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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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 unper- turbated 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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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.

1. Direct link to deposited data

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

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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 + 2.4 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 GeneChip 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        | SPAC24811.06c.S1         | MAP kinase Sty1 | Up |
| mam3        | SPAP1E10.02c.S1          | Cell agglutination protein Mam3 | Up |
| urg2        | SPAC1002.17c.S1          | Uracil phosphoribosyltransferase (predicted) | Down |
| urg1        | SPAC1002.19c.S1          | GTP cyclohydrolase II (predicted) | Down |
| SPAC1039.08 | SPAC1039.18c.S1          | Serine acetyltransferase (predicted) | Down |
| SPAC13C7.12c| SPAC13C7.12c.S1          | Choline kinase (predicted) | Down |
| meu1/mceu2  | 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       | SPAC15F5.07c.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 |
| SPAC27D0.09c| SPAC27D0.09c.S1          | But2 family protein | Down |
| erv1        | SPAC3G6.08.S1            | Sulphydryl oxidase (predicted) | Down |
| mug124      | SPBC19C2.06c.S1          | Sequence orphan (predicted to be involved in meiosis) | Down |
| rec8        | SPBC29A10.14c.S1         | Meiotic cohesin complex subunit Rec8 | Down |
| mug20       | SPBC368.06c.S1           | Sequence orphan (predicted to be involved in meiosis) | Down |
| car1        | SPBP269.02c.S1           | Arginase Car1 | Down |
| SPBP887.05c | SPBP887.05c.S1           | Carbonic anhydrase (predicted) | Down |
| SPCC162.01c | SPCC162.01c.S1           | U4/U6 > US tri-snRNA complex subunit (predicted) | Down |
| aph1        | SPCC4G2.02c.S1           | BscA (5′-nucleosidyl)-tetrabiphosphatase | Down |
| SPCC576.01c | SPCC576.01c.S1           | Sulfate dioxygenase (predicted) | Down |
| meu15       | SPCCP72.03c.S1           | Sequence orphan (predicted to be involved in meiosis) | 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.

### 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        | SPAC1H11.04.S1            | Pheromone p-factor receptor | Up |
| pfs2        | SPAC12G12.14c.S1          | WD repeat protein Pfs2 | Up |
| dad3        | SPAC14C4.16.S1            | DASH complex subunit Dad3 | Up |
| SPC17G6.05c | SPAC17G6.05c.S1           | Vacular protein-sorting protein | Up |
| rgs1        | SPAC22F3.12c.S1           | Regulator of G-protein signaling Rgs1 | Up |
| mre2        | SPAC27D7.03c.S1           | RNA-binding protein involved in meiosis Mre2 | Up |
| spk1        | SPAC13G5.09c.S1           | MAP kinase Spk1 | Up |
| SPAC683.02c | SPAC683.02c.S1            | zf-CCHC type zinc finger protein | Up |
| SPC750.07c  | SPAC750.07c.S1            | GPI-anchored protein (predicted) | Up |
| dak2        | SPAC977.16c.S1            | Dihydroxyacetone kinase Dak2 | Up |
| mam3        | SPAP11E10.02c.S1          | Cell agglutination protein Mam3 | Up |
| mfn1        | SPAPB8E8.05.S1            | M-factor precursor Mfn1 | Up |
| git11       | SPBC215.04.S1             | Heterotrimeric G-protein gamma subunit Git11 | Up |
| cnc1        | SPBC21D10.07c.S1          | Mitochondrial inner protein involved in cytochrome oxidase biogenesis Cnc1 (predicted) | Up |
| mhx2        | SPBC317.01.S1             | MADS-box transcription factor Mhx2 | Up |
| SPBC2H8.05  | SPBC2H8.05.S1             | Conserved fungal protein (predicted nuclear localization) | Up |
| SPBC85S.08  | SPBC85S.08.S1             | Sequence orphan (predicted nuclear localization) | Up |
| mfn3        | SPBP4664.03.S1            | M-factor precursor Mfn3 | Up |
| SPCC569.02c | SPCC569.02c.S1            | Hypothetical protein | Up |
| for3        | SPCC89S.05.S1             | Formin For3 | Up |
| SPCE11.10   | SPCE11.10.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 |
| SPC17F8.08  | SPAC17F8.08.S1            | Sequence orphan (predicted membrane protein) | Down |
| SPC750.08c  | 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 |
| SPC343.13   | SPAC343.13.S1             | Mitochondrial glutamyl-tRNA amidotransferase beta subunit (predicted) | Down |
| SPAC689.02c | SPAC689.02c.S1            | Nitric oxide dioxygenase (predicted) | Down |
| arg7        | SPBC173.14.S1             | Argininosuccinate lyase | Down |
| SPBC237.10c | SPBC237.10c.S1            | NADH-dependent flavin oxidoreductase (predicted) | Down |

### Table 4

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

| Gene symbol | Representative public ID | Description | Nature of differential expression |
|-------------|--------------------------|-------------|----------------------------------|
| cut2        | SPCC84.02.S1              | Cu metalloregulatory transcription factor Cut2 | Up |
| spo6        | SPBC1778.04.S1            | Spo4-Spo6 kinase complex regulatory subunit Spo6 | Up |
| SPC757.02c  | SPBC757.02c.S1            | Hypothetical protein | Up |
| SPBBP2B2.08 | SPBBP2B2.08.S1            | Hypothetical 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 |
| nme1/1///nme2 | SPAC1356.06.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           | fRNA acyltransferase (predicted) | Down |
| car1        | SPBC26F9.02c.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        | SPBC2367.04c.S1           | SEL1 repeat protein Nif1 | Down |
| SPBC21C3.07c| SPBC21C3.07c.S1           | Actin binding methyltransferase (predicted) | Down |
| mfn1        | SPAPB8E8.05.S1            | M-factor precursor Mfn1 | Down |
| aph1        | SPCC43.02.S1              | Bis(5'-nucleosidyl)-tetraphosphatase | Down |
| matmi_1//matmi_2 | SPCC1711.01c.S1       | Mating-type m-specific polypeptide mi 1//mating-type M-specific polypeptide Mi 2 | Down |
| skp1        | SPBC49.05.S1              | SCF ubiquitin ligase complex subunit Skp1 | Down |
| SPCC16G3.20c| SPCC16G3.20c.S1           | Sequence orphan (predicted to be involved in double-strand break repair) | Down |
| rev7        | SPBC12D12.08.S1           | DNA polymerase zeta Rev7 (predicted) | Down |
| SPBC3A2.01c | SPBC3A2.01c.S1            | Nucleic cap-binding complex small subunit | Down |
| SPCC1450.07c| SPCC1450.07c.S1           | o-Amino acid oxidase (predicted) | Down |

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