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Shedding light on the link between the
actin cytoskeleton and stress response
in *Saccharomyces cerevisiae*

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in Biochemistry

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Abstract

Understanding how eukaryotic cells adapt to stress remains a fundamental question in biology. One important stress which affects all cells is amino acid (AA) starvation. Upon perception of AA starvation the Gcn2 protein binds an effector protein complex consisting of Gcn1 and Gcn20. Subsequently, Gcn2 becomes activated and phosphorylates the eukaryotic translation initiation factor 2. This leads to a reduced global protein synthesis and simultaneously to an increased translation of Gcn4, a transcriptional activator of genes necessary for overcoming stress. This signaling pathway is called general amino acid control (GAAC) in yeast. Failing to activate this signaling cascade impairs the starvation response and cellular growth.

Yih1 inhibits Gcn2 by competing with Gcn2 for Gcn1 binding, consequently leading to an impaired stress response. However, Yih1 is not a general inhibitor of Gcn2 but only impedes Gcn2 activation upon release from the cytoskeleton protein actin. Our understanding of the role of actin in Gcn2 signaling and Yih1 itself is limited. Also, the circumstances under which Yih1 is released from actin are unknown. Thus, the scope of this study is to elucidate the link between the actin cytoskeleton and stress response. To achieve this goal, actin mutants were screened for an impaired ability to overcome starvation. Out of 24 mutant strains five exhibited an impaired stress response as indicated by sensitivity to AA analogues, which could be reverted upon Gcn4 induction, and sensitivity to AA imbalance. One of these actin mutations has been proposed to affect the GAAC via an impaired Yih1-actin binding. Another mutation appears to weaken the actin-eEF1A interaction therefore promoting eEF1A mediated Gcn2 inhibition. For the remaining 3 actin mutations the mechanism might be at the transcriptional or translational level. These findings show that actin mutations do affect the GAAC at multiple levels. In addition, the cyclin dependent kinase Cdc28 has been identified as a novel interaction partner of Yih1 and it has been speculated that a link between Yih1 and bud emergence might exist.

The results from this study achieved to shed light on the link between Yih1, the GAAC and the actin cytoskeleton. Yih1 has been placed in the midst of highly regulated and diverse cellular processes, which emphasizes the interconnection that exist between cellular pathways and the likely importance of Yih1 in the cell.

For my family

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Contents

Abstract	iii
Acknowledgements	vii
List of Figures	xx
List of Tables	xxii
List of Abbreviations	xxiii
1 Introduction	1
1.1 The general amino acid control pathway	1
1.1.1 Translation control by phosphorylation of the eukaryotic translation initiation factor 2	2
1.1.2 Selectively increased translation mediated by the transcription activator Gcn4	4
1.2 eIF2 α protein kinases in mammals	5
1.3 The eIF2 α kinase Gcn2	7
1.4 The Gcn2 activator protein Gcn1	7
1.5 The Gcn2 negative regulator protein Yih1	9
1.6 The Yih1 interaction partner actin	12
1.7 Scope of this study	15
2 Materials and Methods	19
2.1 Biological materials	19
2.2 Plasmid construction	21
2.3 Deletion of <i>YIH1</i> in the chromosome	22

2.4	Media	23
2.4.1	Bacterial media	23
2.4.2	Yeast media	23
2.4.3	Media supplements	24
2.5	Growth conditions	25
2.5.1	Bacterial growth conditions	25
2.5.2	Yeast growth conditions	25
2.6	Permanent storage of yeast/bacterial strains	25
2.7	DNA isolation and purification	25
2.7.1	Plasmid DNA isolation	25
2.7.2	Genomic DNA extraction	26
2.8	DNA quantification	26
2.9	Agarose gel electrophoresis	26
2.10	Restriction endonuclease digestion	27
2.11	Polymerase chain reaction	27
2.11.1	Primers	27
2.12	DNA purification	30
2.13	DNA ligation	30
2.13.1	Dephosphorylation	30
2.13.2	Ligation	30
2.14	Transformation of <i>Escherichia coli</i>	30
2.14.1	Preparation of calcium chloride competent <i>E.coli</i> cells	30
2.14.2	Transformation of <i>E.coli</i> using the heat shock method	31
2.15	DNA sequencing	31
2.16	Yeast transformation	31
2.16.1	Preparation of competent <i>Saccharomyces cerevisiae</i> cells	32
2.16.2	Standard yeast transformation	32
2.16.3	Yeast transformation for deleting <i>YIH1</i>	33
2.17	Preparation of yeast whole cell extract	33
2.18	Estimation of protein concentration by Bradford method	34
2.19	Sodium dodecyl sulfate polyacrylamide gel electrophoresis .	34
2.19.1	Gradient gel electrophoresis	34

2.19.2	Straight gel electrophoresis	35
2.20	Staining proteins in polyacrylamide gels	36
2.21	Western blotting	37
2.21.1	Gel transfer	37
2.21.2	Staining proteins on PVDF membranes	37
2.21.3	Immunological detection of proteins	37
2.22	Semi-quantitative growth assay	39
2.23	Growth assay to check for petite mutations	40
2.24	<i>lacZ</i> assay	40
2.24.1	Growing of cells	41
2.24.2	β -Galactosidase assay	41
2.25	Glutathione S-Transferase mediated <i>in vivo</i> pulldown assay	42
2.25.1	Growing and breaking of cells	42
2.25.2	Glutathione S-Transferase mediated pulldown	42
2.26	Purification of His ₆ -Yih1	43
2.26.1	Growing of cells	43
2.26.2	Purification of His ₆ -Yih1 from bacterial whole cell extract	44
2.27	<i>In vitro</i> pulldown with F-actin	44
2.27.1	Protein preparation	44
2.27.2	<i>In vitro</i> F-actin interaction assay	45
2.28	<i>In vitro</i> interaction assay of His ₆ -Yih1 and rabbit G-actin . . .	45
3	Comprehensive screen of actin mutant strains for an impaired GAAC response	47
3.1	Screen of actin mutant strains for sensitivity to sulfometuron methyl	50
3.2	Screen of actin mutant strains for sensitivity to amino acid imbalance	52
3.3	Screen of actin mutant strains for sensitivity to 3-Aminotriazole	54
3.4	Screen of actin mutant strains for sensitivity to SM when the transcription activator <i>GCN4</i> is constitutively expressed . . .	57
3.5	<i>In vivo</i> interaction assay with GST-Yih1 fragment III and actin	60
3.6	Screen of <i>yih1</i> Δ actin mutant strains for sensitivity to SM . .	65

3.7	Screen of actin mutant strains and <i>yih1</i> Δ actin mutant strains for expression of the transcription activator Gcn4	67
3.8	Screen of actin mutant strains overexpressing Yih1 for sensitivity to SM	70
3.9	Actin complementation assay of actin mutant strains	74
3.10	Verification of the actin mutation in actin mutant strain TKY 475	76
3.11	Summary and discussion	77
4	<i>In vitro</i> binding studies with His₆-Yih1 and actin	93
4.1	<i>In vitro</i> binding assay of yeast His ₆ -Yih1 and rabbit muscle filamentous actin	93
4.2	<i>In vitro</i> binding assay of yeast His ₆ -Yih1 and monomeric rabbit muscle actin	95
4.3	Discussion	101
5	Yih1 interacts with the cyclin dependent kinase Cdc28 <i>in vivo</i>	103
5.1	Yeast-2-hybrid screen with Yih1 fragments	104
5.2	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	107
5.3	Mapping the Cdc28 binding site in Yih1	108
5.4	Discussion	114
6	Conclusion	119
A	Result of the semi-quantitative growth assay of TKY 465	123
B	Results of the Gcn4^c semi-quantitative growth assays	125
C	Results of the <i>in vivo</i> interaction assays	135
D	Verification of the deletion of <i>YIH1</i>	143
E	Results of the semi-quantitative growth assays of <i>yih1</i>Δ actin mutant strains	147
F	Results of the <i>lacZ</i> assays	151
G	Results of the <i>ACT1</i> complementation assays	157

H	Verification of the yeast-2-hybrid plasmids	161
I	Results of mapping the Cdc28 binding site in Yih1	167
J	Nature protocol exchange publication	171

List of Figures

1.1	Schematic representation of eukaryotic translation initiation	3
1.2	Schematic representation of <i>GCN4</i> expression	5
1.3	Schematic representation of eIF2 α protein kinases in mammals	6
1.4	Schematic presentation of functional domains in the protein kinase Gcn2	8
1.5	Schematic presentation of functional domains in Yih1	10
1.6	Model for Yih1 mediated Gcn2 regulation	12
1.7	Atomic structure of the actin monomer	13
1.8	Schematic representation of actin filament treadmilling	14
2.1	Schematic representation of location of primers used to delete <i>YIH1</i> in the chromosome	22
2.2	Example of the calculation of a SM ^S reversion	40
3.1	Location of actin mutations investigated	49
3.2	Overview of the screen of actin mutant strains for sensitivity to 3AT	56
3.3	Overview of the screen of actin mutant strains for sensitivity to 3AT continued	57
3.4	Example for a Gcn4 ^C semi-quantitative growth assay	59
3.5	Example of an <i>in vivo</i> interaction assay with GST-Yih1 fragment III and actin	63
3.6	Example of a <i>lacZ</i> assay	68
3.7	Summary of the screen of actin mutant strains overexpressing Yih1 for sensitivity to SM	73
3.8	Test of actin yeast strains for petites mutations	74

3.9	Example of an <i>ACT1</i> complementation assay	75
3.10	Multiple sequence alignment of the wild type actin protein sequence and the actin protein sequence of TKY 475	77
3.11	Location of actin mutations affecting GAAC	85
3.12	Model of the crosstalk between actin and the GAAC and Yih1's involvement in F-actin polymerization	91
4.1	Coomassie blue stain of purified His ₆ -Yih1	94
4.2	<i>In vitro</i> sedimentation assay of yeast His ₆ -Yih1 with rabbit muscle F-actin	96
4.3	<i>In vitro</i> binding assay with rabbit muscle G-actin and yeast His ₆ -Yih1	98
4.4	<i>In vitro</i> binding assay with rabbit muscle G-actin and yeast His ₆ -Yih1	99
4.5	<i>In vitro</i> binding assay with rabbit muscle G-actin and yeast His ₆ -Yih1	100
5.1	Schematic representation of the yeast-2-hybrid system	105
5.2	Schematic representation of full length Yih1 and Yih1 fragments	106
5.3	Verification of the expression of a Yih1 fragment fused to the Gal4 activation domain	106
5.4	Verification of the expression of two Yih1 fragments fused to the Gal4 activation domain	107
5.5	<i>In vivo</i> binding assay with Yih1 fragment III in yeast strain TKY 460	109
5.6	Schematic representation of full length Yih1 and Yih1 fragments	110
5.7	Example of mapping the Cdc28 binding site in Yih1	112
5.8	Expression levels of GST-Yih1 and GST-Yih1 fragments	113
5.9	Result of mapping the Cdc28 binding site in Yih1	114
6.1	Proposed model of Yih1 function	121
A.1	Overview of the screen of TKY 465 for sensitivity to 3AT	124
B.1	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed	126

B.2	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	127
B.3	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	128
B.4	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	129
B.5	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	130
B.6	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	131
B.7	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	132
B.8	Screen of actin mutant strains for sensitivity to SM when the transcription activator Gcn4 is constitutively expressed continued	133
C.1	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	137
C.2	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	138
C.3	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	139
C.4	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	140
C.5	<i>In vivo</i> interaction assay with GST-Yih1 fragment III	141
D.1	Verification of the deletion of <i>YIH1</i> by PCR	144
D.2	Verification of the deletion of <i>YIH1</i> by Western blot analysis	145
E.1	Semi-quantitative growth assay of <i>yih1</i> Δ actin mutant strains	148
E.2	Semi-quantitative growth assay of <i>yih1</i> Δ actin mutant strains continued	149
F.1	Results of <i>lacZ</i> assays	152

List of Figures

F.2	Results of <i>lacZ</i> assays continued	153
F.3	Results of <i>lacZ</i> assays continued	154
F.4	Results of <i>lacZ</i> assays continued	155
G.1	Summary of the <i>ACT1</i> complementation assay	158
G.2	Summary of the <i>ACT1</i> complementation assay continued . .	159
H.1	Verification of pMD02a	162
H.2	Verification of pMD03a	163
H.3	Verification of pMD03a continued	164
H.4	Verification of pMD06a	165
H.5	Verification of pMD06a continued	166
I.1	Mapping of the Cdc28 binding site in Yih1	168
I.2	Mapping of the Cdc28 binding site in Yih1 continued	169
I.3	Mapping of the Cdc28 binding site in Yih1 continued	170

List of Tables

2.1	Plasmids used in this study	19
2.2	Yeast strains used in this study	20
2.3	Antibiotics, drugs and constituents used in this study	24
2.4	Primers used in this study	29
2.5	Inhibitors used in this study	33
2.6	Primary antibodies used in this study	38
2.7	Secondary antibodies used in this study	38
3.1	Overview of the screen of actin mutant strains for sensitivity to SM	51
3.2	Overview of the screen of actin mutant strains for sensitivity to AA imbalance	53
3.3	Overview of the screen of actin mutant strains for sensitivity to 3AT	55
3.4	Summary of the screen of actin mutant strains constitutively expressing the transcription activator Gcn4 for sensitivity to SM	61
3.5	Overview of the <i>in vivo</i> interaction assays with GST-Yih1 fragment III and actin from actin mutant strains	64
3.6	Summary of the screen of <i>yih1</i> Δ actin mutant strains for sensitivity to SM	66
3.7	Overview of the <i>lacZ</i> assays using actin mutant strains and <i>yih1</i> Δ actin mutant strains	69
3.8	Overview of the screen of actin mutant strains overexpressing Yih1 for sensitivity to SM	72

3.9 Overview of the <i>ACT1</i> complementation assay of actin mutant strains	76
3.10 Overview of the comprehensive screen of actin mutant strains for an impaired GAAC	78

List of Abbreviations

In addition to the chemical symbols from the periodic table of elements and the système international d'unités (SI), the following abbreviations are used:

+/-	highly charged region
3AT	3-Aminotriazole
3AT ^S	sensitivity to 3-Aminotriazole
AA	amino acid
AA imbalance ^S	sensitivity to amino acid imbalance
ABC	ATP-binding cassette
APS	ammonium persulfate
ATP	adenosine triphosphate
bp	base pair
BSA	bovine serum albumin
BiFC	bimolecular fluorescence complementation
cak	cdk activating kinase
cdk2	cyclin dependent kinase 2
CIP	calf intestinal phosphatase
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DTT	dithiothreitol
EDTA	ethylenediamine tetra acetic acid
eEF1A	eukaryotic elongation factor 1A
eIF2	eukaryotic initiation factor 2
eIF2 α -P	eukaryotic initiation factor 2 phosphorylated at subunit α
F-actin	filamentous actin
GAAC	general amino acid control

List of Abbreviations

G-actin	globular actin
Gcn	general control non-derepressible
Gcn4 ^C	Gcn4 constitutively expressed
GDP	guanosine diphosphate
GI	Gcn2 and IMPACT
GST	glutathione S- transferase
GTP	guanosine triphosphate
HA	hemagglutinin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His-RS	histidyl-tRNA synthetase
HRI	heme-regulated inhibitor kinase
IMPACT	imprinted and ancient
IPTG	isopropyl- β -D-thiogalactopyranoside
kDa	kilo dalton
LB	luria-bertani
LiOAc	lithium acetate
Ni-NTA	nickel-nitrilo triacetic acid
OD	optical density
OPNG	o-nitrophenyl-beta- β -galactosidase
ORF	open reading frame
p	plasmid
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PERK	PKR-like endoplasmatic reticulum kinase
PKR	protein kinase RNA
PMSF	phenylmethanesulphonyl fluoride
PVDF	polyvinylidene difluoride
RNase	ribonuclease
RWD	RING finger proteins, WD-repeat-containing proteins, yeast DEAD-like helicases
SD	synthetic dropout
SDS	sodium dodecyl sulfate
SM	sulfometuron methyl
SM ^S	sensitivity to sulfometuron methyl
tRNA _i ^{Met}	Methionyl-tRNA
Tris	tris(hydroxymethyl)aminomethane
TAE	tris-acetate EDTA
TBS	tris-buffered saline
TBS-T	TBS-Tween
TC	ternary complex
TE	Tris EDTA

TEMED	N,N,N',N'-Tetramethylethylenediamine
uORF	upstream open reading frame
v/v	volume/ volume
WCE	whole cell extract
w/v	weight/ volume
Yih1	yeast impact homolog
YPD	yeast extract peptone dextrose
YPG	Yeast extract peptone glycerol

