Induction of major heat-shock proteins of *Saccharomyces cerevisiae*, including plasma membrane Hsp30, by ethanol levels above a critical threshold

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Many of the changes induced in yeast by sublethal yet stressful amounts of ethanol are the same as those resulting from sublethal heat stress. They include an inhibition of fermentation, increased induction of petites and stimulation of plasma membrane ATPase activity. Ethanol, at concentrations (4–10%, v/v) that affect growth and fermentation rates, is also a potent inducer of heat-shock proteins including those members of the Hsp70 protein family induced by heat shock. This induction occurs above a threshold level of about 4% ethanol, although different heat-shock proteins and heat-shock gene promoters are optimally induced at different higher ethanol levels. In addition ethanol (6–8%) causes the same two major changes to integral plasma-membrane protein composition that result from a sublethal heat stress, reduction in levels of the plasma membrane ATPase protein and acquisition of the plasma membrane heat-shock protein Hsp30.

Keywords: *Saccharomyces cerevisiae*, stress response, ethanol, heat-shock proteins, plasma-membrane proteins

INTRODUCTION

The increasing ethanol level during batch fermentation of *Saccharomyces cerevisiae* on high sugar substrates acts to cause reductions in the specific growth rate, the fermentation rate and cell viability (see van Uden, 1984a, for literature). These effects become much more severe with increasing temperature, the optimum and maximum temperatures of growth becoming appreciably depressed in the presence of ethanol levels above about 3% (v/v), while thermal death on exposure to extreme temperatures is enhanced (van Uden, 1984a, b). These adverse influences of ethanol on the growth, viability and fermentation of *S. cerevisiae* have been largely explained in terms of its effects on membrane-associated processes (Leao & van Uden, 1984, 1985; Jones, 1989; Rose, 1993). Not only are these toxic effects influenced by the lipid composition of yeast cell membranes but growth in the presence of ethanol causes changes to membrane lipids which are possibly part of an adaptive response (reviewed in Rose, 1993, and Lloyd *et al.*, 1993). The concentration

of ethanol that totally inhibits *S. cerevisiae* growth is also remarkably medium- and strain-dependent (Rose, 1993).

Several of the changes induced in yeast by exposure to stressful ethanol levels are identical to those caused by a heat stress. It is not surprising therefore that ethanol acts in a synergistic way to increase the damage caused by heat (van Uden, 1984a, b; Aguilera & Benitez, 1989). Both heat and ethanol cause membrane disordering and protein denaturation (Casey & Ingledew, 1986; Piper, 1993), besides an inhibition of glycolysis and an enhanced induction of petites (Neves & Francois, 1992; Leao & van Uden, 1982; van Uden, 1984a, b). Ethanol also inhibits glucose transport (Leao & van Uden, 1982, 1985). Both stresses increase the permeability of the plasma membrane, resulting in an increased passive proton influx that acts to dissipate the electrochemical potential gradient that the cell maintains at this membrane. This is reflected in the decline in intracellular pH that is observed both with ethanol addition (Leao & van Uden, 1984) and with heat shock (Coote *et al.*, 1991; Coote, 1993). Ethanol and heat stress will adversely affect those vital functions for which a plasma membrane electrochemical gradient is essential, processes such as nutrient uptake, the main-

Abbreviations: Hsps, heat-shock proteins; 1D, one-dimensional.

Table 1. Yeast strains used in this study

Strain	Relevant genotype
MW109*	<i>a leu2 trp1 ura3 his3 lys2</i>
MW115*	<i>α leu2 trp1 ura3 his3 lys2 ssa3::TRP1 ssa4::URA3</i>
MW116*	<i>α leu2 trp1 ura3 his3 lys2 ssa1::HIS3 ssa2::LEU2 ssa3::TRP1</i>
MW121*	<i>α leu2 trp1 ura3 his3 lys2 ssa1::HIS3 ssa3::TRP1 ssa4::URA3</i>
MW122*	<i>α leu2 trp1 ura3 his3 lys2 ssa4::URA3</i>
MW123*	<i>α leu2 trp1 ura3 his3 lys2 ssa1::HIS3 ssa2::LEU2</i>
MW127*	<i>α leu2 trp1 ura3 his3 lys2 ssa3::TRP1</i>
BJ2168†	<i>a leu2-3,112 trp1 ura3-52 prb1-1122 pep4-3 prc407 gal2</i>
PMY501‡	<i>α leu2-3,112 his4 ura3::pDP501</i>
PMY3(pUP41a)§	<i>α ura3 pUP41a</i>

* These strains are described in Werner-Washburne *et al.* (1987).

† Used for an earlier study on the effects of heat shock on plasma membrane protein composition (Panaretou & Piper, 1992).

‡ A derivative of strain PMY1.1 transformed to uracil prototrophy with the integrative *URA3*-containing vector pDP501, a plasmid bearing a *lacZ* gene under the control of the *UBI4* promoter sequences (−767 to −7 relative to the *UBI4* ATG) (Kirk, 1993).

§ A uracil prototroph transformant of PMY3 carrying the centromeric vector pUP41a which carries *URA3* plus the *HSP12* promoter (−609 to +14 relative to the ATG) fused in-frame to the *lacZ* coding region (U. M. Praekelt & P. Meacock, unpublished).

tenance of potassium balance, and the regulation of intracellular pH (Serrano, 1991).

Heat shock and ethanol induce protective responses in yeast that show a high degree of similarity. Both sublethal heat shock (Coote *et al.*, 1991) and addition of ethanol (Cartwright *et al.*, 1987; Rosa & Sa-Correia, 1991) dramatically stimulate the activity of plasma-membrane ATPase, the enzyme largely responsible for maintaining the proton gradient across the plasma membrane (Serrano, 1991). These increases in ATPase activity cause an enhanced *catalysed* proton efflux that counteracts the dissipation of proton motive force resulting from the stress-induced increase in membrane permeability. It is not surprising, therefore, that mutations that alter plasma-membrane ATPase activity also influence cellular tolerances of both ethanol and heat (Panaretou & Piper, 1990; Coote, 1993). Heat and ethanol also induce heat-shock proteins (Hsps) in both yeast (Plesset *et al.*, 1982) and other organisms (Nover, 1991). These evolutionarily conserved proteins were originally characterized on the basis of their strong induction by heat shock, but they are also induced by a number of chemical agents including ethanol. The trigger for their induction is probably the cytoplasmic accumulation of aberrant or partially denatured protein (Ananthan *et al.*, 1985). Thus ethanol may be an inducer of Hsps through its destabilization of the hydrophobic interactions of protein structure which leads to the association of Hsp chaperones with these destabilized proteins and the concomitant activation of heat-shock genes (Mager & Moradas-Ferreira, 1993).

While it is clear that major Hsps are ethanol-inducible in yeast (Plesset *et al.*, 1982), it is not obvious from published data if this induction is comparable with the induction of these same proteins by heat shock, or if perhaps only a

subset of yeast Hsps are strongly ethanol-inducible. This report collates experiments from a number of laboratories that clarify the ethanol induction of the major *S. cerevisiae* Hsps and the range of ethanol concentrations most effective in this induction. It also reports the first study of the major effects of ethanol on plasma-membrane protein composition.

METHODS

Yeast strains, culture and conditions of ethanol and heat stress. The strains used for this study are listed in Table 1. Cultures were grown with vigorous shaking at 25 °C in standard defined (SD) pH 6.8 medium, with 2% (w/v) glucose as initial carbon source, plus necessary amino acid supplements (Sherman *et al.*, 1983). They were used for ethanol stress experiments when in mid-exponential phase (5×10^6 cells ml^{−1}), by which stage the medium ethanol levels produced by fermentation were less than 0.3%. To heat shock cells, cultures were shifted to 39 °C for 40 min prior to harvesting, this being the optimum temperature for induction of heat-shock element sequences (Kirk & Piper, 1991a, and references cited therein). To ethanol stress cultures the stated amount of additional ethanol was added to cultures and incubation continued at 25 °C.

Pulse-labelling of proteins. Pulse-labelling of proteins with [³H]leucine was as described by Cheng & Piper (1994). For efficient uptake of labelled amino acids in the presence of 4–10% ethanol it is essential that the pH of the medium is greater than 6.0 as ethanol strongly inhibits uptake of labelled amino acids in cultures of lower pH (unpublished observations).

Protein analysis. Samples of total cell protein were isolated as described by Piper *et al.* (1988). Protein determinations were performed using the Bio-Rad Protein Assay Kit and bovine serum albumin as standard. Protein samples were prepared for gel electrophoresis by incubation at 37 °C in SDS-protein gel sample buffer, then analysed on one-dimensional 12.5% or 7%

Fig. 1. Autoradiographs of two 12.5% SDS-gels showing the proteins labelled in *S. cerevisiae* BJ2168 cells during heat and ethanol stress. Cells were pulse-labelled for 40 min at 25 °C, in the absence of ethanol (lanes 1, 5); at 25 °C with 2% (lane 7), 4% (lane 8), 6% (lane 2), 8% (lanes 3, 9) or 10% ethanol (lane 4); or immediately after heat shock to 40 °C (lane 6). Molecular mass marker positions are indicated on the left, and major Hsps on the right of each autoradiograph.

(w/v) SDS-polyacrylamide gels. Gel staining with Coomassie brilliant blue, detection of labelled proteins by fluorography and blotting of proteins onto nitrocellulose membrane was according to standard procedures (Piper *et al.*, 1988; Panaretou & Piper, 1992).

Plasma membrane purification and protein analysis.

Purification of plasma membranes, assessment of the purity of membrane preparations and analysis of plasma-membrane proteins on 12.5% gels was as described by Panaretou & Piper (1992). Blotted plasma-membrane proteins were analysed for Hsp30 as described by Récnacq & Boucherie (1993).

Northern blotting of RNA. Total cell RNA was prepared, Northern blotted onto Hybond N membranes, and probed to *HSP26*, *HSP12* and *ACT1* gene probes as described by Piper *et al.* (1988).

lacZ expression measurements. β -Galactosidase activity, measured as in Kirk & Piper (1991a,b), is presented as fold-induction relative to the low constant level of β -galactosidase in uninduced cultures at 25 °C.

RESULTS

A threshold concentration of approximately 4% ethanol is required for appreciable Hsp induction, subsets of these proteins showing optimal induction at different higher ethanol levels

The addition of 4–10% ethanol to exponentially growing yeast cultures causes strong induction of a few specific proteins. From 1D gel analyses Plesset *et al.* (1982) found that many of these were the same as proteins induced by

heat shock (see also Fig. 1). However there has been no systematic study showing if each major yeast Hsp is inducible to the same extent by ethanol as by heat shock, or indeed whether all Hsps are optimally induced at the same ethanol concentration. Most heat-shock genes of *S. cerevisiae* have now been characterized (Mager & Moradas-Ferreira, 1993; Parsell & Lindquist, 1993) and the availability of several strains with targeted inactivation of these genes facilitates comparison of the ethanol and heat inducibility of most individual Hsps.

We have observed that there is a threshold concentration for appreciable Hsp induction by adding ethanol to vegetative yeast cultures. Appreciable induction started to become detectable by protein pulse-labelling with the addition of approximately 4% ethanol (Fig. 1, lane 8), negligible induction being observed at lower ethanol levels. We also found that different Hsps were optimally induced at different levels of ethanol above 4%; also, while some Hsps appear to be induced as strongly by ethanol as by heat shock, others were not. The induction of Hsp104, Hsp70 and Hsp26 progressively increased as the ethanol addition increased from 4 to 10% (Fig. 1, lanes 1–4). At 8% ethanol (Fig. 1, lanes 3 and 9), Hsp104 and Hsp70 are induced almost as strongly as with heat shock, but this is not the case for Hsp82 and Hsp26 (Fig. 1, lanes 6 and 9).

One of the proteins induced most strongly by ethanol is Hsp104, an important determinant of ethanol tolerance (Sanchez *et al.*, 1992) and a homologue of the *clpA* and

clpB proteases of *E. coli* (Parsell *et al.*, 1991). The form of the Hsp90 protein which is most strongly heat-inducible (Hsp82) was observed to be induced by ethanol to a lesser degree than by heat shock (Fig. 1). *S. cerevisiae* has two functionally equivalent Hsp90 genes (*HSC82* and *HSP82*; Borkovich *et al.*, 1989). *HSC82* displays a high constitutive expression that increases only slightly after heat shock, whereas *HSP82* displays much lower basal expression yet is strongly activated by heat shock. *HSC82* therefore contributes most of the Hsp90 present during normal vegetative growth, whereas *HSP82* expression is mainly responsible for the increase in Hsp90 protein with heat shock (Borkovich *et al.*, 1989). The product of *HSP82* (M_r 81419), distinguishable from that of *HSC82* (M_r 80885) by its slightly slower mobility on 1D gels of stress-induced proteins, is the Hsp90 form induced by ethanol (Fig. 1, lanes 6 and 9; also data not shown).

Induction of small heat shock proteins by ethanol

A recent study has confirmed that several small Hsps are induced by ethanol (Gropper & Rensing, 1993) although loss of at least one of these proteins (Hsp26) does not affect ethanol tolerance (Petko & Lindquist, 1986; Susek & Lindquist, 1989). Northern analysis of the mRNA of strain BJ2168 probed for *HSP26* gene transcripts indicated that Hsp26 mRNA, undetectable with addition of 2% ethanol, is barely detectable after addition of 4% ethanol (Fig. 2b). However it is much more strongly induced with further increases in ethanol concentration, eventually (at 10% ethanol) becoming as strongly induced by ethanol as by heat shock (Fig. 2b). Protein pulse-labelling in this strain also shows the increasing Hsp26 induction with increase in ethanol (Fig. 1, lanes 1–4).

We also analysed the ethanol inducibility of the heat-inducible *HSP12* (Praekelt & Meacock, 1990) and *UBI4* (Finley *et al.*, 1987) promoters using strains containing the appropriate promoter–*lacZ* fusions (Tables 1, 2). An *HSP12* promoter–*lacZ* fusion carried on an episomal centromeric vector [PMY3(pUP41a); Table 1] was optimally induced by 4–6% ethanol. Its induction rapidly decreased to uninduced levels at higher ethanol concentrations (Table 2). This maximal 12-fold induction of the *HSP12* promoter–*lacZ* fusion by ethanol compares to an optimal 61-fold induction of the same sequence by heat shock (Table 2) and 20-fold induction by methanol (U. M. Praekelt and P. Meacock, unpublished results). Northern blotting (Fig. 2c) provided data broadly in agreement with these *lacZ* expression measurements, the Hsp12 mRNA of PMY3(pUP41a) displaying optimal induction at 6% ethanol and declining at higher ethanol levels. The 10% ethanol addition that caused a strong Hsp26 mRNA induction in BJ2168 resulted in no Hsp12 mRNA induction in PMY3(pUP41a) (Fig. 2).

The integrated *UBI4* promoter–*lacZ* fusion of strain PMY501 (Table 1) showed optimal 5.2-fold induction at 4–6% ethanol (Table 2). This induction was more sustained at higher ethanol levels as compared to that of the *HSP12* promoter–*lacZ* fusion (Table 2) and was similar to the maximal four- to fivefold inducibility of the

Fig. 2. (a, b) Northern blots of *S. cerevisiae* BJ2168 RNA samples probed for actin mRNA (a) or Hsp26 mRNA (b). (c) Northern blot of PMY3(pUP41a) RNA probed for Hsp12 mRNA. The RNA samples were from unstressed cells growing at 25 °C (lanes 1), cells treated for 40 min at 25 °C with 2% (lanes 2), 4% (lanes 3), 6% (lanes 4), 8% (lanes 5) or 10% ethanol (lanes 6), and cells heat-shocked to 37 °C (lanes 7) or 40 °C (lanes 8) for 40 min. Ten micrograms of RNA was loaded into each gel lane.

same *UBI4* promoter–*lacZ* fusion by methanol or heat shock (Kirk, 1993).

The Hsp70 genes induced by ethanol are the same as those induced with heat shock

Ethanol causes strong induction of proteins that co-migrate on 1D gels with the heat-induced Hsp70 (Fig. 1). The Hsp70 family proteins of *S. cerevisiae* are encoded by a multigene family comprising no less than 8–9 genes, three of which (*SSA1*, *SSA3* and *SSA4*) are induced by heat shock (Craig & Jacobsen, 1984; Werner-Washburne *et al.*, 1987, 1989). To establish whether the Hsp70 genes induced by ethanol are the same as those induced by heat shock, proteins induced by ethanol in strains with one, two or three disruptions in Hsp70-family proteins (Table 1) were investigated by *in vivo* pulse-labelling at 25 °C in the absence and presence of 6% ethanol. Labelled proteins with an apparent M_r greater than 40000 were then analysed on 1D 7% SDS gels (Fig. 3). This gel system was

Table 2. Ethanol inducibility of *HSP12* or *UBI4* promoter-*lacZ* fusions

Treatment	Induction over 25 °C basal level (-fold)	
	PMY3(pUP41a) (<i>HSP12-lacZ</i>)	PMY501 (<i>UBI4-lacZ</i>)
Ethanol:		
4%, 40 min	12	5.2
6%, 40 min	12	5.2
8%, 40 min	3	3.5
10%, 40 min	0	2.5
Heat shock:		
25–39 °C, 40 min	61	4.5

Fig. 3. Analysis on a 7% gel of the proteins with M_r greater than 40000 labelled in a 40 min pulse-labelling of the Hsp70 gene disruption strains listed in Table 1, in the absence (–) and presence (+) of 6% ethanol.

employed since the products of genes *SSA1–4* are not clearly resolved from each other even on two-dimensional gels (Werner-Washburne *et al.*, 1989).

Fig. 3 shows that the Hsp70 induction by ethanol is due almost entirely to one or more of genes *SSA1*, *SSA3* and *SSA4*, since it was completely abolished in a strain (MW121; Table 1) disrupted in all three of these genes. There was however substantial Hsp70 induction in strain

MW115 that lacks the *SSA3* and *SSA4* products, so *SSA1* is clearly strongly ethanol-inducible. There was also strong Hsp70 induction in a strain that lacked both the *SSA1* and *SSA2* products (MW123; Fig. 3), and in a strain which lacked the *SSA1*, *SSA2* and *SSA3* products (MW116). Clearly therefore *SSA4* is also ethanol-inducible. Fig. 3 therefore shows the strong Hsp70 induction by ethanol to be due to the induction of *SSA1*, *SSA4* and possibly *SSA3*. These are the three *S. cerevisiae* Hsp70 genes which are also strongly activated by heat shock, although one of them (*SSA1*) shows significant expression in unstressed cells (Craig & Jacobsen, 1984; Werner-Washburne *et al.*, 1987, 1989). Fig. 3 also shows a high synthesis of Hsp104, Hsp82 and Hsp70 in the absence of ethanol in those strains that lack functional *SSA1* and *SSA2* genes (strains MW123 and MW116 labelled in the absence of ethanol). This constitutive expression of heat-shock genes in *ssa1 ssa2* strains has been previously noted by Craig & Jacobsen (1984) and Werner-Washburne *et al.* (1987).

Ethanol causes the two major changes to plasma-membrane protein composition also seen with heat shock, a rapid decline in plasma-membrane ATPase levels and acquisition of Hsp30

Damage to the plasma membrane is thought to be a major cause of ethanol toxicity (see Introduction). While the effects of ethanol on the lipids of this membrane have been the subject of considerable study (see Introduction), there has been no investigation of whether ethanol influences plasma-membrane protein composition. At least one activity of this membrane (plasma membrane ATPase) influences both heat and ethanol tolerance (Panaretou & Piper, 1990). Both heat stress (Coote *et al.*, 1991; Coote, 1993) and ethanol (Cartwright *et al.*, 1987; Rosa & Sa-Correia, 1991) cause stimulation of this ATPase activity, the resulting enhancement of catalysed proton efflux from the cell helping to counteract the depolarization of the plasma membrane resulting from the stress-induced increases in membrane permeability (see Introduction).

With heat shock a single integral membrane Hsp (Hsp30) is targeted to the plasma membrane (Panaretou & Piper, 1992; Récnacq & Boucherie, 1993). Also, levels of the proton-pumping plasma-membrane ATPase decline (Panaretou & Piper, 1992). Since Hsp30 is a membrane protein its total cellular abundance is considerably less than that of major soluble Hsps such as Hsp104, Hsp82, Hsp70 or Hsp26. It is therefore not prominent on gels of total cell heat- or ethanol-induced proteins (Fig. 1), its analysis requiring the preparation of membrane fractions.

Brief treatment of BJ2168 cells with 6 or 8% ethanol resulted in a reduction in levels of the most abundant plasma-membrane protein, plasma-membrane ATPase (Fig. 4a) and induction of Hsp30 (Fig. 4b). These are the same two changes to plasma-membrane protein composition previously shown to result from sublethal heat shock of this same strain (Panaretou & Piper, 1992). Hsp30 was induced to similar levels by heat shock and treatment with 6% ethanol, although its induction was

Fig. 4. Major changes to the protein composition of the plasma membranes of BJ2168 cells following a 40 min heat shock at 40 °C or a 40 min ethanol treatment at 25 °C. (a) Coomassie-brilliant-blue-stained gel of plasma-membrane protein samples; (b) an identical gel fractionation of the same samples, blotted and probed for Hsp30 using an anti-Hsp30 antiserum. Lanes: 1, exponentially growing cells; 2, cells heat shocked for 40 min at 40 °C; 3, cells treated with 6% ethanol; 4, cells treated with 8% ethanol. Thirty micrograms of total plasma membrane protein was loaded in each gel lane.

appreciably less with 8% ethanol (Fig. 4b). The ATPase loss with heat shock appears to reflect protein turnover in the absence of *de novo* synthesis since it still occurs in cells subjected to these stresses in the presence of cycloheximide (K. Talreja & P. W. Piper, unpublished results). This progressive and relatively rapid loss of ATPase from the plasma membrane with heat or ethanol stress almost certainly influences the capacity of the cells for sustained maintenance of homeostasis during the stress. Using the appropriate mutants future studies can determine whether this reduction in plasma-membrane ATPase, or the acquisition of Hsp30, contribute significantly to ethanol or heat tolerance.

DISCUSSION

The results described here provide further evidence of the strong similarity between the cellular responses to ethanol and to heat shock. The forms of Hsp90 and Hsp70 induced by ethanol are shown to be the same as those induced by heat (Figs 1 and 3). Also these two stresses are shown to have similar effects on the levels of two major integral plasma-membrane proteins, the ATPase and Hsp30 (Fig. 4). While it has long been known that cells alter the lipid composition of their membranes in response to ethanol, this is the first demonstration that they also rapidly alter the protein composition of the plasma membrane.

There is a critical threshold (4%) for induction of heat-shock proteins by ethanol (Figs 1 and 2). Maximal induction is seen at higher ethanol levels which are different for individual heat-shock proteins and heat-shock promoters (Figs 1, 2 and 4 and Table 2). Increasing ethanol levels from 4 to 10% increases the synthesis of

Hsp104, Hsp70 and Hsp26 in strain BJ2168 (Figs 1, 2b). However Hsp30 induction declines in this strain as ethanol levels increase above 6% (Fig. 4), as does Hsp12 in strain PMY3(pUP41a) (Fig. 2c, Table 2). This suggests that the level of induction of different heat shock genes is regulated by factor(s) that sense different sublethal ethanol concentrations.

The effects of alkanols on cell inactivation relate to their lipid solubility, indicating that they act in a non-specific way to disorder the membrane lipids (Leao & van Uden, 1982; Van Uden, 1984a). Methanol is the least toxic, inactivating cells only above 18–20%, even though the heat-shock element sequences of yeast promoters are induced maximally at about 10% methanol (Kirk & Piper, 1991b). Ethanol is cytotoxic at lower levels (van Uden, 1984a), inducing most Hsps maximally at 4–10% (Fig. 1). Further increases in the length of the aliphatic chain and lipophilic character of the alcohol increase its cytotoxicity, presumably due to the increased membrane solubility (Jones, 1989). They also increase the potential for induction of heat-shock protein (Gropper & Rensing, 1993), presumably due to an increased capacity for the alcohol to destabilize the hydrophobic interactions that maintain protein conformations.

In vegetative cells tolerances of potentially lethal heat and ethanol are highest in cells with low cAMP-dependent protein kinase activity, increasing as cultures progress from exponential to early-stationary phase (Casey & Ingledew, 1986; Iida, 1988; Piper, 1993). Increased ethanol tolerance is inducible, frequently occurring under the same conditions as those leading to increased thermo-tolerance (Plesset *et al.*, 1982; Watson, 1990; Sanchez *et al.*, 1992; Costa *et al.*, 1993). With the close parallels between the physiological states leading to ethanol

tolerance and thermotolerance in yeast it is not surprising that determinants of thermotolerance are also important influences on ethanol tolerance. Thus loss of Hsp104 has been shown to reduce both ethanol tolerance, tolerance to heat, and heat induced tolerance to ethanol (Parsell *et al.*, 1991; DeVirgilio *et al.*, 1991; Sanchez *et al.*, 1992). Besides Hsps, several physiological changes resulting from heat or ethanol exposure are also important in determining cellular tolerances to these stresses (Mager & Moradas-Ferreira, 1993; Piper, 1993; Parsell & Lindquist, 1993). The rapid loss of plasma membrane ATPase protein with ethanol stress (Fig. 4a) contrasts with the ethanol stimulation of the activity of this enzyme (see Introduction). Both these influences on the ATPase will affect its role in maintenance of homeostasis (Serrano, 1991) and should therefore have important effects on tolerance to prolonged exposure to ethanol. Other factors that influence homeostasis will also influence tolerance to ethanol and heat. Thus cells survive heat stress best at pH 6–7 (Cheng & Piper, 1994), when their extracellular pH approximates to intracellular pH. The same probably applies to exposure to alkanols and other agents that increase membrane permeability.

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