellular component
Molecular function
PTM
others
Biological processes
Cellular component
Molecular function
PTM
others

Fig.4

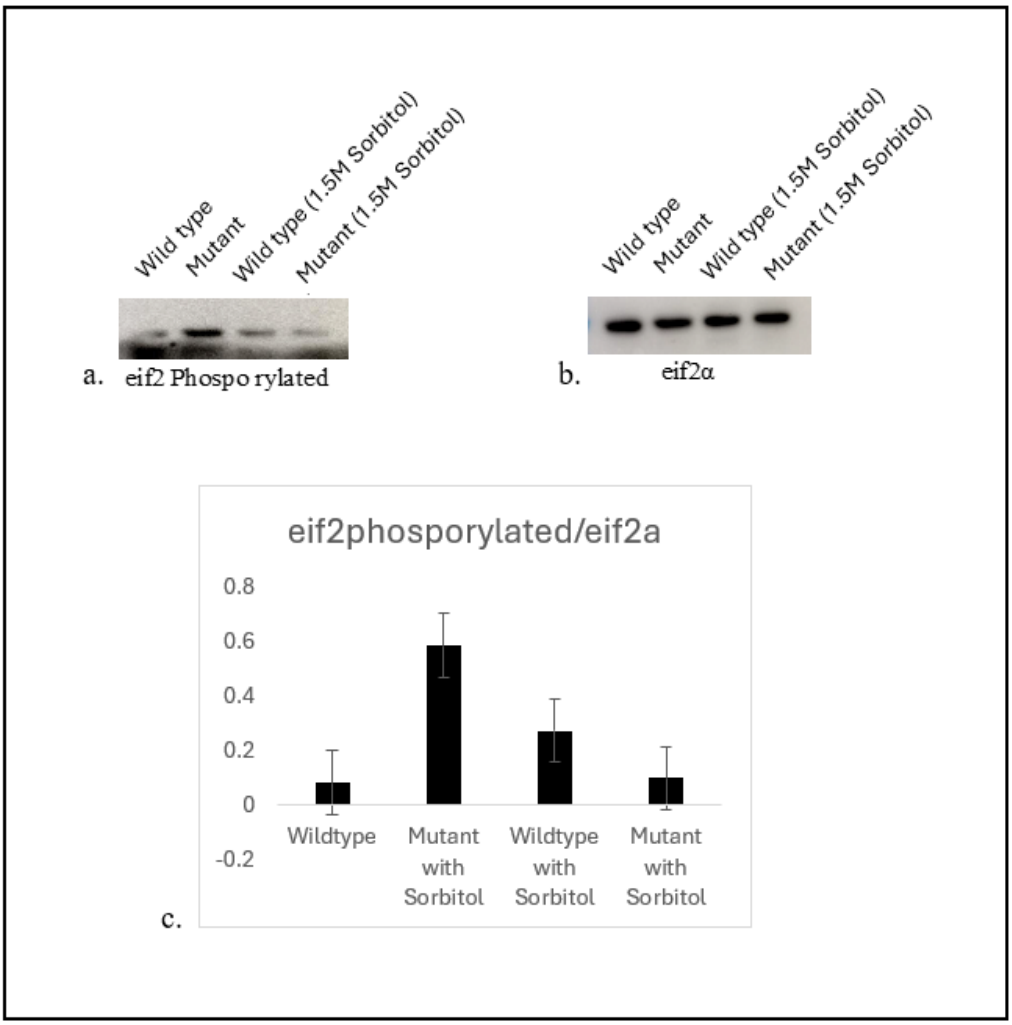
Wild type
Mutant
Wild type (1.5M Sorbitol)
Mutant (1.5M Sorbitol)
a. eif2 Phospo rylated
Wild type
Mutant
Wild type (1.5M Sorbitol)
Mutant (1.5M Sorbitol)
b. eif2α
eif2phosporylated/eif2a
0.8
0.6
0.4
0.2
0
-0.2
Wildtype
Mutant with Sorbitol
Wildtype with Sorbitol
Mutant with Sorbitol
c.

Fig.5

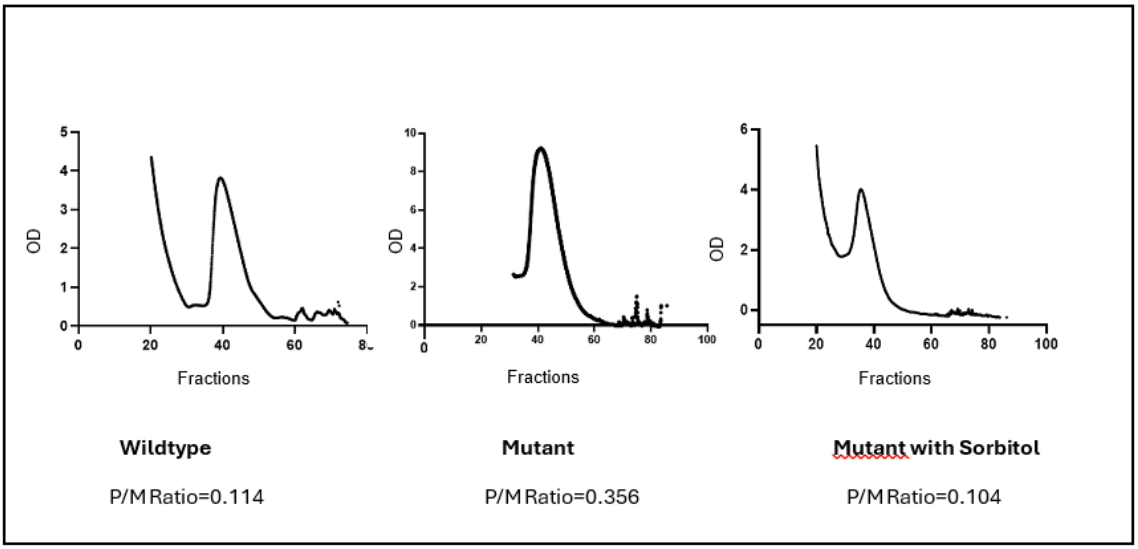
OD
Fractions
Wildtype
P/M Ratio=0.114
Mutant
P/M Ratio=0.356
Mutant with Sorbitol
P/M Ratio=0.104

Fig.6

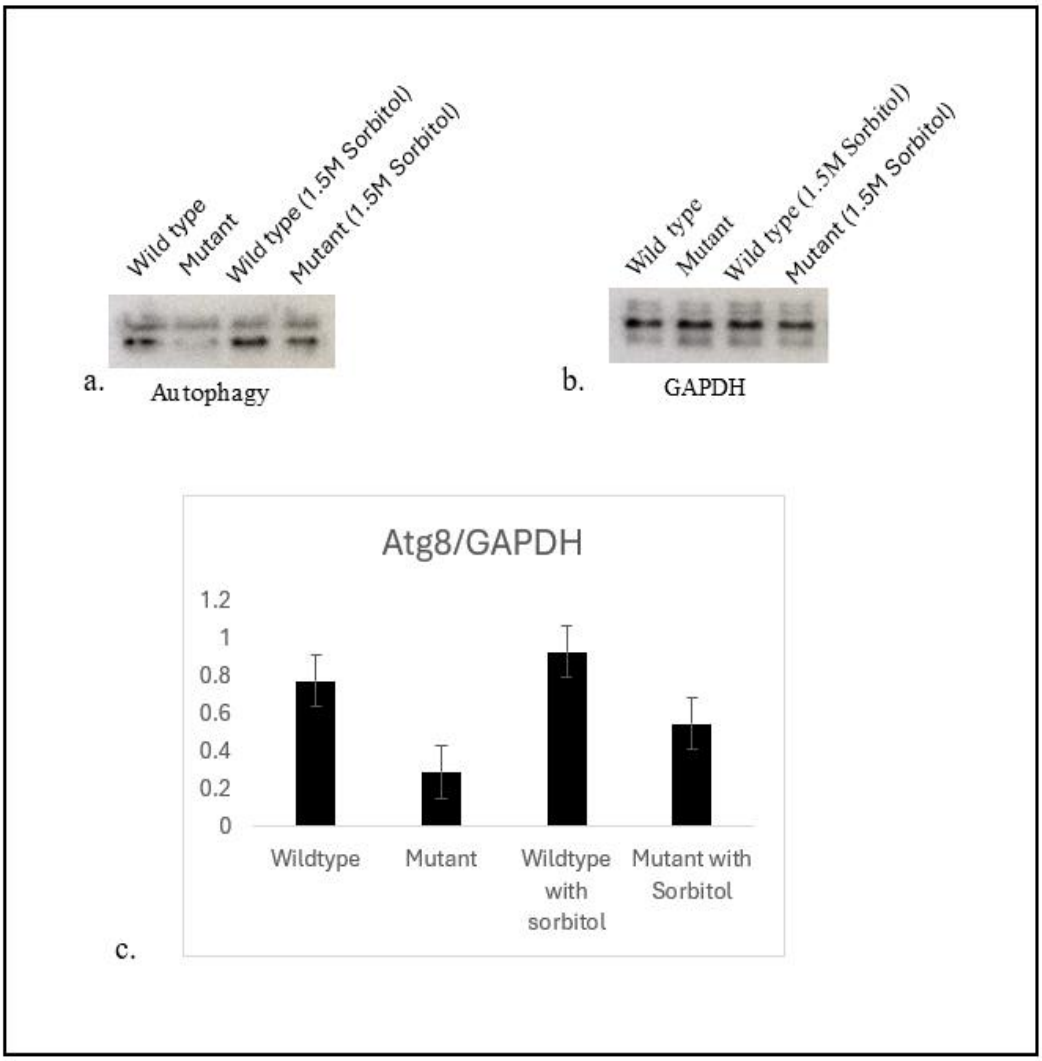
Wild type
Mutant
Wild type (1.5M Sorbitol)
Mutant (1.5M Sorbitol)
a.
Autophagy
b.
GAPDH
Atg8/GAPDH
1.2
1
0.8
0.6
0.4
0.2
0
Wildtype
Mutant
Wildtype with sorbitol
Mutant with Sorbitol
c.

Fig.1(a) Effect of sorbitol on the growth of wild type and mutant (*eco1W216G*) at 37°C (serial dilution assay)

Wild type and mutant (*eco1W*216*G*) strains of *Saccharomyces cerevisiae* were grown, and serially diluted and spotted onto YPD medium containing 0.5M,1M,1.5M and 2M sorbitol respectively. Data shown here is a representative of three independent biological replicates.

Fig.1(b) Effect of sorbitol on the growth of wild type and *eco1W216G* at 37°C (Growth Curve Assay)

48hrs growth curve assay of wild type and eco1W216G treated with 1.5 M Sorbitol and wild type and mutant (eco1W216G) without sorbitol as control. The O.D was measured at 30 min intervals. Data shown is obtained from 3 independent biological replicates. The blue line(•) indicates wild type with 1.5 M sorbitol, red line(▲) indicates eco1W216G treated with 2M Sorbitol, Green line(◆) indicates wild type, purple line(●) indicates eco1W216G. Graphs for growth curve were prepared using GraphPad prism 9 software.

Fig.2 One Spot Two Spot Assay to check the effect of sorbitol on chromosome cohesion

Cohesinopathy mutants show defects while chromosome segregation. Wild type and mutant strains of *Saccharomyces cerevisiae* with 50 and 100 copies of rDNA were grown in YPD broth medium with and without 1.5M sorbitol at 30°C and collected at log phase . The cells were nocodazole treated to arrest them at metaphase and checked for the presence of one spot and two spot. Images for live cells were collected using confocal microscopy with 100x objective. In order to quantify fluorescence intensity, approximately 100 cells of each type were counted. Graphs were prepared using GraphPad prism 9 software. T-test was used to calculate P values , all values are <0.05.

Fig.3(a) Effect of sorbitol on stress response gene

Data denoting fold change expression levels of stress response gene (kar1). RT-q PCR was performed on RNA from treated and untreated wild type and mutant cells . cDNA was prepared from RNA extracted from wild type and mutant cells grown at 30°C temperature in YPD medium with and without 1.5M and 2M sorbitol. Graphs were plotted from data obtained using GraphPad Prism 9 software. P values were calculated, all p-values are statistically significant.(P value<0.005*), act1 was used as control.

Fig3(b) Heatmap showing gene expression in presence of sorbitol

Heatmap showing the RNA sequencing based gene expression pattern. Red and blue values represent up and downregulated genes respectively. Color density indicates levels of fold change was displayed.

Fig3(c) Effect of sorbitol on gene expression using gene ontology study

Wild type and mutant strains were collected from cells grown in YPD with and without 1.5M sorbitol at 30°C temperature. RNA from these cells were collected and RNA sequencing was performed with NGSA method. Analysis was done based on log2 fold change between the groups which was further used to prepare graph using DAVID software.

Fig.4 Effect of sorbitol on protein translation using western blot analysis

Whole cell extracts of wild type and mutant cells were made from cells grown in YPD broth with and without sorbitol in the medium and grown at 30°C temperature. Extracts were then used for western blotting to measure levels of eif2α protein and phospo- eif2α,which is an indicator of translational inhibition. **Fig4a.** Western blot showing levels of phosphorylation of eif2α where wild type and *eco1W216G* untreated samples were used as controls and wildtype and *eco1W*216*G* cells treated with 1.5M sorbitol. **Fig4b.** shows levels of eif2α, as loading control**. Fig4c.** Graph showing densitometry data.

Fig.5 Effect of Sorbitol on protein translation using polysome profiling

Polysome profiles of wild type and mutant strains were collected from cells grown in YPD with and without 1.5M Sorbitol at 30°C temperature. Ratio of polysomes to monosome (80S) is shown. **Fig5a.** Polysome profile of wild type **Fig5b.** Polysome profile of mutant (*eco1W*216*G*) with higher 80S peak than 80S peak in wildtype; reduced polysome to monosome ratio than wild type. **Fig5c.** Polysome profile of sample treated with 1.5M sorbitol with polysome to monosome ratio almost restoring to wild type levels.

Fig.6 Effect of sorbitol on protein translation and autophagy using western blot analysis

Whole cell extracts of wild type and mutant cells were made from cells grown in YPD broth with and without 1.5M sorbitol in the medium and grown at 30°C temperature. Extracts were then used for western blotting to measure levels of autophagy using ATG8 as a marker for autophagy. **Fig 6a.** Western blot showing levels of ATG8-GFP with wild type (W303a) and mutant (*eco1W*216*G*) untreated samples used as controls and wild type (W303a) and mutant

(*eco1W*216*G*) cells treated with 1.5M sorbitol used as samples. **Fig 6b.**Western blot of loading control using with GAPDH. **Fig 6c.** Graph showing densitometry data.