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Data in Brief

Genome wide transcription profiling of the effects of overexpression of Spc1 and its kinase dead mutant in *Schizosaccharomyces pombe*

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ABSTRACT

The Mitogen Activated Protein Kinase Spc1 (p38 homolog) is a major player in stress responses of the unicellular fission yeast *Schizosaccharomyces pombe*. This pathway is therefore also known as the SAPK or Stress Activated Protein Kinase pathway. Spc1 is a known activator of transcription factors that control gene expression in response to extracellular stimuli and is also known to interact with the translation machinery [1–8]. Spc1 has also been implicated in cell cycle regulation and meiosis in *S. pombe* [1,2,9,10]. Given its documented role in modulating gene expression, we performed a microarray based identification of genes whose expression in unperturbed cells (absence of stress stimuli) is dependent on Spc1. For this we overexpressed Spc1 in *S. pombe*. Additionally we also overexpressed Spc1K49R (a kinase dead mutant of Spc1) to understand the contribution of Spc1's kinase activity towards the observed gene expression changes. The microarray data are available at NCBI's Gene Expression Omnibus (GEO) Series (accession number GSE73618). Here we report the annotation of the genes whose expression get altered by Spc1/Spc1K49R overexpression and also provide details related to sample processing and statistical analysis of our microarray data.

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Specifications	
Organism/cell line/tissue	*Schizosaccharomyces pombe*
Sex	Not applicable
Sequencer or array type	Affymetrix — GeneChip® Yeast Genome 2.0 Array
Data format	Raw
Experimental factors	Spc1/Spc1K49R were separately overexpressed in wild type *S. pombe* cells and gene expression in such cells was compared with that of control cells (which are transformed with the empty vector).
Experimental features	Cells were grown to saturation in the presence of 20 μM Thiamine. They were then harvested, washed (to remove Thiamine) and resuspended in fresh media and incubated for 24 h before proceeding with RNA isolation.
Consent	Not applicable
Sample source location	Not applicable

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73618.

2. Experimental design, materials and methods

2.1. Experimental design

We overexpressed Spc1/Spc1K49R in *S. pombe* cells, and then looked at the changes in the transcriptional profile of the cells. Earlier reports on identification of Spc1 dependent gene expression do exist [11]. However in those screens transcriptional changes were identified after deleting Spc1. Spc1 is known to have contrasting effects on cellular physiology (especially cell division) in a dose dependent manner. We argued that deletion and overexpression of Spc1 may therefore represent two extremes of such dose dependent effects and therefore overexpression may identify newer targets of Spc1. We also overexpressed Spc1K49R to check whether these transcriptional changes were entirely dependent on the kinase activity or not.

2.2. Strains, media and growth conditions

S. pombe strain used in this study was a wild type strain GSY001 (*h-leu1-32 ura4-D18, a gift from Paul Russell*). Cells were grown as described by S. Moreno et al. [12]. All cells were grown at 30 °C in Edinburgh's Minimal Medium (EMM)-Leucine.

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Table 1
Summary of differential gene expression analysis.

Groups compared	Total no. of differentially expressed genes (up/down)	No. of upregulated genes	No. of downregulated genes
Spc1-OP/control	42	20	22
Spc1K49R-OP/control	132	68	64
Spc1-OP/Spc1K49R-OP	60	36	24

2.3. *S. pombe* transformations

One milliliter of an overnight *S. pombe* culture in YES was harvested and then resuspended in 0.5 ml PEGLET (10 mM Tris [pH 8], 1 mM EDTA, 0.1 M lithium acetate, 40% polyethylene glycol [PEG]). Five microliters of denatured salmon sperm DNA (10 mg/ml) was added to it. One microgram of the purified plasmid DNA was then added to this mixture and allowed to stand overnight at room temperature, after which the cells were resuspended in 150 μl YES and spread onto appropriate selection plates.

2.4. Overexpression of Spc1/Spc1K49R

Wild type *S. pombe* cells were transformed separately with the plasmids pGS017 (empty vector pREP41; control) or pGS023 (pREP41 + Spc1; for Spc1 overexpression) or pGS041 (pREP41 + Spc1K49R; for Spc1K49R overexpression). pGS023 (or pGS041) contain the full length Spc1 gene (or the Spc1K49R mutant) cloned downstream of the nmt1 promoter which is fully repressed in the presence of Thiamine. Single colonies were inoculated in liquid media and grown to saturation in EMM-Leucine + 20 μM Thiamine. The cells were then harvested, washed to remove Thiamine and resuspended in fresh EMM-Leucine media and incubated with shaking at 30 °C for 24 h to allow derepression of the nmt1 promoter and consequent overexpression of Spc1/Spc1K49R.

2.5. Sample preparation and hybridization

The quality of RNA isolated was analyzed in an Agilent 2011 Bioanalyzer with an RNA LabChip kit according to the manufacturer's protocol. The array used in this microarray was Affymetrix — Gene Chip Yeast Genome 2.0 (Affymetrix, Santa Clara, CA). The array format was 100 midi. This array contained probes for both *S. pombe* and *Saccharomyces cerevisiae*. For each sample total RNA was isolated and then used for first strand cDNA synthesis which was followed by a second strand cDNA synthesis. This was done according to the protocol in Affymetrix GeneChip 3′ IVT Express Manual (Affymetrix 2008). Biotin labeling was performed for 16 h at 40 °C. The fragmented and biotin labeled cDNA was hybridized to the arrays. The hybridization was done for 16 h at 10 rpm at 65 °C. The hybridized arrays were scanned using Affymetrix Scanner G 300 7G.

2.6. Microarray data analysis

2.6.1. Normalization and quality control

After scanning of slides, raw data sets were extracted from scanned CEL files and analyzed using GeneSpring GX12.6 software. Raw data was processed using RMA (Robust Multi-array Average) normalization algorithm that consists of three steps: a background adjustment, quantile normalization and finally summarization. Genes of low intensity information content in each data set were filtered by excluding probes corresponding to intensities less than the 10.0 percentile in the raw data. Quality control of the data was done by Principal component analysis method.

2.6.2. Differential gene expression analysis

Statistical analysis was performed for the identification of differentially expressed genes. The moderated t-test method was applied for assessing the statistically significant differentially expressed genes between the control sample (not overexpressing Atf1) and the sample in which Atf1 was overexpressed. The p-value cut-off 0.05 was considered statistically significant.

3. Results and discussion

Differential gene expression was observed for genes corresponding to 3445 probes. This data was further refined by setting a ≧1.5 fold change cut-off for differential gene expression. Only 42 genes were found to exhibit differential expression after Spc1 overexpression, while 132 genes were found to be differentially expressed after Soc1K49R overexpression (see Table 1). The Yeast Genome 2.0 Array contains probes for both *S. pombe* as well *as S. cerevisiae*.

Table 2
List of genes differentially expressed after Spc1 overexpression (compared with empty vector controls).

Gene symbol	Representative public ID	Description	Nature of differential expression
sty1	SPAC24B11.06c.S1	MAP kinase Sty1	Up
mam3	SPAP11E10.02c.S1	Cell agglutination protein Mam3	Up
urg2	SPAC1002.17c.S1	Uracil phosphoribosyltransferase (predicted)	Down
urg1	SPAC1002.19.S1	GTP cyclohydrolase II (predicted)	Down
SPAC1039.08	SPAC1039.08.S1	Serine acetyltransferase (predicted)	Down
SPAC13G7.12c	SPAC13G7.12c.S1	Choline kinase (predicted)	Down
meu1///meu2	SPAC1556.06b.S1	Sequence orphan///sequence orphan (predicted to be involved in meiosis)	Down
SPAC19A8.14	SPAC19A8.14.S1	Aminoacyl-tRNA hydrolase (predicted)	Down
hem14	SPAC1F5.07c.S1	Protoporphyrinogen oxidase (predicted)	Down
SPAC1F8.08	SPAC1F8.08.S1	Sequence orphan (predicted membrane protein)	Down
SPAC750.08c	SPAC212.09c.S1	NAD-dependent malic enzyme	Down
SPAC27D7.09c	SPAC27D7.09c.S1	But2 family protein	Down
erv1	SPAC3G6.08.S1	Sulfhydryl oxidase (predicted)	Down
mug124	SPBC19C2.06c.S1	Sequence orphan (predicted to be involved in meiosis)	Down
rec8	SPBC29A10.14.S1	Meiotic cohesin complex subunit Rec8	Down
mug20	SPBC36B7.06c.S1	Sequence orphan (predicted to be involved in meiosis)	Down
car1	SPBP26C9.02c.S1	Arginase Car1	Down
SPBP8B7.05c	SPBP8B7.05c.S1	Carbonic anhydrase (predicted)	Down
SPCC162.01c	SPCC162.01c.S1	U4/U6 × U5 tri-snRNP complex subunit (predicted)	Down
aph1	SPCC4G3.02.S1	Bis(5′-nucleosidyl)-tetraphosphatase	Down
SPCC576.01c	SPCC576.01c.S1	Sulfonate dioxygenase (predicted)	Down
meu15	SPCPJ732.03.S1	Sequence orphan (predicted to be involved in meiosis)	Down

Table 3
List of genes differentially expressed after Spc1K49R overexpression (compared with empty vector controls).

Gene symbol	Representative public ID	Description	Nature of differential expression
mam2	SPAC11H11.04.S1	Pheromone p-factor receptor	Up
pfs2	SPAC12G12.14c.S1	WD repeat protein Pfs2	Up
dad3	SPAC14C4.16.S1	DASH complex subunit Dad3	Up
SPAC17G6.05c	SPAC17G6.05c.S1	Vacuolar protein-sorting protein	Up
rgs1	SPAC22F3.12c.S1	Regulator of G-protein signaling Rgs1	Up
mei2	SPAC27D7.03c.S1	RNA-binding protein involved in meiosis Mei2	Up
spk1	SPAC31G5.09c.S1	MAP kinase Spk1	Up
SPAC683.02c	SPAC683.02c.S1	zf-CCHC type zinc finger protein	Up
SPAC750.07c	SPAC750.07c.S1	GPI-anchored protein (predicted)///GPI-anchored protein	Up
dak2	SPAC977.16c.S1	Dihydroxyacetone kinase Dak2	Up
mam3	SPAP11E10.02c.S1	Cell agglutination protein Mam3	Up
mfm1	SPAPB8E5.05.S1	M-factor precursor Mfm1	Up
git11	SPBC215.04.S1	Heterotrimeric G-protein gamma subunit Git11	Up
cmc1	SPBC21D10.07.S1	Mitochondrial inner membrane protein involved in cytochrome oxidase biogenesis Cmc1 (predicted)	Up
mbx2	SPBC317.01.S1	MADS-box transcription factor Pvg4	Up
SPBC32H8.05	SPBC32H8.05.S1	Conserved fungal protein (predicted nuclear localization)	Up
SPBC685.08	SPBC685.08.S1	Sequence orphan (predicted nuclear localization)	Up
mfm3	SPBPJ4664.03.S1	M-factor precursor Mfm3	Up
SPCC569.02c	SPCC569.02c.S1	Hypothetical protein	Up
for3	SPCC895.05.S1	Formin For3	Up
SPCP1E11.10	SPCP1E11.10.S1	Ankyrin repeat-containing protein	Up
SPAC11D3.09	SPAC11D3.09.S1	Agmatinase (predicted)	Down
SPAC11D3.10	SPAC11D3.10.S1	Hypothetical protein (predicted to have pyridoxal phosphate binding activity)	Down
gsk3	SPAC1687.15.S1	Serine/threonine protein kinase Gsk3	Down
SPAC1F8.08	SPAC1F8.08.S1	Sequence orphan (predicted membrane protein)	Down
SPAC750.08c	SPAC212.09c.S1	NAD-dependent malic enzyme	Down
mug62	SPAC22F3.04.S1	AMP binding enzyme (predicted)	Down
sap49	SPAC31G5.01.S1	RNA-binding protein Sap49	Down
SPAC343.13	SPAC343.13.S1	Mitochondrial glutamyl-tRNA amidotransferase beta subunit (predicted)	Down
SPAC869.02c	SPAC869.02c.S1	Nitric oxide dioxygenase (predicted)	Down
arg7	SPBC1773.14.S1	Argininosuccinate lyase	Down
SPBC23G7.10c	SPBC23G7.10c.S1	NADH-dependent flavin oxidoreductase (predicted)	Down

Given the high degree of homology of the genome sequence of both these organisms, positive hybridization results were obviously observed for probes designed against *S. cerevisiae* genes also. Tables 2, 3 and 4 list the differentially expressed genes. For better clarity, only the *S. pombe* specific matches are included in these tables.

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Table 4
List of genes differentially expressed after Spc1 overexpression (compared with Spc1K49R overexpression).

Gene symbol	Representative public ID	Description	Nature of differential expression
cuf2	SPCC584.02.S1	Cu metalloregulatory transcription factor Cuf2	Up
spo6	SPBC1778.04.S1	Spo4-Spo6 kinase complex regulatory subunit Spo6	Up
SPCC757.02c	SPCC757.02c.S1	Hypothetical protein	Up
SPBPB2B2.08	SPBPB2B2.08.S1	Conserved fungal protein	Up
SPAC13G6.13	SPAC13G6.13.S1	Sequence orphan	Up
SPBC800.11	SPBC800.11.S1	Inosine–uridine preferring nucleoside hydrolase (predicted)	Up
mug131	SPBC1861.06c.S1	hypothetical protein (predicted to be involved in meiosis)	Up
klp8	SPAC144.14.S1	Kinesin-like protein Klp8	Up
SPAC3H1.02c	SPAC3H1.02c.S1	Metallopeptidase	Up
meu1///meu2	SPAC1556.06b.S1	Sequence orphan///sequence orphan (predicted to be involved in meiosis)	Down
urg2	SPAC1002.17c.S1	Uracil phosphoribosyltransferase (predicted)	Down
SPAC14C4.01c	SPAC14C4.01c.S1	DUF1770 family protein	Down
SPBC25H2.10c	SPBC25H2.10c.S1	tRNA acetyltransferase (predicted)	Down
car1	SPBP26C9.02c.S1	Arginase Car1	Down
sro1	SPBC1347.11.S1	Stress Responsive Orphan 1	Down
SPBC365.04c	SPBC365.04c.S1	RNA-binding protein, involved in ribosome biogenesis (predicted)	Down
SPBC1604.09c	SPBC1604.09c.S1	exoribonuclease Rex4 (predicted)	Down
nif1	SPBC23G7.04c.S1	SEL1 repeat protein Nif1	Down
SPBC21C3.07c	SPBC21C3.07c.S1	Actin binding methyltransferase (predicted)	Down
mfm1	SPAPB8E5.05.S1	M-factor precursor Mfm1	Down
aph1	SPCC4G3.02.S1	Bis(5′-nucleosidyl)-tetraphosphatase	Down
matmi_1///matmi_2	SPBC1711.01c.S1	Mating-type m-specific polypeptide mi 1///mating-type M-specific polypeptide Mi 2	Down
skp1	SPBC409.05.S1	SCF ubiquitin ligase complex subunit Skp1	Down
SPCC16C4.20c	SPCC16C4.20c.S1	Sequence orphan (predicted to be involved in double-strand break repair)	Down
rev7	SPAC12D12.09.S1	DNA polymerase zeta Rev7 (predicted)	Down
SPBC13A2.01c	SPBC13A2.01c.S1	nUclear cap-binding complex small subunit	Down
SPCC1450.07c	SPCC1450.07c.S1	D-Amino acid oxidase (predicted)	Down

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