Activation of salt shock response leads to solubilisation of mutant huntingtin in *Saccharomyces cerevisiae*

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Abstract Formation of cytoplasmic and nuclear aggregates is a hallmark of Huntington's disease (HD). Inhibition of aggregation of mutant huntingtin has been suggested to be a feasible approach to slow down the progress of this neurodegenerative disorder. Exposure to environmental stimuli leads to the activation of the stress response machinery of the cell. In this work, we have investigated the effect of salt shock on the aggregation of mutant huntingtin (103Q-htt) in a yeast model of HD. We found that at an optimum concentration of NaCl, the protein no longer formed aggregates and existed in the soluble form. This led to lower oxidative stress in the cell. Salt shock resulted in the synthesis of the osmolyte glycerol, which was partially responsible for the beneficial effect of stress. Surprisingly, we also found increase in the synthesis of another osmolyte, trehalose. Using deletion strains, we were able to show that the effect on solubilisation of mutant huntingtin is due to the synthesis of optimum amounts of both osmolytes. Stress-induced effect was monitored on gene expression. Genes related to proteins of the osmosensory pathway were upregulated on exposure to salt while those coding for stress response proteins were downregulated when solubilisation of mutant huntingtin occurred. Our study shows that activation of stress response elements can have beneficial effect in the solubilisation of huntingtin in a yeast model of HD.

Keywords Glycerol · Huntingtin · Huntington's disease · Salt shock · Trehalose

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Introduction

Huntington's disease (HD) is a devastating neurodegenerative disorder characterized by motor, cognitive and psychiatric symptoms. The highlight of the disease is the formation of intracytoplasmic and intranuclear inclusions of huntingtin, in which the natively occurring polyglutamine stretch towards the N terminus of the protein is expanded abnormally. Although debatable, increasing evidence points to a positive correlation between aggregation of mutant huntingtin (in which the polyglutamine tract exceeds a threshold value) and cell death in HD (Krobitsch and Lindquist 2000; Muchowski et al. 2000). Efforts to inhibit protein aggregation have yielded promising results in disease models and transgenic animals (Tanaka et al. 2004; Cashikar et al. 2005; Vacher et al. 2005). As such, inhibition of protein aggregation is recognized as a viable strategy to slow down the progress of the disease. One of the more promising approaches to restore loss of proteostatic balance due to protein misfolding and aggregation is by activating the stress response machinery of the cell (Rodriguez et al. 2013). For example, administration of activators of heat shock factor 1 (Hsf1) like geldanamycin has led to reduction in toxicity and improved survival in mammalian cells expressing an elongated polyQ stretch (Sittler et al. 2001). Overexpression of Hsp104 has resulted in inhibition of huntingtin inclusion formation in yeast (Cashikar et al. 2005), *Caenorhabditis elegans* (Satyal et al. 2000) and higher models (Carmichael et al. 2000; Vacher et al. 2005).

The N-terminal fragment of mutant huntingtin, harbouring the elongated polyQ stretch, has been expressed in yeast, and this transformed organism has been used extensively to study protein misfolding in Huntington's disease (Krobitsch and

Lindquist 2000; Muchowski et al. 2000; Sittler et al. 2001; Cashikar et al. 2005). Yeast responds to changes in extracellular concentrations of various solutes such as sugars and NaCl through a stress response pathway known as osmotic stress response (Hohmann 2002). To cope up with increased osmolarity, yeast cells undergo a rapid shift in transcriptional and translational response along with synthesis and retention of compatible solutes such as glycerol. These adaptive responses are directed by the high osmolarity glycerol (HOG) signalling pathway, which is governed by Hog1 MAP kinase (MAPK) cascade (Hohmann 2002, 2009). This cascade is well conserved and is present in higher organisms including humans. The activation of Hog1 MAPK leads to its translocation inside the nucleus, causing changes in gene expression and cell cycle patterns. This osmoadaptive response has short- and long-term effects on cell physiology (Saito and Posas 2012). Transcription and translation of the genome serve as long-term adaptation while synthesis of compatible solutes such as glycerol and trehalose are termed as short-term response of the cell to osmotic stress condition (Albertyn et al. 1994; Proft and Struhl 2004). In this work, we have studied the effect of activation of osmoregulatory response in yeast on the aggregation of mutant huntingtin protein. Since glycerol and trehalose are classical protein stabilizers, the presence of these osmolytes is expected to have some beneficial effect on the aggregation of the mutant protein and overall stress levels in yeast cells.

Materials and methods

Materials

Saccharomyces cerevisiae BY4742 (MAT α *his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0*, [RNQ1⁺]) deletion strains were products of Open Biosystems and were purchased from Saf Labs Pvt. Ltd., India. Luria Bertani broth, agar, ampicillin, acrylamide and mouse anti-FLAG antibody were purchased from Sigma-Aldrich, India. Goat anti-mouse horseradish peroxidase (HRP)-conjugated monoclonal antibody and tetramethyl benzidine/hydrogen peroxide substrate were obtained from Bangalore Genei, India. Amino acids for amino acid dropout mixture were obtained from SRL Pvt. Ltd., India. Yeast nitrogen base (without amino acids) was purchased from HiMedia Laboratories Pvt. Ltd., India. Dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased from Cayman Chemical Company, USA. Oligo dT₁₈ primer was purchased from Fermentas Inc., USA. MMLV reverse transcriptase was purchased from Promega Corporation, USA. SYBR Premix Ex TaqTM (Perfect Real Time) kit was purchased from Takara Bio, Inc., Japan. Free glycerol detection kit was purchased from Abcam plc, UK. All other reagents and chemicals used were of analytical grade or higher.

Methods

Expression of FLAG-103Q-htt-EGFP

S. cerevisiae BY4742 Δ *nth1* (neutral trehalase-deletion mutant) and Δ *tps1* (trehalose-6-phosphate synthase-deletion mutant) cells were transformed with pYES2-FLAG-Htt-103Q-EGFP harbouring gene insert for mutant length huntingtin (exon 1, 103Q) using the lithium acetate-PEG method (Gietz et al. 1992). Cells were grown in SC-URA medium containing 2 % (*w v*⁻¹) dextrose at 30 °C till O.D.₆₀₀=0.8. Protein expression was induced with 2 % (*w v*⁻¹) galactose for 7 h at 30 °C. Cells were pelleted and washed with autoclaved double distilled water. Expression of proteins was confirmed using fluorescence microscopy (E600 Eclipse, Nikon), native PAGE and western blotting. For salt shock, transformed yeast cells were incubated in 0.25, 0.5, 0.75 and 1 M NaCl during induction.

Native PAGE analysis

Cell pellets were lysed using acid-treated glass beads (Einhauer et al. 2002). Lysates were set aside for 1 h for the glass beads and debris to settle down. Total lysates were centrifuged at 15,000×*g* for 10 min. Supernatant was collected and protein was estimated in the supernatant by Bradford dye binding method (Bradford 1976), using bovine serum albumin as the standard protein. The samples were analysed by 12 % native PAGE and scanned using Typhoon Trio (GE Healthcare) in the EGFP mode at λ_{ex} =488 nm and λ_{em} =526 nm.

Western blotting analysis

Supernatant fractions were run on 12 % SDS PAGE gel, and protein bands were transferred electrophoretically to a nitrocellulose membrane (0.45 μ m). Expression of 103Q-htt was confirmed using FLAG antibody (1:1,000) as the primary antibody and anti-mouse HRP-conjugated antibody (1:2,500) (raised in goat) as the secondary antibody.

Estimation of glycerol

Frozen cell pellets were extracted twice with 0.5 M trichloroacetic acid (Ferreira et al. 1997). The cell suspensions were centrifuged at 15,000×*g* for 2 min at 4 °C. The amount of glycerol was estimated in the samples using free glycerol kit as per the manufacturer's instructions. The absorbance of the coloured product was monitored at 570 nm.

Estimation of trehalose

Trehalose was extracted following the same protocol as for glycerol. The amount of trehalose produced by the cells was

determined by HPLC (SCL-10A VP, Shimadzu, Japan) (Lillie and Pringle 1980). Samples were injected into a Zorbax carbohydrate analysis column (Agilent Technologies, USA), and the eluate was monitored using a refractive index detector (RID-10A, Shimadzu, Japan). The mobile phase was a mixture of acetonitrile and water in the ratio of 70:30 (v/v), at a flow rate of 1 ml/min.

Measurement of intracellular reactive oxygen species

Cells were washed with 10 mM phosphate-buffered saline, pH 7.4 (PBS) and counted using a Neubaur chamber. Cells (1×10^7) were aliquoted into a microcentrifuge tube, and DCFH-DA (10 mM, dissolved in dimethyl sulphoxide) was added at a final concentration of 10 μ M followed by addition of H_2O_2 at a final concentration of 1 mM (Wong et al. 2002). The final reaction mixture was made up to 1 ml with PBS and incubated at room temperature for 60 min. The emission intensity of dichlorofluorescein (de-esterified and oxidised metabolite of DCFH-DA) was recorded using a spectrofluorimeter (RF-5301PC, Shimadzu) with λ_{ex} =504 nm and λ_{em} =519 nm. The emission intensity of dichlorofluorescein in the absence of cells was recorded as a control.

Gene expression analysis

The level of expression of various genes in transformed *S. cerevisiae* $\Delta nth1$ cells subjected to salt shock (0.5 M NaCl) was measured by real-time PCR. Total RNA was isolated using the hot phenol method (Collart and Oliviero 2001) and treated with DNase I. RNA was reverse transcribed using oligo dT₁₈ primer and MMLV reverse transcriptase according to the manufacturer's protocol. Primers were designed using Primer3 (v. 0.4.0) software (Rozen and Skaletsky 2000). cDNA (1:10 dilution) was used with SYBR® Premix Ex Taq™ (Perfect Real Time) kit according to the manufacturer's protocol. Cyclings were performed on an Eppendorf Mastercycler® ep realplex thermal cycler using SYBR Green detection. Data were analysed using realplex 2.2 software (Eppendorf) to calculate cycle threshold (C_t) values. *Actin1* gene was used as the housekeeping gene (internal control). Relative fold change in gene expression was calculated by comparative C_t method ($2^{-\Delta\Delta C_t}$ method) (Schmittgen and Livak 2008). PCR reactions were carried out in triplicate each from three different cDNA samples.

Results and discussion

Effect of salt shock on solubility of 103Q-htt

In the absence of added salt in the induction medium, FLAG-103Q-htt-EGFP was expressed in *S. cerevisiae*

BY4742 $\Delta nth1$ cells in the aggregated form (Fig. 1a). With increase in the concentration of NaCl, the extent of pin-pointed aggregates decreased and the fluorescence intensity of FLAG-103Q-htt-EGFP increased, becoming more diffused. The maximum diffused fluorescence was seen at 0.5 M NaCl (Fig. 1a). With increase in the concentration of NaCl to 0.75 M and beyond, the fluorescence intensity declined, indicating damage to the protein synthetic machinery due to osmotic shock. Exposure of yeast cells to osmotic shock has been reported to increase the level of oxidative stress and hence cell death, most probably by apoptosis (Silva et al. 2005; Alves et al. 2011). Native PAGE analysis showed the presence of aggregates in the well even in the soluble fraction of cells grown in the absence of added salt (Fig. 1b). The fluorescence intensity of the band for monomeric 103Q-htt increased with increase in the concentration of NaCl, as expected from the fluorescence micrographs. Maximum solubilisation of 103Q-htt was observed at 0.5 M NaCl beyond which the solubility showed a diminishing trend. Immunoblotting with FLAG antibody confirmed that the band observed in native PAGE was for 103Q-htt. The intensity of the band on the blot for the monomeric protein increased with increasing concentration of NaCl, reaching maxima at 0.5 M NaCl and declining thereafter (Fig. 1c). Thus, salt shock was able to solubilise 103Q-htt, but this effect was concentration dependent.

Salt shock increases the production of intracellular osmolytes

Salt shock leads to the activation of the high osmolarity glycerol (HOG) pathway in yeast cells (Hohmann 2002, 2009). The major product of this pathway is glycerol, which has protein stabilization properties (Knubovets et al. 1999; Mishra et al. 2007). The amount of glycerol produced by the cells was found to increase with increase in the concentration of NaCl up to 0.75 M (Fig. 2a). Beyond this concentration, the level of glycerol decreased although it was still significantly higher than the non-treated cells. The increased production of glycerol may have resulted in solubilisation of 103Q-htt when cells were subjected to salt shock with 0.5 M NaCl, as seen above (Fig. 1). The amount of trehalose produced by the cell was found to increase with increase in the concentration of NaCl up to 1 M. Since these experiments were carried out with $\Delta nth1$ cells which cannot degrade trehalose, the level of trehalose builds up in these cells (Fig. 2b). At a higher level, the osmolyte competes with and inhibits the folding of newly translated polypeptide (Singer and Lindquist 1998), which was reflected in the failure of any functional protein being formed at higher concentration of NaCl (Fig. 1). The combination of elevated levels of glycerol and trehalose may account for the solubilisation of 103Q-htt when exposed to an optimum concentration of salt.

Fig. 1 Solubilisation of 103Q-htt subjected to salt shock. **a** Fluorescence micrographs of *Saccharomyces cerevisiae* $\Delta nth1$ cells subjected to salt shock. Images were visualised under 100 $\times$ objective and 10 $\times$ eyepiece. $Bar=10\ \mu m$. **b** Native PAGE analysis of soluble fractions of yeast cell lysates. The gel was scanned under fluorescence mode, excitation at 488 nm and emission at 526 nm. **c** Western blotting of soluble fractions of yeast cell lysates. Blots were probed with FLAG antibody as the primary antibody and HRP-conjugated mouse antibody as the secondary antibody

Measurement of oxidative stress

Expression of mutant huntingtin in the aggregated form leads to enhanced oxidative stress in the cell (Hands et al. 2011). The level of reactive oxygen species (ROS) was found to be the highest for $\Delta nth1$ cells grown in the absence of NaCl and was found to decrease with increasing concentration of the salt (Fig. 3a). The solubility of 103Q-htt had been found to increase in $\Delta nth1$ cells with increase in concentration of NaCl (Fig. 1b). Thus, the level of oxidative stress is inversely related to the solubility of the mutant huntingtin protein and confirms that the aggregation of protein leads to production of reactive oxygen species in the cell (Hands et al. 2011). The reduction in oxidative stress in $\Delta nth1$ cells correlates with the increase in the amount of trehalose inside the cell, leading to solubilisation of 103Q-htt. Since levels of both glycerol and trehalose showed an upward trend on exposure to a high concentration of NaCl, oxidative stress was also measured in cells unable to synthesize trehalose ($\Delta tps1$, deficient in trehalose-6-phosphatase synthase 1) to delineate the effects of

trehalose and glycerol on the solubility of mutant huntingtin under salt shock conditions. In *Candida albicans*, salt shock led to increased content of stored glycerol while the level of trehalose remained virtually unaffected (Sánchez-Fresneda et al. 2013). This was because the synthesis of trehalose is independent of Hog1 kinase, the major protein of the osmoregulatory HOG pathway in the organism (Sánchez-Fresneda et al. 2013). Although no report is available to the contrary in *S. cerevisiae*, the level of the disaccharide was influential in the salt shock response of this organism against stress due to expression of mutant huntingtin. In the presence of 0.25 M or 0.5 M NaCl, the levels of ROS were found to remain unaltered as compared to untreated cells in case of $\Delta tps1$ strain (Fig. 3a). In the absence of trehalose, the mutant protein did not undergo solubilisation (Fig. 3b), which correlated with a higher level of oxidative stress in $\Delta tps1$ cells. The results show that although glycerol has traditionally been considered to be the major osmolyte produced during salt shock in yeast, trehalose also exerts a beneficial effect in ameliorating oxidative stress under these conditions.

Fig. 2 Estimation of levels of intracellular glycerol (**a**) and trehalose (**b**) in *S. cerevisiae* $\Delta nth1$ cells subjected to salt shock. Cells were lysed using TCA, as described in “Methods”. The level of glycerol was estimated using free glycerol kit, as per the manufacturer’s instructions. The level of trehalose was analysed by HPLC. Lysed cells were dried in a hot air oven

until a constant dry cell weight was obtained. Values shown are mean $\pm$ standard error of mean (SEM) of three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.005$ against cells expressing 103Q-htt without salt shock

Fig. 3 **a** Measurement of oxidative stress using DCFH-DA in transformed *S. cerevisiae* parental (empty bars), $\Delta nth1$ (filled bars) and $\Delta tps1$ (grey bars) strains, following salt shock. Fluorescence intensity of the metabolised probe was measured following induction of expression of 103Q-htt for 7 h. Samples were excited at 504 nm and the emission intensity of the dye was measured at 519 nm. Values shown are mean $\pm$ SEM of three independent experiments. *, # $p < 0.01$ against parental and $\Delta nth1$ strains expressing 103Q-htt without salt shock, respectively, ## $p < 0.05$ against $\Delta nth1$ strain expressing 103Q-htt without salt shock. **b** Fluorescence micrograph of *S. cerevisiae* $\Delta tps1$ cell expressing 103Q-htt. Images were visualised under 100 $\times$ objective and 10 $\times$ eyepiece. Bar = 10 μ m

Gene expression analysis

Relative fold-change in levels of gene expression in cells expressing 103Q-htt in the presence of 0.5 M NaCl as compared with cells expressing 103Q-htt without salt shock was calculated (Table 1). The levels of the master regulator *Hsf1* and the larger heat shock proteins, *Hsp104* and *Hsc82*, remained unaltered, while upregulation of smaller chaperones, viz. *Ssa1*, *Ydj1* and *Sis1*, was seen in the absence of NaCl (Table 1). Higher expression of these chaperones is a cellular defence response to correct excessive protein misfolding (Merienne et al. 2003). Downregulation of these genes in osmotically stressed cells

Table 1 Change in gene expression levels in *S. cerevisiae* $\Delta nth1$ cells expressing 103Q-htt following salt shock (0.5 M NaCl) as compared to cells expressing 103Q-htt in the absence of salt shock

Target gene	Fold change	<i>p</i> value
<i>Hsp104</i>	-1.08 ± 0.07	0.2878
<i>Hsc82</i>	-1.07 ± 0.35	0.8574
<i>Ssa1</i>	-2.11 ± 0.08	0.0001
<i>Sis1</i>	-2.02 ± 0.18	0.0047
<i>Ydj1</i>	-2.44 ± 0.22	0.0029
<i>Hsf1</i>	-1.19 ± 0.11	0.3252
<i>Tps1</i>	1.40 ± 0.04	0.0030
<i>Tps2</i>	-1.09 ± 0.08	0.3373
<i>Hor2</i>	2.63 ± 0.07	0.0011
<i>Gpp1</i>	2.24 ± 0.09	0.0033
<i>Hog1</i>	-1.30 ± 0.02	0.0011
<i>Sch9</i>	1.25 ± 0.10	0.1071
<i>Ptp2</i>	1.38 ± 0.03	0.0007
<i>Ptc1</i>	1.44 ± 0.10	0.0405
<i>Ptp3</i>	2.84 ± 0.03	3.84×10^{-5}
<i>Sho1</i>	1.47 ± 0.04	0.0015

PCR reactions were carried out in triplicate from three different cDNA samples. Negative values indicate downregulation of genes in salt-treated cells. Values shown are mean $\pm$ SEM

may regulate their levels in such a manner that their role in protein folding is not compromised. The results also indicate the marginal role of the heat shock proteins in osmoprotection leading to stabilization of 103Q-htt in yeast cells. A similar trend was seen in case of yeast cells expressing 103Q-htt in the presence of 0.75 M NaCl as compared with cells expressing 103Q-htt without salt shock (Table 2). Significant downregulation of the components of the chaperone network of the cell was seen, demonstrating their insignificant role during osmoprotection.

On the other hand, genes for the synthesis of osmolytes were significantly upregulated in response to exposure to salt. *Tps1* and *Tps2* encode for trehalose-6-phosphate synthase enzymes 1/2 and are required for synthesis of trehalose. The level of *Tps1* was upregulated in $\Delta nth1$ cells treated with 0.5 M NaCl compared to cells not exposed to salt shock (Table 1), which correlated with increase in the amount of trehalose in $\Delta nth1$ cells treated with 0.5 M NaCl. This also shows that the build up of trehalose in these cells was not entirely due to their inability to degrade the disaccharide but also to the ability of the cellular machinery to increase the synthesis of the osmolyte. In the presence of 0.75 M NaCl, the levels of *Tps1* and *Tps2* were upregulated even further (Table 2), indicating that the synthesis of osmolytes, rather than upregulation of heat shock proteins (chaperones), is the predominant cellular defence response to salt stress in case of yeast cells. Most of the genes involved in glycerol biosynthesis, except *Hog1* and *Sch9*, were upregulated when yeast cells were exposed to 0.5 M NaCl (Table 1). *Gpp1* is a constitutively expressed glycerol-1-phosphatase which is involved in glycerol biosynthesis and is induced in response to osmotic stress (Pahlman et al. 2001). Upregulation of *Gpp1* in response to osmotic stress (Table 1) explains the increased production of glycerol under these conditions (Fig. 2a). *Hor2* (hyperosmolarity responsive) is involved in glycerol biosynthesis and is again

Table 2 Change in gene expression levels in *S. cerevisiae* $\Delta nth1$ cells expressing 103Q-htt following salt shock (0.75 M NaCl) as compared to cells expressing 103Q-htt in the absence of salt shock

Target gene	Fold change	<i>p</i> value
<i>Hsp104</i>	-1.84 ± 0.19	0.0106
<i>Hsc82</i>	-1.61 ± 0.04	0.0006
<i>Ssa1</i>	-2.31 ± 0.08	0.0032
<i>Sis1</i>	-1.77 ± 0.07	0.0037
<i>Ydj1</i>	-2.32 ± 0.10	0.0049
<i>Hsf1</i>	2.08 ± 0.15	0.0020
<i>Tps1</i>	2.11 ± 0.36	0.0362
<i>Tps2</i>	4.54 ± 0.99	0.0233
<i>Hor2</i>	3.75 ± 0.65	0.0131
<i>Sch9</i>	1.39 ± 0.19	0.1132
<i>Ptp2</i>	1.73 ± 0.32	0.0852
<i>Ptc1</i>	-13.06 ± 0.01	3.36×10^{-7}
<i>Ptp3</i>	1.08 ± 0.25	0.7717
<i>Sho1</i>	1.34 ± 0.32	0.3429

Negative values indicate downregulation of genes in salt-treated cells. Values shown are mean $\pm$ SEM

induced in response to hyperosmotic condition (Pahlman et al. 2001). Its expression has also been shown to increase in response to oxidative stress. This is consistent with the results that expression of 103Q-htt increases oxidative stress in the cells which may cause upregulation of *Hor2* (Pahlman et al. 2001). In the presence of 0.5 M NaCl, upregulation of *Hor2* was seen (Table 1). *Hor2* is linked with increase in glycerol synthesis. Upregulation of *Hor2* in cells treated with 0.5 M NaCl may facilitate increased production of glycerol, thus ameliorating toxicity due to the expression of 103Q-htt. Hog1 is a MAP kinase involved in upregulation of about 600 genes by phosphorylation of various osmoresponsive transcription factors (Westfall et al. 2004). Phosphorylation, rather than overexpression, determines its role in the osmoregulatory cascade (Westfall et al. 2004). This explains why its expression is not altered significantly upon exposure to salt shock (Table 1). When exposed to a higher concentration of NaCl (i.e. 0.75 M), the expression of the glycerol synthetic gene, *Hor2*, was further upregulated. This points to increased synthesis of glycerol by the cell in response to a stronger osmotic stress.

Various phosphatases, viz. Ptc1 (type 2C protein phosphatase) (Warmka et al. 2001), Ptp2 and Ptp3 (phosphotyrosine-specific protein phosphatases) (Winkler et al. 2002), exert a tight control over the activation of the HOG pathway. They are involved in dephosphorylation of Hog1 and, thus, inactivation of the osmoregulatory pathway. Ptp2 and Ptp3 are also involved in the cellular localization of Hog1 (Winkler et al. 2002). Constitutive phosphorylation of Hog1 is deleterious to the survival of the yeast cell (Wurgler-Murphy et al. 1997). Upregulation of the genes coding for these phosphatases (Table 1) signifies a concerted effort by the yeast cell to regulate the osmosensing pathway. The transmembrane protein Sho1 acts as an osmosensor and is phosphorylated by Hog1 (O'Rourke and Herskowitz 2004). It is involved in the HOG pathway, and its upregulation (Table 1) is a part of the response of the yeast cell to osmoregulation. The expression of the phosphatase *Ptc1* was, however, significantly downregulated when yeast cells were exposed to 0.75 M NaCl (Table 2). As mentioned above, downregulation of *Ptc1* inhibits dephosphorylation of Hog1 (Warmka et al. 2001), which exerts a deleterious effect on yeast cell survival (Wurgler-Murphy et al. 1997). This probably explains why no expression of 103Q-htt was seen when the concentration of the salt in the medium is increased beyond 0.5 M (Fig. 1a).

The results presented above show that mild osmotic stress induces overexpression of stress response elements which help to solubilise mutant huntingtin, thereby decreasing oxidative stress in the cell. Accumulation of trehalose and glycerol in response to osmotic stress, rather than activation of the cellular chaperone network, leads to solubilisation of mutant huntingtin. This indicates the potential therapeutic benefit of these molecules.

Conclusion

The study explores the possibility of upregulating the synthesis of pseudochaperones by cells leading to the formation of soluble protein or correcting the folding of misfolded proteins. Higher concentration of osmolytes was not beneficial for expression or solubilisation of proteins. This correlates with earlier observation that trehalose is an inhibitor of protein folding. Since at lower concentration, trehalose and glycerol showed a beneficial effect, the reported role of trehalose as an inhibitor of protein folding is likely to be concentration dependent. Interestingly, the disaccharide trehalose was established to have a major role in protecting proteins against misfolding and aggregation during exposure to salt stress. Most of the reports available in the literature focus only on glycerol as an osmoprotectant. Gene expression analysis did not show any change in the expression of Hsp104, indicating its non-participation during osmotic stress. However, downregulation of other molecular chaperones such as Ssa1, Sis1 and Ydj1 during stress conditions indicates that lower expression of such proteins results in solubility of the misfolded protein. The possible explanation of downregulation may be their role in 'seed' formation and propagation of the misfolded protein. Thus, two potent osmolytes, viz. glycerol and trehalose, stabilise the misfolded protein species in yeast cells exposed to osmotic shock.

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