MINIREVIEW

Stressed out! Effects of environmental stress on mRNA metabolism

Ursula Bond

Microbiology Department, Moyne Institute for Preventive Medicine, Trinity College, University of Dublin, Dublin, Ireland

Correspondence: Ursula Bond, Microbiology Department, Moyne Institute for Preventive Medicine, Trinity College, University of Dublin, Dublin 2, Ireland. Tel.: +353 1 608 2578; fax: +353 1 679 9294; e-mail: ubond@tcd.ie

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Abstract

Exposure of yeast cells to environmental stresses can disrupt essential intracellular processes, especially those carried out by large macromolecular complexes. The production of mature, translatable mRNAs is most sensitive to stress owing to the inhibition of messenger RNA splicing and alterations in the export of mRNA from the nucleus. Changes in the cytoplasmic pools of mRNAs also occur following exposure to stress conditions. Messenger RNAs accumulate in discrete cytoplasmic foci such as processing bodies and stress granules. These dynamic changes in RNA metabolism, following exposure to stress, ensure the preferential production and export of heat-shock mRNAs and the sequestering of general cellular mRNAs in the nucleus or in cytoplasmic foci, thus allowing for a redirection of the translational machinery to encode stress proteins, which aid in cellular recovery following stress. Stress proteins, such as Hsp70p and Hsp104p, have been shown to play a direct role in the repair of macromolecular complexes involved in RNA metabolism in yeast cells, thus ensuring that the cell returns to homeostasis.

Introduction

Like all living cells, the unicellular yeasts can maintain normal growth within a reasonably broad range of physiological conditions, such as temperature, pH and solute concentrations; however, exposure to conditions outside this range can be considered a stress to the cell. Exposure to environmental stress leads to the disruption of many intracellular processes, in particular those carried out by macromolecular complexes which are extremely sensitive to perturbation by stress conditions. Cells can respond to environmental stress by activating a specific set of genes, referred to as heat-shock genes, which encode a set of proteins, the heat-shock proteins (Hsps). These genes, in addition to being activated in response to elevated temperatures, are induced in response to other stresses, such as exposure to ethanol, respiratory poisons, protein synthesis inhibitors, heavy metals and being under nutrient-limiting conditions. Thus, Hsps are more accurately referred to as stress proteins (SPs). Hsps play important roles in the repair of intracellular damage caused by exposure to stress through their biological activities as molecular chaperones.

The structure and function of Hsps in the yeast *Saccharomyces cerevisiae* have been thoroughly characterized over the past 25 years. Because there are many excellent reviews describing the heat-shock genes in *S. cerevisiae*, the molecular mechanisms leading to their transcriptional activation by specific stress transcription factors, the stress-activated signal transduction pathways and the biological roles of Hsps, these topics will not be discussed herein. The reader is referred to a number of excellent reviews covering these topics (Craig *et al.*, 1993; Becker & Craig, 1994; Piper, 1998; Toone & Jones, 1998; Estruch, 2000; Gasch & Werner-Washburne, 2002; Hashikawa & Sakurai, 2004). Instead, this review examines the perturbations caused by environmental stress on one essential intracellular process, namely mRNA production. Firstly, the mechanisms that lead to disruption of the normal processing and export of mRNAs from the nucleus and alterations in the steady state pools of cytoplasmic RNAs are discussed. Secondly, the role played by Hsps in restoring homeostasis in RNA metabolism following exposure to stress is examined.

Heat-shock proteins in *Saccharomyces cerevisiae*

The major Hsps in *S. cerevisiae* are Hsp104, Hsp90, Hsp70, Hsp60, Hsp30 and Hsp26 (Parsell *et al.*, 1991; Craig *et al.*, 1993, 1995; Nathan *et al.*, 1997) and are commonly referred to as chaperone proteins. In stressed cells, Hsp70 proteins inhibit or prevent protein aggregation through the binding of hydrophobic surfaces of denatured proteins. Hsp70 can

also aid in the reactivation of heat denatured proteins with the help of its cochaperone protein Hsp40 (Ydj1) (Glover & Lindquist, 1998).

The Hsp104 protein plays a vital role in stress tolerance in *S. cerevisiae* (Parsell *et al.*, 1991; Sanchez *et al.*, 1992; Seppa *et al.*, 2004). Hsp104 has been shown to play an essential role in the solubilization of stress-induced protein aggregates upon the return of cells to normal growing conditions. A functional relationship between members of the Hsp70 family and Hsp104 in stress tolerance has been demonstrated whereby, following heat stress, a molecular complex consisting of Hsp70, Hsp40 and Hsp104 is formed (Glover & Lindquist, 1998). This complex works to prevent aggregation of proteins damaged by the stress and to disaggregate large protein aggregates upon return to normal growing temperatures. The small Hsp26 facilitates the disaggregation of aggregated proteins by Hsp104. Coassembly of Hsp26 with misfolded proteins appears to generate a 'reactivation-competent' aggregate and improves access of the Hsp104/Hsp70/Hsp40 disaggregation complex (Cashikar *et al.*, 2005).

The role of Hsps in thermotolerance

The role of Hsps in the ability of cells to survive extreme high temperatures was first demonstrated in experiments where cells were preincubated at temperatures at which Hsps are induced prior to exposure to the more extreme temperature. Preincubation of yeast cells at 37 °C prior to an exposure to a 55 °C temperature increases cell survival significantly (Piper, 1996, 1998). The induction of thermotolerance is directly correlated with the presence of Hsps in the cell and in particular with Hsp104. Because Hsps can be induced by other forms of stress, such as exposure to high concentrations of ethanol, it is not surprising that cross-protection by different types of stress can occur. Thus pre-exposure of cells to ethanol concentrations capable of inducing Hsps can induce protection against a subsequent exposure to heat stress and vice versa (Piper, 1995).

Macromolecular changes in thermally damaged cells

Because one of the major roles of Hsps in the cell is to protect against intracellular damage and to repair damage following exposure to thermal stress when cells are returned to normal physiological conditions, one might ask the following question: what cellular processes are particularly sensitive to thermal stress? Examination of a variety of eukaryotic cells using light, phase contrast and electron microscopy reveals many visible changes in the cells architecture following exposure to stress. Visible nuclear changes include chromatin rearrangements and nucleolar disruption as well as the appearance of granular bodies (Tartakoff, 1996; Sandqvist & Sisttonen, 2004; Tartakoff *et al.*, 2004). Changes in the cytoplasmic architecture are also evident in stressed cells (Anderson & Kedersha, 2002) (see section on Alterations in the cytoplasmic pools of mRNA in stressed cells). Similarly, biochemical analysis of cellular processes in stressed cells indicate that processes carried out by large macromolecular complexes appear to be particularly sensitive to thermal and other forms of stress (Bond, 1988; Welch *et al.*, 1991; Collier *et al.*, 1993; Liu *et al.*, 2005). Therefore, it is not surprising that the production of mature mRNAs will be stress-sensitive because many macromolecular complexes are required for the synthesis, processing, export and translation of mRNAs.

mRNA splicing in thermally stressed cells

One of the largest macromolecular complexes in the nucleus is the spliceosome (Nilsen, 2003). This complex structure, similar in size to ribosomes, is assembled in a step-wise manner on nascent pre-mRNA transcripts. Recent analysis, by mass spectrometry of spliceosomal complexes assembled onto pre-mRNAs, identified at least 300 unique proteins, making the spliceosome the most complex molecular machine so far characterized (Neubauer *et al.*, 1998; Jurica *et al.*, 2002; Zhou *et al.*, 2002; Hartmuth *et al.*, 2004). The most abundant components of the spliceosome are the small nuclear ribonucleoprotein complexes (snRNPs). Each snRNP in the spliceosome contains a unique small uridine-rich RNA, a conserved set of proteins referred to as Sm proteins and, in most cases, some snRNP-specific proteins. Three unique snRNPs are required for pre-mRNA splicing: U1, U2 and the tri-snRNP U4/U5/U6. The tri-snRNP preassembles from the di-snRNP U4/U6 and the U5 snRNP. Recently, a 45S penta-snRNP consisting of U1, U2, U4/U6/U5 snRNPs has been identified in yeast cells, suggesting that all snRNPs may preassemble into a single complex prior to formation of the spliceosome (Stevens *et al.*, 2002).

The first indication that the yeast splicing machinery was sensitive to thermal stress came from an observation that exposure of cells to a heat shock of 42 °C leads to an accumulation of a pre-mRNA species containing unspliced introns (Yost & Lindquist, 1991). Under these conditions, there is a complete inhibition of pre-mRNA splicing, as illustrated by the presence of only actin pre-mRNAs and the absence of mature mRNA (Fig. 1a). This inhibition of splicing was also observed in a variety of species from yeast to humans (Yost & Lindquist, 1986, 1991; Bond, 1988; Bond & James, 2000).

An analysis of the major components of the spliceosome in heat-stressed yeast cells revealed alterations in the snRNP components of the spliceosome, the most sensitive component being the U4/U5/U6 tri-snRNP (Bracken & Bond, 1999). In yeast, levels of the tri-snRNP decrease by approximately 90% following a 1 h exposure to 42 °C (Fig. 1b). The

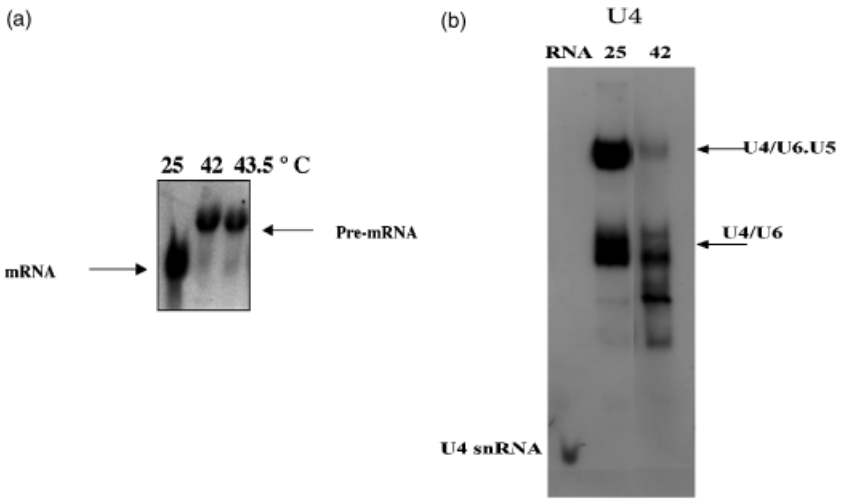

Fig. 1. Adapted from Bracken & Bond (1999). (a) mRNA splicing is inhibited in heat-stressed cells. Northern blot of RNA isolated from *Saccharomyces cerevisiae* cells heat-shocked at the indicated temperatures for 1 h and probed for actin mRNA. Mature actin mRNA is only observed in nonheat-treated cells. Actin pre-mRNA accumulates in heat-treated cells. (b) snRNP complexes are destabilized in heat-stressed cells. snRNP complexes from cells treated for 1 h at the indicated temperatures, separated in nondenaturing cells and probed for U4 snRNA. The major U4-containing complexes are indicated by arrows.

levels of the U4/U6 snRNP also decrease and distinct subparticles containing U4 and U6 snRNAs accumulate (Fig. 1b). Changes in the other snRNP components of the spliceosome are also observed: the levels of U1 snRNP decrease dramatically following heat shock (A. P. Bracken & U. Bond, unpublished), whereas there are less dramatic effects on the two remaining snRNP complexes, U5 and U2 (Bracken & Bond, 1999).

Recent experiments have revealed additional subtle changes in the spliceosome in heat-stressed mammalian cells (Shin *et al.*, 2004). The SR protein SRp38 is a general repressor of splicing. In its dephosphorylated state, SRp38 inhibits splicing in M-phase cells. SRp38 is also dephosphorylated following exposure of HeLa cells to heat-shock conditions that also lead to the disruption of snRNPs. The dephosphorylated SRp38 interacts with the 70 kDa U1 snRNP protein, leading to interference of the interaction of U1 with the 5′ splice site. Currently, it is unclear if similar alterations in SRp38 occur in yeast cells. Thus, it appears that gross perturbations and subtle biochemical alterations in spliceosome components contribute to the inhibition of splicing in heat-shocked cells.

The inhibition of splicing in yeast cells is in fact reversible; mRNA splicing returns to normal within 30 min of replacing cells at the normal growing temperature (Vogel *et al.*, 1995; Bracken & Bond, 1999). The return of splicing is accompanied by a restoration of all snRNP complexes. Both Hsp104 and Hsp 70 proteins are required for this restoration of snRNP complexes (Bracken & Bond, 1999). In HeLa cells, the dephosphorylated form of SRp38 also reverts to the phosphorylated form during recovery from splicing.

Splicing thermotolerance

Preincubation of cells under mild stress conditions at which Hsps are induced can confer protection of the cells to a subsequent exposure to a more lethal temperature. The implication of these findings is that Hsps function as insulator molecules to protect intracellular components from the damage of lethal stress. Because the macromolecular complexes, the snRNPs, have been identified as a target of thermal stress, it is likely that these complexes may be protected by Hsps in thermotolerant cells. In fact, cells made thermotolerant by a preincubation at 37 °C completely protect snRNP complexes from disruption when subsequently exposed to a more severe stress at 42 °C. Surprisingly, the protection of the complexes was independent of the presence of Hsp104 but requires at least one member of the Hsp 70 SSA gene family (Bracken & Bond, 1999).

What role might Hsp70 play in snRNP protection in thermotolerant cells? One clue to a possible function of Hsp70 in the splicing pathway came from an analysis of snRNP particles in cells exposed to a heat stress (42 °C) with or without a prior induction of Hsps at 37 °C. The migration of the U4/U6 snRNP on nondenaturing polyacrylamide gels is slightly retarded in thermotolerant cells and from cells incubated at 37 °C compared with the U4/U6 snRNP from cells heat-shocked at 42 °C alone (Fig. 2a). Similar gel retardation was observed in tri-snRNPs (Fig. 2a) and in U1 and U2 snRNPS (A. Bracken, unpublished) from thermotolerant cells, indicating that additional proteins and/or RNAs are associating with snRNPs under these conditions. Coimmunoprecipitation with an antibody directed against Prp4p, a constituent component of both U4/U6 and U4/U6/U5 snRNPs, reveals that Hsp70 does indeed associate with these snRNPs under heat-shock conditions and in thermotolerant cells (Fig. 2b) (Bracken & Bond, 1999). Interestingly, Hsp70 has been found in association with the penta-snRNP complex (Stevens *et al.*, 2002), suggesting that Hsps may play a role in the assembly of *de novo* snRNP particles.

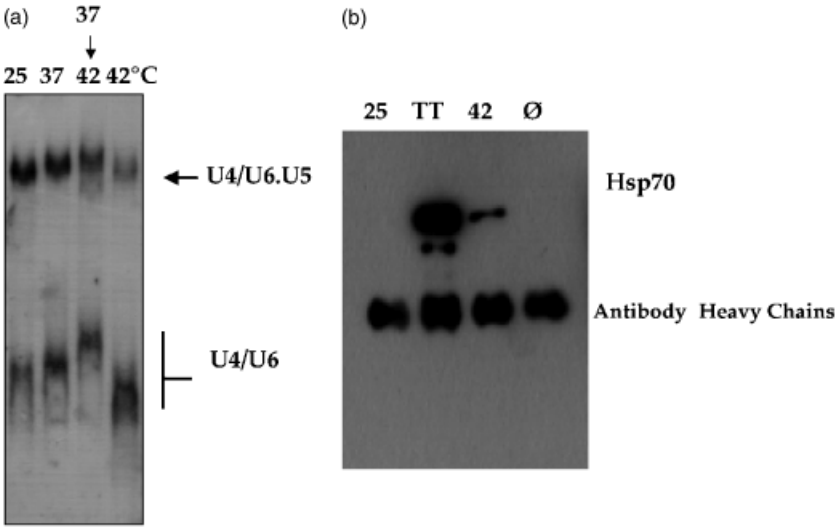

Fig. 2. Adapted from Bracken & Bond (1999). (a) The mobility of snRNP complexes are altered in heat-stressed cells. snRNP complexes from *Saccharomyces cerevisiae* cells, treated at the temperatures indicated, were separated on nondenaturing gels and probed for U4 snRNA. The major U4-containing complexes are indicated with an arrow. (b) Hsp70 associates with U4-containing snRNPs in heat-stressed cells. Cell extracts from *S. cerevisiae* cells heat-stressed as indicated were immunoprecipitated with anti-Prp4 antibodies. The immunoprecipitated samples were then Western blotted using an anti-Hsp70 antibody. U4-containing snRNPs from cells made thermotolerant (TT) by a pre-incubation at 37 °C, prior to an incubation at 42 °C, contain Hsp70, as do U4-containing snRNPs from cells directly heat-stressed at 42 °C. Untreated cells (25 °C) show no Hsp70 associated with U4-containing snRNPs. The lane indicated by ∅ contains no cell extract.

Nuclear export of mRNAs

Export of mRNA from the nucleus is a complex and elaborate process that is coupled to the transcription and subsequent processing of the primary transcript. Over the past several years, the picture emerging of mature mRNA biogenesis in the nucleus is one in which the various processes of transcription, 5′ end capping, mRNA splicing, 3′ end cleavage, polyadenylation and mRNA export, are seamlessly connected (Maniatis & Reed, 2002; Moore, 2005). Subsequent to transcription initiation, the emerging transcript is coated with a variety of RNA binding proteins and their associated protein complexes to form a messenger ribonucleoprotein (mRNP). The complex pool of mRNPs in the nucleus is often referred to as heterogeneous ribonucleoprotein particles (hnRNPs). These protein complexes serve to process the transcript (5′ capping, mRNA splicing, 3′ end cleavage and polyadenylation) and to chaperone the mRNP to the nuclear pore complex (NPC). The chaperone complexes such as THO (Tho2p, Hpr1p, Mft1p and Thp2p) are loaded onto the mRNP cotranscriptionally and interact with the export adaptor proteins, Yra1p and Sub2p, to form a transcription/export complex (TREX) (Strasser *et al.*, 2002). Other export chaperones, such as the Sus1p/Thp1/Sac3p complex, can also interact with the mRNP (Fischer *et al.*, 2002). The chaperone complexes direct the mRNP to the nuclear pore where they interact with the mRNP export receptor Mex67p-Mtr2p (Vinciguerra & Stutz, 2004).

Nuclear-cytoplasmic shuttling proteins, belonging to the SR protein family, including Npl3p, Nab2p, Hrp1p, Gbp2p and Hrb1p, also associate with mRNAs cotranscriptionally and accompany the mRNP through the NPC into the cytoplasm (Lee *et al.*, 1996; Windgassen & Krebber, 2003; Hacker & Krebber, 2004). Npl3p interacts with the export receptor Mex67p/Mtr2p and directs the mRNP to the NPC (Lee *et al.*, 1996). Other protein complexes such as the exon-junction complex (EJC) (Maquat, 2004), which is deposited onto the mRNP following intron removal, and the Rat8p, a DEAD-box (single letter amino acid code) helicase (Estruch & Cole, 2003), accompany the matured mRNP from the nucleus. The latter is proposed to aid in translocation of the mRNP through the NPC. Thus, a series of RNA–protein and protein–protein interactions direct the processed mRNA transcript from its point of synthesis to the nuclear pore, ensuring that the mRNA is exported from the nucleus as an mRNP particle.

Recent evidence indicates that cycles of phosphorylation and dephosphorylation of shuttling proteins are required for mRNA export from the nucleus. Npl3p is recruited to the emerging mRNAs in its phosphorylated form but only interacts with Mex67p in its unphosphorylated state (Gilbert *et al.*, 2001; Gilbert & Guthrie, 2004; Izaurralde, 2004). Upon exiting the nucleus, Npl3p is phosphorylated by the kinase Sky1p, which promotes dissociation from the mRNA and its reimportation to the nucleus. The phosphatase responsible for dephosphorylation of Npl3p in the nucleus, to complete the export/import cycle, appears to be Glc7p because temperature-sensitive mutants of *glc7* result in assembly of aberrant mRNP complexes (Gilbert & Guthrie, 2004). Likewise, in mammalian cells, the phosphorylation status of two other SR proteins, 9G8 and SF2, which play a role in mRNA export, determines their interaction with the nuclear export receptor [TAP/NXF1(Mex67p in yeast)]. Specifically, 9G8 and SF2 associate with TAP in the

dephosphorylated form and this dephosphorylation is thought to be a mechanism for the selective export of spliced mRNA vs. unspliced pre-mRNA (Huang *et al.*, 2004; Huang & Steitz, 2005).

The interesting possibility of the existence of multiple mRNA export pathways has been raised by Duncan *et al.* (2000), who have recently shown that Tom1p, a protein with ubiquitin ligase activity, can differentially affect the export of hnRNPs associated with mRNAs. Mutations in *tom1* block efficient export of Nab2p, leading to the accumulation of Nab2p-mRNA complexes at the NPC; however, mRNPs containing Np13p are unaffected by this mutation and are efficiently exported from the nucleus.

In the absence of correct assembly and export of mRNPs, for example in cells lacking components of the 3′ end processing machinery or in TREX mutants, the mRNPs are targeted for degradation by the nuclear exosome, a large complex of 3′ to 5′ exonucleases (Jensen *et al.*, 2003). The aberrant mRNPs appear to accumulate in discrete foci at the sites of mRNA transcription. Deletion of Rrp6p, a unique nuclear component of the exosome, releases the transcripts from the nuclear foci, indicating that the transcripts are made but retained and degraded by the nuclear exosome. This nuclear mRNP surveillance mechanism ensures that incorrectly processed or packaged mRNAs do not exit the nucleus.

Nuclear export of mRNAs in heat-stressed cells

RNA export is regulated following heat shock and other forms of stress in *S. cerevisiae* (Saavedra *et al.*, 1996). Following heat or ethanol stress, poly(A)$^+$ RNAs accumulates within yeast nuclei; however, mRNAs encoding Hsps are efficiently exported from the nucleus (Saavedra *et al.*, 1996; Cole & Saavedra, 1997; Rollenhagen *et al.*, 2004). The retention of mRNAs in the nucleus following exposure to stress is a consequence of alterations in the chaperone proteins associated with mRNA in the nucleus and the inhibition of receptor-mediated, energy-dependent nucleocytoplasmic transport of mRNAs via the NPC.

Analysis of the export of mRNAs encoding Hsps in mutant cells lacking specific proteins required for mRNA export revealed that most of proteins required for export under nonstress conditions are required for export in heat-stressed cells, with the exception of the hnRNP protein Npl3p, which is dispensable under stress conditions (Saavedra *et al.*, 1997). It should be noted, however, that the status of all currently known proteins required for mRNA export, including the TREX and Sus1p/Thp1/Sac3p complexes, has not been examined in stressed cells. Upon exposure to stress, Npl3p is rapidly but transiently exported from the nucleus (Krebber *et al.*, 1999; Nanduri & Tartakoff, 2001). This finding raises the possibility that the hnRNP composition of mRNAs encoding Hsps may differ from that of nonheat-shock mRNAs (Fig. 3). It is interesting to speculate that, like the subtle changes in the phosphorylation state of some spliceosome proteins, alterations in the phosphorylation of hnRNP proteins like Npl3p may contribute to the disruption of mRNA export in heat-stressed cells. In addition to being phosphorylated, Npl3p and other hnRNP proteins such as Hrp1p (see next paragraph), are posttranslationally methylated on arginine residues. The nuclear export of hnRNPs is influenced by the methylation state of Npl3p (Xu & Henry, 2004). The methylation status of these hnRNPs in stressed cells has currently not been examined.

Alterations in other general hnRNPs have previously been observed in stressed yeast cells. Upon exposure to osmotic stress, the hnRNP-like protein Hrp1p/Nab4p rapidly relocalizes from the nucleus to the cytoplasm (Henry *et al.*, 2003). Hrp1p is an essential RNA binding protein which functions both in the nucleus and the cytoplasm. In the nucleus, Hrp1p is required for correct mRNA 3′ end formation, whereas in the cytoplasm this protein plays a role in mRNA decay via the nonsense-mediated decay pathway (see section on Alterations in the cytoplasmic pools of mRNA stressed cells). Similar redistributions and alterations in hnRNPs have been observed in heat-stressed HeLa cells (Jolly & Morimoto, 1999), therefore it is likely that alterations in hnRNP composition may play an important role in the retention of mRNAs in the nucleus in heat-stressed yeast cells (Fig. 3).

The association of pre-mRNAs with RNA binding proteins can be detected *in vitro* by nondenaturing gel electrophoresis. Such mRNP complexes are referred to as H-complexes. Interestingly, Hsp70 has been identified as a component of H-complexes (Zhou *et al.*, 2002), perhaps indicating a role for Hsps in the reorganization of hnRNPs following stress.

Alterations in the nuclear pore complex in stressed cells

Alterations in components of the NPC have also been identified in heat-stressed yeast cells. The NPC in yeast is a 50 to 60 MDa macromolecular complex consisting of the structural components of the pores, the nucleoporins or Nups. Other proteins, such as soluble transport receptors, a GTPase, Gsp1, its GTP exchange factor, Prp20, and a GTPase activating protein, Rna1, associate with the NPC (Cole & Saavedra, 1997; Rout *et al.*, 2003; Rodriguez *et al.*, 2004). Biochemical analysis indicates that specific NPC subcomplexes exist, such as the Nup84p and Nup82p-containing subcomplexes (Suntharalingam & Wente, 2003). The Nup82p-containing complex is only found on the cytoplasmic face of the NPC and contains, in addition to Nup82p, the proteins Rat7p, Nsp1p, Gle1p and Rip1p. Both Rat7p

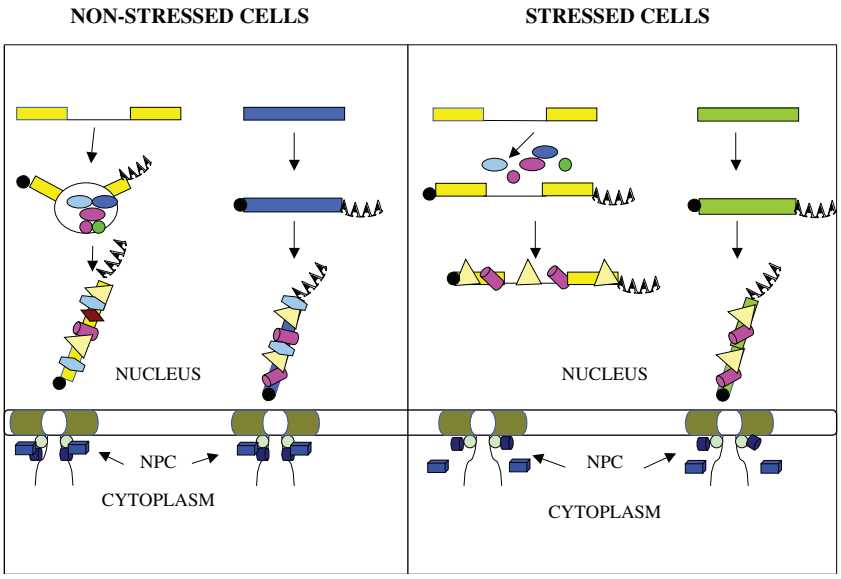

Fig. 3. Schematic representation of RNA processing in heat-stressed and normal cells. Splicing of intron-containing mRNAs (exons: yellow) is inhibited in heat-stressed cells owing to the disruption of the spliceosome (white circle and oval shapes; snRNPs). mRNAs encoding Hsps (green) do not contain introns and therefore are unaffected by the inhibition of mRNA splicing. The hnRNP composition of heat-stressed cells differs from that of non-stressed cells, so the pool of proteins associating with mRNAs encoding stress proteins (green) may differ from those associated with mRNAs in nonstressed cells (blue); for example, Npl3p (blue hexagon) is not required for mRNA export in heat-stressed cells. Alterations in the nuclear pore complex (NPC) also occur in heat-stressed cells (see section on Alterations in the nuclear pore complex in stressed cells).

and Gle1p have been shown to interact with the DEAD-box RNA helicase protein Rat8p, an essential export factor which shuttles between the nucleus and the cytoplasm (Rollenhagen *et al.*, 2004).

Perturbations in the Nup82 subcomplex are observed in heat-stressed cells (42 °C). Analysis of mRNA export in strains lacking components of the Nup82 subcomplex revealed that the export of mRNAs encoding Hsps was completely dependent on Rip1p following heat or ethanol stress (Fig. 4). The Nup82p complex associated protein, Rat8p, has also been shown to be essential for nuclear export in heat-stressed cells. Interestingly, a unique 6-amino acid motif, found only in Rat8p homologues but not in other DEAD-box proteins, has been shown to enhance the ability of Rat8p to function in heat-stressed cells (Rollenhagen *et al.*, 2004). Rat8p has been shown to play a crucial role in the remodelling and release of mRNPs into the cytoplasm (Snay-Hodge *et al.*, 1998). Under stress conditions, in *rip1* mutants cells, components of the Nup82 complex, such as Rat8p and Gle1p, appear to dissociate, thereby reducing the efficiency of the complex and leading to a reduction in mRNA export (Rollenhagen *et al.*, 2004). The following model has been proposed by Rollenhagen *et al.* (2004) to explain the role of the Nup82p complex in heat-stressed cells. The Nup82p complex is stabilized by the presence of Rip1p under stress conditions, thus allowing for export of mRNAs encoding Hsps. It is still unclear why mRNAs encoding Hsps are preferentially exported under heat stress conditions, but this may simply be a consequence of the increase in molar mass of these mRNAs in the nucleus owing to the rapid activation of heat-shock genes and the redirection of RNA polymerase complexes to heat-shock gene loci. The alterations in NPC-associated proteins in heat-stressed cells may significantly reduce the traffic of mRNA from the nucleus (Vainberg *et al.*, 2000). It still remains possible that Hsp mRNAs associate with specific export factors that facilitate their export in heat-stressed cells.

Nuclear export of mRNAs is also inhibited by exposure of cells to 10% ethanol. Interestingly, under these stress conditions, the essential NPC component Rat8p undergoes a rapid and reversible change in localization and accumulates in the nucleus, again indicating that the NPC is extremely susceptible to cellular stress (Takemura *et al.*, 2004). It is interesting to note that this accumulation of Rat8p in the nucleus is not observed following exposure of cells to heat stress. Thus, while both stresses result in a similar outcome, inhibition of mRNA export, there may be subtle differences in the manner in which this is achieved.

Protection of nuclear export in thermotolerant cells

As with the mRNA splicing machinery, nuclear export under thermal stress conditions can be restored by a preincubation of cells under conditions that induce Hsps (Rollenhagen *et al.*, 2004). As mentioned above, the protein Rip1p is required for nuclear export of mRNAs under heat-stress conditions. However, if cells are pretreated at 37 °C for 1 h prior to exposure to the heat-stress conditions of 42 °C, nuclear export of mRNAs can be partially preserved in cells lacking Rip1p (Fig. 4). This thermoprotection of nuclear export is directly correlated with the retention of Rat8p but surprisingly not Gle1p at the NPC. The protection of the integrity of the NPC in thermotolerant cells again points to a

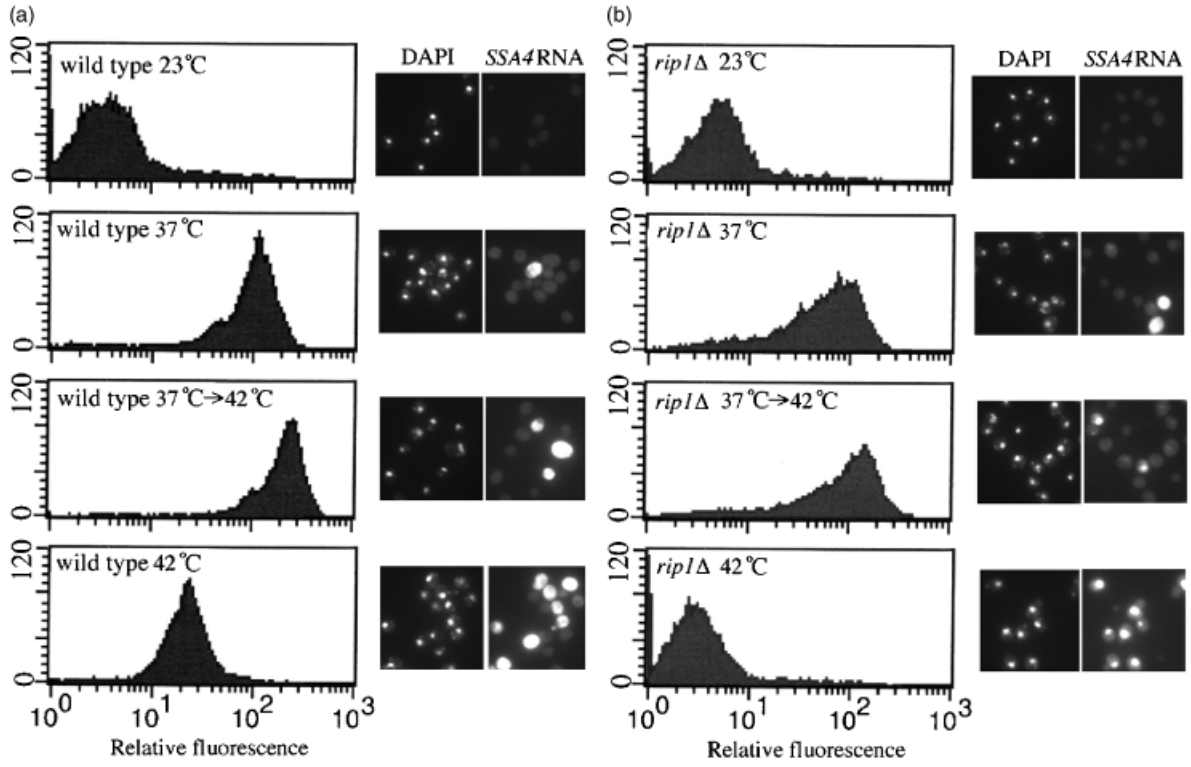

Fig. 4. Adapted from Rollenhagen *et al*. (2004). Export of mRNAs in heat-stressed cells requires Rip1. *In situ* hybridization was performed to examine the localization of SSA4 mRNA and Ssa4p protein production was monitored using a Ssa4p-GFP fusion protein. GFP-fusion protein was monitored by flow cytometry. (a) wild-type cells; (b) *rip1*Δcells. SSA4 mRNA is retained in the nucleus in *rip1*Δcells at 42 °C but is exported to the cytoplasm in wild-type cells (wild-type 42 °C). Little or no Ssa4p-GFP is produced in *rip1*Δheat-treated cells. (Compare relative fluorescence *rip1*Δ42 °C to wild-type 42 °C and wild-type 23 °C.)

role for Hsps in maintaining the structure of macromolecules under thermal stress conditions; however, a direct role for Hsps in this function as not yet been shown.

The fate of retained mRNAs in the nucleus in stressed cells

As mentioned above, in ethanol- and heat-stressed cells, the bulk of poly A+mRNAs accumulates in the nucleus, whereas mRNAs encoding Hsps are preferentially exported (Takemura *et al*., 2004). The bulk poly (A)+RNA retained in the nucleus localizes primarily to the nuclear rim (Rollenhagen *et al*., 2004). As shown in Fig 1(a), intron-containing mRNAs accumulate in severely heat-shocked cells (42 °C), whereas the pre-existing cytoplasmic mRNAs appear to be rapidly degraded. Taken together, these studies suggest that the mRNA retained in the nucleus following exposure to stress are stabilized and not degraded and raises the question as to whether the macromolecular complex, the nuclear exosome, which is responsible for degrading aberrantly or unprocessed mRNAs in the nucleus, is functional in stressed cells.

Alterations in the cytoplasmic pools of mRNA in stressed cells: P-bodies, stress granules and mRNA decay in stressed cells

A number of studies have uncovered a dramatic redistribution of cytoplasmic mRNAs in cells exposed to certain stress conditions. Cytoplasmic mRNAs are mostly associated with polyribosomes; however, under conditions where mRNA translation initiation is inhibited, the cytoplasmic pool of mRNA is redirected to granular cytoplasmic foci. The fate of the mRNA in these granular foci is subject to much investigation. Two possible fates for mRNAs in these foci exists: the mRNAs may be targeted for degradation or alternatively stored in a nontranslatable form until the stress is removed. Evidence supporting both possibilities has been obtained and it may be that the fate of the cytoplasmic pool may vary from species to species.

In yeast, the major pathway for mRNA degradation involves a shortening of the polyA+ tail, in a process termed deadenylation. These mRNAs now become substrates for the Dcp1p/Dcp2p decappping complex, which removes the 5′ cap structure, thereby exposing the transcript to degradation

by the 5′ to 3′ exonuclease, Xrn1p (for review, see Hilleren & Parker, 1999). A second mechanism of cytoplasmic mRNA degradation involves the 3′ to 5′ exonuclease complex, the cytoplasmic exosome. These two pathways represent the primary pathways for mRNA degradation in yeast cells; however, a number of specialized mRNA degradation pathways have also been described. These include the nonsense-mediated mRNA decay pathway, which degrades aberrant mRNAs containing stop codons (nonsense mutations) within the ORF (Alonso, 2005), and the initiation-mediated mRNA decay pathway, which comes into play under conditions when translation initiation is slowed down (Heikkinen *et al*., 2003).

Under conditions of protein synthesis inhibition in yeasts, cytoplasmic mRNAs are found in discrete cytoplasmic foci termed processing bodies (P-bodies) (Teixeira *et al*., 2005). Similar foci have also been identified in mammalian cells. P-bodies have been shown to contain the Dcp1p/Dcp2p decapping enzymes, the cytoplasmic DExD/H-box helicase, Dhh1p, which stimulates mRNA decapping, the Topoisomerase II-associated deadenylation-dependent mRNA-decapping factor, Pat1p, and the 5′ to 3′ exonuclease Xrn1p. The presence of these factors in P-bodies has led to the suggestion that P-bodies are sites of mRNA degradation. Teixeira *et al*. (2005) have recently shown that P-bodies increase in number in cells placed under osmotic stress. This stress leads to a concomitant decrease in protein synthesis. In contrast, this study showed that exposure of yeast cells to heat stress or oxidative stress leads to neither an increase in P-bodies nor a decrease in protein translation rates. However, it should be noted that the heat-stress conditions used to examine for the presence of P-bodies were quite mild (37 °C), a stress that does not lead to thermal inactivation of other macromolecular processes such as splicing and export from the nucleus. It would therefore be interesting to examine the degree of P-body formation under more severe stress conditions where thermal stress has led to the inactivation of other macromolecular processes.

A number of questions arise regarding the fate of the cytoplasmic pool of mRNAs in stressed cells. As shown in Fig. 1, clearly the steady state pool of mature mRNAs, as exemplified by actin mRNA, is extremely low in heat-stressed yeast cells. This must result from a decrease in transcription, processing and export from the nucleus and a concomitant increase in the degradation of pre-existing mRNAs. What is not clear is whether the rate of mRNA degradation is increased in stressed cells. The increase in P-bodies in the cytoplasm of cells stressed under some conditions suggests that this might be the case. Recent data from the Parker Laboratory (University of Arizona, AZ) indicate that mRNAs sequestered in P-bodies can return to the ribosomes following restoration of translation, suggesting that P-bodies, in addition to being sites of mRNA degradation, serve as storage sites for mRNAs during certain physiological conditions (Brengues *et al*., 2005).

Other cytoplasmic granular bodies have been identified in higher eukaryotes exposed to stress. In particular, discrete cytoplasmic phase-dense particles, referred to as stress granules, are observed in cells exposed to heat, oxidative, hyperosmolarity and UV stress (Anderson & Kedersha, 2002). Stress granules are repositories of mRNA pools, in particular mRNAs encoding constitutively expressed 'housekeeping' proteins, whereas mRNAs encoding Hsps are selectively excluded from these granules. Thus, stress granules store nontranslating mRNAs, enabling a redirecting of the translational apparatus to produce Hsps exclusively. Stress granules appear to be cytoplasmic foci distinct from P-bodies because both mRNP complexes contain a different set of proteins. Stress granules contain translation initiation factors, small ribosomal proteins and the RNA binding proteins TIA and TIA-R, whereas, P-bodies contain proteins associated with mRNA degradation. Furthermore, direct visualization of P-bodies and stress granules in mammalian cells indicates that these foci are physically distinct and spatially separate. Recent data from the laboratory of Charles Cole (Dartmouth University, NH), indicate that stress granules also exist in yeast cells. Cytoplasmic mRNAs can be visualized in cytoplasmic foci in certain genetic backgrounds, in particular in cells mutant in proteins that interact with the NPC (C. Cole, pers. commun.). These foci have been referred to as stress granules because they increase in number under stress conditions. It is currently not clear whether these foci are similar or different in composition to the stress granules identified in mammalian cells. However, one may predict, based on the different rates of mRNA decay in mammalian and yeast cells, that different mechanism may prevail in these two systems. Because yeast mRNAs appear to have much shorter half-lives compared with their mammalian counterparts, it may not be necessary to invoke stress granules to sequester nontranslating but stable mRNAs in yeast cells. The turnover rate of mRNAs in yeast may simply be increased following stress by increasing the number of P-bodies in the cytoplasm.

Fate of mRNAs encoding heat-shock proteins in stressed cells

What of the fate of mRNAs encoding Hsps in stressed cells? Recent experiments suggest that the steady-state levels of Hsp-encoding mRNAs are selectively regulated depending on the level of stress imposed on the cell, and that this fine-tuning is controlled through mRNA degradation (Heikkinen *et al*., 2003). The specialized pathway termed the initiation-mediated decay pathway is activated under conditions when translation initiation is inhibited. This pathway appears to play a pivotal role in regulating the levels of

Hsp-encoding mRNAs. Previous analysis of mRNA levels in yeast strains harbouring mutations in *PRT1*, a component of the eIF3 translation factor, revealed that mRNAs encoded by the Hsp70 genes *SSA1* and *SSA2* are degraded by the initiation-mediated decay pathway. Subsequent analysis indicated that this regulation extended to other mRNAs encoding Hsps such as mRNAs encoding Hsp90. mRNAs encoding non-Hsps appear not to be degraded by this pathway. Selective degradation of Hsp-encoding mRNAs occurs, depending on the status of mRNA translation in the cells. When mRNA translation is partially inhibited to approximately 30% of the wild type rate, by growing specific mutant alleles of *prt1* under restrictive conditions, Hsp-encoding mRNAs are selectively degraded. However, under conditions where mRNA translation is virtually abolished (< 5% of wild-type rates), the Hsp-encoding mRNAs are stabilized. This suggests that the level of Hsps produced during stress conditions can be finely adjusted by altering the specific rate of decay of Hsp-encoding mRNAs. Under severe stress conditions, such as exposure to temperatures above 41 °C, Hsp-encoding mRNAs are stabilized and are available for protein translation upon removal of the stress condition and on return to normal growing conditions. Under less severe stress conditions, such as exposure to mild heat-shock temperatures of 37 °C, the steady-state levels of Hsp-encoding mRNAs are regulated through initiation-mediated decay to allow a low but consistent level of Hsp production during the stress. These findings are consistent with what is known about Hsp production and function in mild and severely heat-stressed cells: in severely stressed cells, Hsp synthesis and repair of macromolecular structures are limited to the period following return to normal growth conditions, whereas in mildly stressed cells Hsps are synthesized during the stress and macromolecular complexes are protected from disruption by the presence of Hsps.

Concluding remarks

From an initial analysis of the effects of stress on macromolecular structures required for the production of mature mRNA, it would appear that stress simply leads to a disruption in mRNA production owing to the general instability of macromolecules under stress conditions. However, a more focussed analysis has revealed that stress, rather than simply imposing destructive forces, does in fact lead to subtle changes in macromolecular structures that result in a redirection of the cells energy to allow the synthesis of Hsps. Even the gross apparent destructive changes such as those observed in the spliceosome are ultimately advantageous to the cell. Thus, changes in mRNA synthesis, processing, export and degradation all contribute to the production of Hsps under stress conditions which themselves function in restoring homeostasis to RNA metabolism upon recovery from the stress.

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