An investigation of the relative contributions conveyed by heat shock proteins, trehalose, carbon source and gene expression to hyperthermia in *Saccharomyces cerevisiae*

By

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This thesis is dedicated to Michelle Deegenaars,

my mentor, my best friend, my soul mate.

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

(Claudia Gross)

Errata

Changes to list of references:

p. 154 Baroni et al. Call aycle should be Cell cycle
p. 154 Barraclough Proein should be Protein
p. 163 Eleutherio et al. strews should be stress
p. 166 Gounalaki et al. yast should be yeast
p. 171 Lagunas et al. *Saccharomycec* should be *Saccharomyces*
p. 178 Neumann et al. intracellulat should be intracellular
p. 179 Nishida et al. sakai should be Sakai
heatt should be heat
p. 179 Orita et al. elctrophoresis should be electrophoresis
p. 185 Schumacher et al. 1996 renaturaton should be renaturation
p. 189 Utans et al. Pron. should be Proc.
p. 190 Von Meyenberg *cerevisiae*during should be *cerevisiae* during

The following references were listed but not cited in the thesis:

Beckman et al., 1992
Bettany et al., 1995
Cchandrashekhar et al., 1995
Duncan, 1995
Halberg & Hallberg, 1996
Hansen et al., 1994
Jacob & Buchner, 1994
Saavedra et al., 1996
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Abstract

Intrinsic and heat shock induced thermotolerance of *Saccharomyces cerevisiae* was investigated in cells grown on glucose (repressed) and acetate (derepressed) supplemented media. Heat shocked cells (37°C/30 min), in either medium, exhibited induced synthesis of heat shock proteins (hsps) and trehalose. In all cases, with the notable exception of repressed cells of a relatively thermosensitive strain (Ysen), heat shock acquisition of thermotolerance to a 50°C stress also occurred in the absence of protein synthesis and coincident decrease in trehalose accumulation. Results indicated that a marked increase in thermotolerance exhibited by derepressed cells compared with repressed cells was not closely correlated with levels of hsps or trehalose. It was concluded that mechanisms for intrinsic and induced thermotolerance appear to be different and that growth on acetate endows cells with a biochemical predisposition, other than hsps or trehalose, which confers intrinsic tolerance.

Patterns of heat shock gene transcription and translation as well as trehalose content were investigated in both repressed and derepressed *S. cerevisiae* cells during heat shock, return of cells to 25°C (recovery) and subsequent exposure to a second heat shock (re-heat shock). Heat shocked cells, grown in either glucose or acetate supplemented media, initially acquired high thermotolerance to a 50°C heat stress, which was progressively lost when cultures were allowed to recover at 25°C and subsequently exposed to heat stress at 50°C. In all cases, with the notable exception of repressed cells of thermosensitive Ysen, inhibition of protein synthesis, and coincident decrease in trehalose accumulation, during the heat shock had little effect on the kinetics of loss of thermotolerance. Heat shock at 37°C elicited a marked increase in transcription and translation of genes encoding major hsps. During recovery at 25°C, both metabolic activities were suppressed followed by a gradual increase in hsp mRNA transcription to levels observed prior to heat shock. *De novo* translation of hsp mRNAs, however, was no longer observed during the recovery phase, although immuno-detection analyses demonstrated persistence in cells of high levels of hsps 104, 90, 70 and 60 throughout the 240 min recovery period. In addition, while heat shock-induced trehalose was rapidly degraded during recovery in repressed cells, levels remained high in derepressed cells. Results therefore indicated that the progressive loss of induced thermotolerance exhibited by glucose and acetate grown cells was not closely correlated with levels of hsp or trehalose. It was concluded that both

constitutive and *de novo* synthesized hsps require heat shock associated activation in order to confer thermotolerance and that this modification is progressively reversed upon release from the heat shocked state.

Data generated for re-heat shock analyses suggested that induction of the heat shock response may be modified by the previous thermal history of the cell. Exposure of cells to a second heat shock resulted in hsp supplementation to the pre-existing pool induced by a prior heat treatment. Protein synthesis inhibition prior to and during re-heat shock in pre-heat shocked, recovered cells (that contained elevated levels of hsps) did not suppress induction of thermotolerance in strain Ysen. It was therefore concluded that pre-existing hsps might be re-activated following a subsequent re-heat shock treatment. However, the extent of thermotolerance induced upon successive treatments decreased with increased recovery periods. Consequently, it was proposed that hsps are either progressively deactivated and/or saturated in chaperone function, resulting in increased requirement for newly synthesised hsps or *de novo* proteins. Evidence presented collectively suggested that trehalose was not a vital factor in tolerance. Support for these observations was derived from examination of thermotolerance characteristics, trehalose content and hsp profiles of a *S. cerevisiae* mutant deficient in trehalose accumulation. Results clearly indicated that hsps play a more predominant role in conveying thermotolerance.

Differential display of PCR amplified reverse transcribed mRNA (DDRT-PCR) was employed to survey changes in gene expression profiles induced by heat shock and carbon catabolite derepression. Analyses of three cell types, repressed control, repressed heat shocked and derepressed of Ysen, as well as cells of a relatively thermoresistant strain (Yres), yielded 30 differentially displayed cDNA fragments common to heat shocked and derepressed cells of both strains. Eighteen of these generated signals on Northern blots, of which three were confirmed as regulated. Five amplicons were cloned and sequenced. Three exhibited homology to *S. cerevisiae* genes with well characterized protein products: *HSP 90*, *HXK1* and *STA1*. The remaining two amplicons showed nucleotide identity to *YTIS11*, a homolog of the mammalian *TIS11* and putative transcriptional activator, and an orphan gene encoding a hypothetical transmembrane protein belonging to the multi-drug resistance translocase family. Novel application of DDRT-PCR in this manner identified new and known genes that may be further evaluated as factors involved in stress regulation and demonstrated the potential of the technique to systematically analyze gene expression in yeast.

Publications arising from this thesis

1. Gross, C. and Watson, K. (1996) Heat shock protein synthesis and trehalose accumulation are not required for induced thermotolerance in derepressed *Saccharomyces cerevisiae*. *Biochem Biophys Res Comm* **220**, 766-772.

2. Gross, C and Watson, K. (1998) Application of mRNA differential display to investigate gene expression in thermotolerant cells of *Saccharomyces cerevisiae*. *Yeast* **14**, in press.

3. Gross, C and Watson, K. (1998) Transcriptional and translational regulation of major heat shock proteins and patterns of trehalose mobilization during hyperthermic recovery in repressed and derepressed *Saccharomyces cerevisiae*. *Can J Microbiol* in press.

4. Gross, C and Watson, K. *De novo* protein synthesis is essential for thermotolerance acquisition in a *Saccharomyces cerevisiae* trehalose synthase mutant. In preparation.

5. Gross, C and Watson, K. Heat shock proteins may require heat shock-associated activation to confer thermotolerance. In preparation.

Copies of publications 1 and 2 are presented in Appendix 2.

Aspects of thesis presented at conference proceedings

1. Gross, C. and Watson, K. (1994) Growth on acetate evokes thermoresistance in *Saccharomyces cerevisiae*. *Proc Aust Soc Biochem Mol Biol* **26**, POS-2-29.

2. Gross, C. and Watson, K. (1995) Differential display driftnetting – fishing for genes that confer thermotolerance. *Proc 7th FACBMB Congress* **27**, POS-1-63.

3. Gross, C. and Watson, K. (1996) A catalogue of differentially expressed genes from thermotolerant and derepressed *Saccharomyces cerevisiae*. *Proc 9th Int Symposium on Yeasts* **44**, POS-P4-4.

4. Watson, K., Gross, C. and Arasanilai, J. (1996) The transient nature of heat shock induced thermotolerance and oxytolerance in yeast. *Proc 9th Int Symposium on Yeasts* **13**, SYM-S3-2.

Abbreviations

aa:	amino acid
bp:	base pairs
ADPG:	adenosine diphosphate glucose
AMP:	adenosine monophosphate
AMPK:	AMP-activated protein kinase
AMPS:	ammonium peroxodisulphate
ATPase:	adenosine triphosphatase
BSA:	bovine serum albumin
CaM:	Ca^{2+}-dependent calmodulin
CaM kinase II:	calmodulin-dependent protein kinase II
cAMP:	cyclic adenosine monophosphate
cDNA:	complementary DNA
cfu ml^{-1}:	colony forming units per ml
dATP:	2'-deoxyadenosine 5'-triphosphate
dCTP:	2'-deoxycytosine 5'-triphosphate
DDRT-PCR:	differential display reverse transcriptase PCR
DEPC:	diethylpyrocarbonate
DNA:	deoxyribonucleic acid
dGTP:	2'-deoxyguanosine 5'-triphosphate
DNase:	deoxyribonuclease
dNTP:	2'-deoxynucleoside 5'-triphosphate
DTT:	dithiothreotol
dTTP:	2'-deoxythymidine 5'-triphosphate
ECL:	enhanced chemiluminescence
EDTA:	ethylenediaminetetraacetic acid
ER:	endoplasmic reticulum
EST(s):	expressed sequence tag(s)
g-1-p:	glucose-1-phosphate
g-6-p:	glucose-6-phosphate
HSC:	heat shock cognate gene

hsc:	heat shock cognate protein
HSE:	heat shock element
HSF:	heat shock transcription factor
hsp(s):	heat shock protein(s)
HSP(s):	heat shock protein gene(s)
IPTG:	isopropylthiogalactoside
kb:	kilobases
kDa:	kilodaltons
LB:	Luria-Bertani
LM:	Luria medium
MAP:	mitogen activated protein
M_r:	relative mass
mRNA:	messenger RNA
MOPS:	3-[*N*-morpholino]propanesulfonic acid
OD:	optical density
oligo-dT:	oligo deoxythymidine
ORF:	open reading frame
PBS:	phosphate buffered saline
PBS-T:	PBS-Tween 20
PCR:	polymerase chain reaction
pgm:	phosphoglucomutase
PMSF:	phenylmethylsulphonylfluoride
polyA:	poly adenylic acid
polyT:	poly thymidylic acid
RNA:	ribonucleic acid
RNase:	ribonuclease
rubisco:	ribulose bisphosphate carboxylase-oxygenase
SDS:	sodium dodecyl sulphate
SDS-PAGE:	SDS-polyacrylamide gel electrophoresis
SMP:	skim milk powder
SSC:	saline sodium citrate
STRE:	stress response element
t-6-P (t-6-p):	trehalose-6-phosphate

TAE:	Tris acetate EDTA
***Taq* polymerase:**	*Thermus aquaticus* DNA polymerase
TBE:	Tris borate EDTA
TCA:	trichloroacetic acid
TE:	Tris EDTA
TEMED:	N,N,N'N'-tetramethylethylenediamine
TPN:	tetrachloroisophthalonitrile
tRNA:	transfer RNA
UDP:	uridine diphosphate
UDPG:	uridine diphosphate glucose
YEP:	yeast extract peptone
YEPA:	YEP with 1% potassium acetate
YEPG:	YEP with 2% glucose
ygp:	yeast glycogen phosphorylase
YNB:	yeast nitrogen base
YNBA:	YNB with 1% potassium acetate
YNBG:	YNB with 2% glucose

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Prologue

Thinking _ _ _ _ _ _ where to start?

Oh, "let's start at the very beginning, a very good place to start _ _ _ _ _ _."

Do - to yeast is very dear,

Ra - I've done RNA analysis by the tonne,

Me - is for the MOPS and EDTA on the shelf,

Fa - it's been so long since I've begun,

So - this simple organism stood me in good stead,

La - I see visions of LM agar in my head,

Te - a buffer we've all used to make our daily bread ($)

and that brings us back to '**Do**' ($)!

anyway, _ _ _ _ _ _

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