Osmostress-induced changes in yeast gene expression

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Summary

When Saccharomyces cerevisiae cells are exposed to high concentrations of NaCl, they show reduced viability, methionine uptake and protein biosynthesis. Cells can acquire tolerance against a severe salt shock (up to 1.4 M NaCl) by a previous treatment with 0.7 M NaCl, but not by a previous heat shock. Two-dimensional analysis of [3H]-leucine-labelled proteins from salt-shocked cells (0.7 M NaCl) revealed the elevated rate of synthesis of nine proteins, among which were the heat-shock proteins hsp12 and hsp26. Northern analysis using gene-specific probes confirmed the identity of the latter proteins and, in addition, demonstrated the induction of glycerol-3-phosphate dehydrogenase gene expression. The synthesis of the same set of proteins is induced or enhanced upon exposure of cells to 0.8 M sucrose, although not as dramatically as in an iso-osmolar NaCl concentration (0.7 M).

Introduction

Exposure of living cells to solutions with high osmolarity causes a rapid efflux of water. In order to adapt to the reduced water activity of their environment and the consequent decrease in turgor pressure, cells respond by synthesizing and/or accumulating osmoprotective compounds (Brown, 1978; Brown and Edgley, 1980; Le Rudulier et al., 1984; Higgins et al., 1987; Booth and Higgins, 1990; Csonka, 1989). Upon osmotic upshift of exponentially growing Saccharomyces cerevisiae cells, glycerol seems to be the major compatible solute (Brown, 1978; Brown and Edgley, 1980; Trollimo et al., 1988; Blomberg and Adler, 1989). In addition trehalose — presumed to be involved in stabilizing membranes (Crowe et al., 1984; Wiemken, 1990) — accumulates, particularly when yeast cells growing on non-fermentable carbon sources approach stationary phase (Mackenzie et al., 1988; André et al., 1991).

Apart from adaptation processes, a stress response is likely to be evoked in salt-treated cells to protect them against the unfavourable conditions. Our studies aim to identify yeast genes involved in osmo-adaptation and osmostress-protection.

To date, studies concerning osmostress-induced changes in gene expression are almost entirely limited to bacteria (Clark and Parker, 1984; Higgins et al., 1987; Eshoo, 1988; Booth and Higgins, 1990; Hecker et al., 1988; Bhagwat and Apte, 1989; Csonka, 1989; Jung et al., 1990; Hagemann et al., 1990; Jenkins et al., 1990; Mizuno and Mizushima, 1990; Lamarck et al., 1991; Repoila and Gutierrez, 1991). With respect to yeast, previous investigations were mainly focused on physiological processes (Reed et al., 1987; André et al., 1991; Yagi, 1988; Blomberg et al., 1988; Meikle et al., 1988; Trollimo et al., 1988; Blomberg and Adler, 1989; Uschio and Nakata, 1989; Larsson et al., 1990; van Zyl et al., 1990). We started to analyse the osmostress response of the yeast S. cerevisiae. In this paper we present data regarding cell viability, nutrient uptake, protein biosynthesis and gene expression in salt- and sucrose-treated yeast cells.

Results

Cell viability and osmotolerance upon osmotic upshift

Addition of NaCl to exponentially growing yeast cells, up to a final concentration of 0.7 or 1.4 M, leads to a reduced viability relative to control (unshocked) cells (see Fig. 1). Cells are able to resume growth in liquid medium after 1 h at 0.7 M NaCl, but not at 1.4 M NaCl (Fig. 1A). When yeast cells are first grown in liquid medium containing 0.7 M NaCl, a larger fraction of the cell population (relative to control cells) can subsequently grow onYPD (yeast extract/bacto-peptone/glucose) agar plates containing 1.4 M NaCl (Fig. 1B). This phenomenon was observed although the starting number of viable cells in liquid medium with 0.7 M NaCl was lower than that of the controls (Fig. 1A).

The increased number of control cells that were able to grow on agar plates with 1.4 M NaCl 1–2 h after dilution (Fig. 1B) is obviously due to an increase in the number of viable cells in liquid medium prior to plating. As a matter of fact, the osmotolerant fraction (i.e. number of colonies on
YPD + 1.4 M NaCl (number of colonies on YPD) of the control cell population remained around 50% during the entire incubation period (Fig. 1C). However, when cells are preconditioned in liquid medium with salt, either at 0.7 M or 1.4 M NaCl, more than 80% of the viable cell population is able to grow on 1.4 M NaCl YPD agar plates. This result not only demonstrates an induction of osmotolerance, which reaches a maximum by 30 min after the onset of the osmotic shock, but also indicates that this acquired osmoterance is maintained, at least up to 3 h.

**Uptake and incorporation of [35S]-methionine upon osmotic upshift**

Recent evidence indicates that full induction of tolerance to high salt depends on de novo protein synthesis (Blomberg and Adler, 1989). This prompted us to perform a two-dimensional analysis of protein extracts of yeast subjected to an osmotic challenge. Pulse-labelling using [3H]-leucine during 30 min, however, led to remarkably low incorporation levels, especially when cells were exposed to the highest NaCl concentration (1.4 M). Therefore we determined whether amino acid uptake and/or protein synthesis are affected by the addition of high salt. This was carried out by pulse-labelling yeast cells for 10 min with [35S]-methionine at different time points (Fig. 2). Exposure of yeast to increasing salt concentrations appeared to lead to a significant reduction in the rate of methionine uptake and incorporation relative to controls (Fig. 2A and B). Uptake at NaCl concentrations equal to and higher than 0.7 M is dramatically diminished during the first 60 min (Fig. 2A). For an NaCl concentration of 0.7 M, uptake recovers up to 90% of control values at 2 h, whereas at 1.4 M NaCl no recovery takes place at all. It should be noted, however, that if cell viability is taken into account, the uptake recovery in yeast exposed to 0.7 M NaCl might reach values higher than 100%, as fewer viable cells (Fig. 1A) attain values comparable with the respective controls (Fig. 2A).

Similar results were obtained with respect to methionine incorporation (Fig. 2B). One could argue that the lower rates of incorporation are solely due to a decrease in uptake rates. This may be true for yeast cells exposed to 0.1 M NaCl, since the incorporation/uptake ratios of salt-treated and control cells are similar under these conditions (Fig. 2, C and D). At 0.7 M NaCl, however, the proportion of methionine present in the cells that is incorporated into protein is about half of the control values during the first 10–30 min, and reaches control values after 1 h. On the other hand, at 1.4 M NaCl only a partial recovery can be observed after 3 h (Fig. 2D). Therefore, it is likely that some step of protein synthesis is directly affected.

When yeast cells are preconditioned at 0.7 M NaCl for 30 min or 2 h, and subsequently shifted to 1.4 M NaCl, methionine uptake recovers up to 40% of control values (unshocked cells) after 2 h (Fig. 3A). The severe inhibitory effect on uptake exerted by 1.4 M NaCl cannot be overcome by a previous heat shock from 28→40°C for 30 min. The same holds for methionine incorporation (Fig. 3B). Incorporation/uptake ratios show that a somewhat higher proportion of methionine taken up by salt-treated cells is incorporated into protein (Fig. 3, C and D). In the first 60 min an opposite effect is evident with heat-preconditioned cells that are subsequently exposed to 1.4 M NaCl, but after 2–3 h the values are similar to those of control (Fig. 3C) and salt-preconditioned cells (Fig. 3D). Comparison of non-preconditioned with preconditioned cells shows that at that time a previous heat shock is as beneficial for the rate of protein synthesis as a pretreatment with salt (Fig. 3D).

**Two-dimensional analysis of protein extracts from salt- and sugar-stressed yeast**

Because of the temporary inhibition of amino acid uptake
and protein synthesis during the first 60 min of exposure to high salt (Fig. 2), a prolonged labelling period for two-dimensional analysis was needed. Therefore yeast cells were exposed to different stresses and labelled with $[^35]S$-methionine for 90 min. Total protein extracts were prepared and subsequently the same amount of counts was loaded on each gel. As can be judged from Figs 4A and 4B, these analyses showed the rate of synthesis of several proteins to be decreased upon exposing yeast cells to 0.7 M NaCl, whereas the synthesis of another set of proteins is induced. The latter proteins range in molecular mass between 73 and 14 kDa: 73, 63, 45, 35, 33, two 29, 26 and 14 kDa. Notably, the 26 and 14 kDa proteins comigrate with the previously identified heat-shock proteins hsp26 (Petko and Linquist, 1986) and hsp12 (Preakelt and Meacock, 1990; see Fig. 4C). Apparently, osmotic shock does not induce the synthesis of other heat-shock proteins like hsp70 and hsp60. The 45 kDa protein found to be synthesized at high rates might represent glycerol-3-phosphate dehydrogenase a synthetic oligomer was designed on the basis of published sequences (see the Experimental procedures; Pidoux et al., 1990; Sleep et al., 1991). The results presented in Fig. 5 confirm the very high level of induction of HSP26- and in particular HSP12-gene expression upon exposure of cells to 0.7 M NaCl. High levels of both mRNAs coding for these hsp's can be observed from 30 to

Fig. 2. $[^35]$S-methionine uptake and protein synthesis rates upon osmotic shock. Exponentially growing cells exposed to the indicated salt concentrations were pulse-labelled for 10 min according to the Experimental procedures. For every salt shift a corresponding dilution control was performed. A, methionine uptake of salt-treated cells relative to control values; B, incorporation of salt-treated cells relative to control values; C and D, incorporation/uptake ratios of control (non-shocked) and salt-treated cells, respectively. Data represent average values of three independent experiments.

Therefore the osmotic upshift experiment was repeated using 0.8 M sucrose. This sucrose concentration corresponds to an osmolality similar to 0.7 M NaCl as measured using a cryoscopic osmometer. As can be concluded from Fig. 4D, the same set of proteins is synthesized at a higher rate upon sucrose addition. However, this induction is apparently not as dramatic as that seen in salt-stressed cells, especially with regard to hsp12 and hsp26.

Analysis of mRNA from salt-treated yeast cells

In a first analysis of the yeast genes whose transcription rates might be enhanced upon osmotic upshift, we used gene-specific probes in a Northern analysis. As probes for the HSP12 and HSP26 mRNAs, recombinant DNA fragments were used (see the Experimental procedures); for the mRNA encoding glycerol-3-phosphate dehydrogenase a synthetic oligomer was designed on the basis of published sequences (see the Experimental procedures; Pidoux et al., 1990; Sleep et al., 1991). The results presented in Fig. 5 confirm the very high level of induction of HSP26- and in particular HSP12-gene expression upon exposure of cells to 0.7 M NaCl. High levels of both mRNAs coding for these hsp's can be observed from 30 to
60 min after the onset of salt shock (Fig. 5, lanes 9 and 10). After 2 h, the concentration of both transcripts declines, particularly HSP26 mRNA, which drops to barely detectable levels (lanes 11 and 12). After 3 h, again an increase in HSP12 mRNA occurs (lane 12). Also, in the control sample weak signals of HSP12 and HSP26 transcripts are visible (lane 6) in agreement with data reported on the expression pattern of these genes during the growth cycle (Kurtz et al., 1986; Praekelt and Meacock, 1990), which may reflect cells approaching stationary phase. Thus it is likely that the second rise in HSP12 mRNA concentration is the result of a combined effect of salt stress and early stationary phase signals, such as depletion of nutrients.

Upon salt exposure the cellular concentration of the mRNA for glycerol-3-phosphate dehydrogenase (GPD) is also increased, displaying an expression pattern similar to the hsp messengers during the first half hour (lanes 8 and 9). However, unlike HSP12 and HSP26 mRNAs, 1 h after the onset of salt stress the GPD transcript (1.5 kb) is hardly visible (lanes 10–12). We did not detect GPD mRNA in control cells (lanes 1–6). André et al. (1991) reported that the osmotically regulated cytoplasmic GPD (NAD*-linked) isoform, involved in glycerol biosynthesis, is only present at low levels in yeast grown on glucose as carbon source. The same holds for the FAD-linked mitochondrial isoform (GUT2; Sprague and Cronan, 1977). Apparently, therefore, under non-stressful conditions and in the presence of glucose GPD mRNA is kept at very low levels because of catabolite repression. Upon osmotic stress this repression is transiently overcome.

Upon salt shock the concentration of actin mRNA also rises but stays at a fairly constant level throughout the salt treatment (lanes 8–12). However, this apparent induction does not follow the pattern of HSP12, HSP26 or GPD gene expression and therefore possible RNA loading artefacts can be excluded.

Discussion

Upon a sudden decrease of the water activity of the medium, both liquid and solid, S. cerevisiae cells display a reduced viability (Fig. 1). Similar results have been reported for other yeast strains (MacKenzie et al., 1988; Trolmo et al., 1988; Blomberg and Adler, 1989). These authors, however, described a more drastic negative
effect on the survival potential of yeast cells upon moderate or severe osmotic shock. These differences essentially may be due to the use of different strains, as we could also observe a 10000-fold drop in viability on YPD plates containing 1.4 M NaCl with other yeast strains (data not shown).

The strain used by us showed a 20% reduction in cell viability upon a shift to 0.7 M NaCl and a restoration of growth 1–2 h after the beginning of the osmotic challenge (Fig. 1). Interestingly, this parallels the recovery of methionine uptake and protein biosynthesis after a transient decrease during the first 60 min of incubation at high salt (Figs 2 and 3). In contrast, the induction of osmotolerance in salt-preconditioned cells seems to be an earlier event, since it reaches its maximum value by 30 min of induction at an intermediate salt concentration (Fig. 1C; see also Blomberg and Adler, 1989). These results suggest that survival and ability to grow in media with lowered water activity are two different phenomena, the latter only being possible after recovery of nutrient uptake and protein synthesis (Roth et al., 1985b; this paper).

Fig. 4. Two-dimensional analysis of protein extracts of yeast cells exposed to 0.7 M NaCl (B), heat shock (28–42°C; C), and 0.8 M sucrose (D) as well as of control cells (A). Cells were labelled with L-[3H]-leucine for 90 min. 3 x 10^6 c.p.m. were loaded on each gel. In C, heat-shock proteins are indicated by arrows and their molecular masses.

As stated in the Introduction, upon osmotic shock yeast cells synthesize and accumulate glycerol, a so-called compatible solute or osmolyte which may stabilize soluble enzymes and restore the cell turgor pressure required for growth (Brown, 1978; Brown and Edgley, 1980). It is also likely, therefore, that some of the proteins showing increased synthesis rates upon osmotic shock (Fig. 4) are enzymes involved in glycerol metabolism. This is also suggested on the basis of the experiments of Trollmo et al. (1988), Blomberg and Adler (1989) and André et al. (1991), who showed that some enzyme activities for glycerol production are induced upon a shift to lower water activity. Indeed, we were able to show an increase in a glycerol-3-phosphate dehydrogenase (GPD) transcript (Fig. 5). The temporary nature of this salt-induced increase may indicate that glycerol-3-phosphate dehydrogenase is a very stable enzyme and that increases in its specific activity can be accomplished by short-lived accumulations of the GPD mRNA.

The failure of methionine uptake to recover in heat-preconditioned cells (Fig. 3A) is in agreement with the finding that a previous heat shock does not induce osmotolerance in S. cerevisiae (Trollmo et al., 1988). In contrast, protein synthesis seems to recover in heat-preconditioned cells after 2 h at 1.4 M NaCl, being similar to the rate (corrected for a lower uptake) in salt-preconditioned cells (Fig. 3D). These data suggest that heat-shock proteins may play a part in protecting the protein biosynthetic machinery. On the other hand, a previous heat shock is unable to induce key enzymes for glycerol synthesis...

Fig. 5. Glycerol-3-phosphate dehydrogenase, HSP26 and HSP12 gene expression upon exposure of yeast cells to 0.7 M NaCl. RNA was extracted from cells grown on SYE (lanes 1–6) or exposed to SYE containing 0.7 M NaCl (lanes 7–12). Aliquots were taken at 0 (lanes 1 and 7), 10 (lanes 2 and 8), 30 (lanes 3 and 9), 60 (lanes 4 and 10), 120 (lanes 5 and 11) and 180 min (lanes 6 and 12) after salt addition. For Northern blot analysis, 32P-labelled HSP26 DNA, HSP12 cDNA, glycerol-3-phosphate dehydrogenase (GPD) oligomer (see the Experimental procedures), and actin DNA were used as probes, the latter as a control for RNA loading.
(Trollmo et al., 1988) and fails to restore nutrient uptake (Fig. 3A). This might be the reason why only salt-preconditioning yeast cells acquire osmotolerance.

A notable difference was observed between a treatment of yeast cells with salt as compared with sucrose (see Fig. 4, B and D). Whereas exposure of cells to high salt concentrations strongly, but transiently, induces the synthesis of the heat-shock proteins hsp12 and hsp26, these proteins are apparently not synthesized at such high rates in 0.8 M sucrose-treated cells. This finding seems to indicate that high salt is more stressful than sucrose. On the other hand, preliminary results obtained by exposure of yeast to 0.7 M KCl and 0.85 M maltose (not shown) resulted in an increase in HSP12 and HSP26 mRNA levels similar to that for cells treated with an iso-osmolar NaCl concentration (0.7 M). Further studies are needed to unravel the molecular basis for the observed differences. The results obtained to date indicate that, upon osmotic challenge, yeast cells seem to induce, in addition to osmoprotective events, a stress response which has similarities with that observed in heat-shocked cells. This may be due to the denaturation of proteins upon cell dehydration or to injury of the active transport systems across the membrane (Roth et al.; 1985a;b; Meury and Kohiyama, 1991). Induction of heat-shock protein synthesis by exposure to salt is in agreement with previous reports showing that a salt treatment can render yeast cells thermostable (Trollmo et al., 1988).

In order to be able to establish a direct connection between the induction of hsp12 and hsp26 upon salt stress and osmotolerance induction, we also carried out viability studies with hsp12, hsp26 and hsp12 hsp26 disruption mutants (Praekelt and Meacock, 1990; data not shown). We did not find any significant difference in cell viability, however (either in solid or liquid medium), between mutant and wild-type cells, which confirms previous results obtained by Petko and Lindquist (1986) for hsp26 strains. Remarkably, the presence (or absence) of the marker URA3 used to select for hsp12 mutants (Praekelt and Meacock, 1990) seemed to be the most important factor for tolerance to high salt, suggesting that uracil uptake is greatly impaired at NaCl concentrations around 1.4 M.

Experimental procedures

Yeast strain, media and growth conditions

S. cerevisiae YT6-2-1 L rDNA: pMIRY2 (CIP2, a, his4-519, can1; Lopes, 1990) was maintained on YPD agar plates (1% yeast extract; 2% bacto-peptone; 2% glucose; 1.5% agar). Liquid cell cultures were grown in minimal medium SYE (0.67% yeast nitrogen base without amino acids, Difco; 1% succinic acid; 0.6% NaOH; 1.0% glucose; 0.025% yeast extract; adapted from Hartwell, 1970) supplemented with 0.004% L-histidine, and incubation was performed in a rotary shaker at 30°C. Osmotic shock was carried out by adding 0.5x SYE containing 5 M NaCl (nSYE) or 2 M sucrose (sSYE) up to the indicated final salt/sugar concentrations to samples of an exponentially growing cell culture at 0.5 OD600nm according to the equation y = Vi fiCi/CF x 1, in which y is the volume of nSYE/sSYE added, Vi is the initial sample volume, Ci is the initial salt/sugar concentration, and CF is the final salt/sugar concentration. As controls, identical volumes of 0.5x SYE without stress solute were added to another set of samples of the same starting culture.

Cell viability tests

In order to test the cell viability upon osmotic shock, samples were taken at indicated time points after the addition of 0.5x SYE with or without salt. Each sample was diluted in duplicate to allow a countable number of cells to be spread on YPD agar plates either with or without 1.4 M NaCl. The plates were incubated at 30°C in sealed plastic bags and the number of colonies counted after 2 or 6 d, depending upon the absence or presence of salt in the plate. The osmotic tolerant fraction of the cell population was determined by dividing the number of colonies able to grow on YPD + 1.4 M NaCl plates by the number of colonies present on YPD plates without salt.

Uptake and incorporation of [35S]-methionine upon osmotic shock

To estimate uptake and protein synthesis rates upon osmotic shock, 2-ml samples of cell suspensions exposed to the indicated salt concentrations were pulse-labelled with 0.45 MBq l-[35S]-methionine (>37 TBq mmol-1, Amersham) for 10 min at 30°C. Samples were immediately put in an ethanol-ice bath pre-cooled at -20°C and divided into two 1-ml aliquots. These aliquots were either (i) directly filtered through Whatman GF-C filters (uptake), which retain whole yeast cells, and washed with fresh minimal medium SYE; (ii) or treated with glass beads, followed by the addition of 0.5 ml NaOH/H2O2 (4 NaOH pellets + 1.33 ml H2O2 to a final volume of 10 ml completed by bi-distilled water), incubation at 37°C for 10 min, precipitation using 2 ml of cold 25% trichloroacetic acid (TCA) for 15 min, filtration through Whatman GF-C filters, and washing with cold 10% trichloroacetic acid (protein synthesis).

Two-dimensional gel electrophoresis of total protein extracts

In order to determine which proteins are induced by lowering the water activity of the medium, 22 MBq of l-[4,5-3H]-leucine (5.18 TBq mmol-1; Amersham) was added to 1-ml samples of control and salt-treated cell suspensions. After labelling, the cells for 90 min at 30°C, samples were washed three times with 1 ml of TC (20 mM Tris-HCl, pH 8.8; 2 mM CaCl2). Cell pellets were resuspended in 200 μl of lysis buffer (20 mM Tris-HCl, pH 8.8; 2 mM CaCl2; 0.1% sodium dodecyl sulphate (SDS); 15 mM dithiothreitol; 1 mM PMSF; 6 μM leupeptin; 2 μM pepstatin A; 2% Triton X-100; the latter four added just before use) and broken by glass beads (diameter = 0.5 μm; Sigma) for 15-1 min cycles of stirring and cooling on ice. The samples were...
boiled for 3 min and the proteins solubilized by adding 160 mg of Urea (Ultra-Pure, BRL). Before loading, 16.0 µl of amphiolys 3–10 (Serva) was added to the samples. The two-dimensional protein analysis of the protein extracts was made according to Galego and Rodrigues-Pousada (1985) with the following exceptions: (i) 2% Triton X-100 (Serva) and 4.5% amphiolys 3–10 (Serva) were added instead of Nonidet NP40 and a 2% mix prepared from amphiolys batches of different pH ranges; and (ii) the running voltage of the first dimension was increased to 500 V. Alterations of protein synthesis rates as cited in the text were mainly judged by appearance/disappearance of a given spot on the two-dimensional gel. Minor fluctuations in the intensity of protein spots already present in control gels were ignored.

Preparation and Northern analysis of RNA

Isolation of RNA from yeast cells was carried out as described by Zitomer et al. (1979). Samples containing 10 µg of total cellular RNA were fractionated on 1.5% agarose gels after denaturation in 1 M glyoxal and 50% dimethylsulphoxide (McMaster and Carmichael, 1977) and blotted onto Hybond-N (Amersham) membrane. As gene-specific probe for HSP12 mRNA a 0.5 kb EcoRI-generated cDNA fragment (Praekelt and Meacock, 1990) and a 0.7 kb BglII–BamHI DNA fragment, which encompasses the entire coding region of the HSP26 gene (−6 to +676; Bossier et al., 1989; Susek and Lindquist, 1989), were used, respectively. To probe for the glyceral-3-phosphate dehydrogenase (GPD) mRNA a 33-mer, single-stranded oligodeoxynucleotide (5’-CTTGGCAATAGTACCCGATTTCCGATAC-3’) was synthesized. This oligomer is complementary to a highly conserved nucleotide sequence near the 5’ end of genes coding for both cytoplasmic (NAD∗-linked) and mitochondrial (FAD-linked) isoforms of this enzyme among eukaryotic cells (Pidoux et al., 1990; Sleep et al., 1991). HSP12 and HSP26 probes were prepared by the random priming method (Feinberg and Vogelstein, 1983) and the GPD 33-mer was labelled by phosphorylation using T4 polynucleotide kinase (Boehringer Mannheim). Hybridization and washing were carried out in accordance with the recommendations of the membrane manufacturer. For the oligomer GPD, the hybridization temperature was lowered to 55°C; washing was performed at the hybridization temperature with 5x SSPE (0.9 M NaCl; 50 mM Na2HPO4, pH 7.7; 5 mM EDTA) for 15 min and 1x SSPE + 0.1% SDS for 15 min.

References


Acknowledgements

We would like to thank Drs P. A. Meacock and U. Praekelt (University of Leicester, UK) for providing us with the hsp12::URA3 and hsp26::HIS3 disruption mutants and the HSP12 cDNA clone, and Dr M. Tuite (University of Kent, UK) for his gift of the HSP26 DNA clone. In addition, we are indebted to Dr T. Karpova (St. Petersburg) for her contribution to the start of these investigations. This work was supported by a Junta Nacional de Investigación Científica e Tecnológica (Portugal) grant to J.C.S.V.


