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Data in Brief

Genome wide transcription profiling reveals a major role for the transcription factor Atf1 in regulation of cell division in *Schizosaccharomyces pombe*

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ABSTRACT

The mechanism underlying stringently controlled sequence of events in the eukaryotic cell cycle involves periodic transcription of a number of genes encoding important regulators of cell cycle, growth, proliferation and apoptosis. Deregulated activities of transcription factors that contribute to this programmed gene expression, are associated with many diseases including cancer. A detailed mechanistic understanding of the transcriptional control associated with cell division is, therefore, important. We have reported earlier that the transcription factor Atf1 in *Schizosaccharomyces pombe* can regulate G2–M transition by directly controlling the expression of the mitotic cyclin Cdc13 (1).To gain a better understanding of the role of Atf1 in cell cycle, we performed a microarray based identification of cell cycle related targets of Atf1. The microarray data are available at NCBI's Gene Expression Omnibus (GEO) Series (accession number GSE71820). Here we report the annotation of the genes whose expression get altered by Atf1 overexpression and also provide details related to sample processing and statistical analysis of our microarray data.

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	*Atf1 was overexpressed in wild type S. pombe cells and gene expression in such cells was compared to that of control cells (which do not overexpress Atf1)*
Experimental features	*Cells were grown to saturation in the presence of 20* μM Thiamine. *Thwy 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=GSE71820

2. Experimental design, materials and methods

2.1. Experimental design

Atf1 is a bZIP domain containing transcription factor in *Schizosaccharomyces pombe* and is known to be a major player in orchestration of the cellular transcriptional response to diverse stress conditions (2). It has also been implicated in many important events during cell division. Atf1 is important for activation of the spindle orientation checkpoint (3) as well as for accumulation of cells in G1 after nitrogen starvation. It is also an activator of the Anaphase promoting complex and facilitates degradation of the mitotic cyclin Cdc13 and the securin Cut2 (4,5). We have earlier reported it to be important for expression of Cdc13 as well (1). Evidently, Atf1 plays a major role in regulation of cell division in *S. pombe*. So we investigated whether Atf1 can affect the expression of any other cell cycle related genes apart from *cdc13*$^{+}$. For this we overexpressed Atf1 in *S. pombe* cells, and then looked at the changes in the transcriptional profile of the cells. Earlier reports on identification of Atf1 dependent gene expression do exist (6,7). However in those screens transcriptional changes were identified after deleting Atf1. In such a strategy, genes whose expression is controlled redundantly by an Atf1 dependent mechanism may fail to get identified. We, therefore, used the overexpression approach to facilitate identification of such genes.

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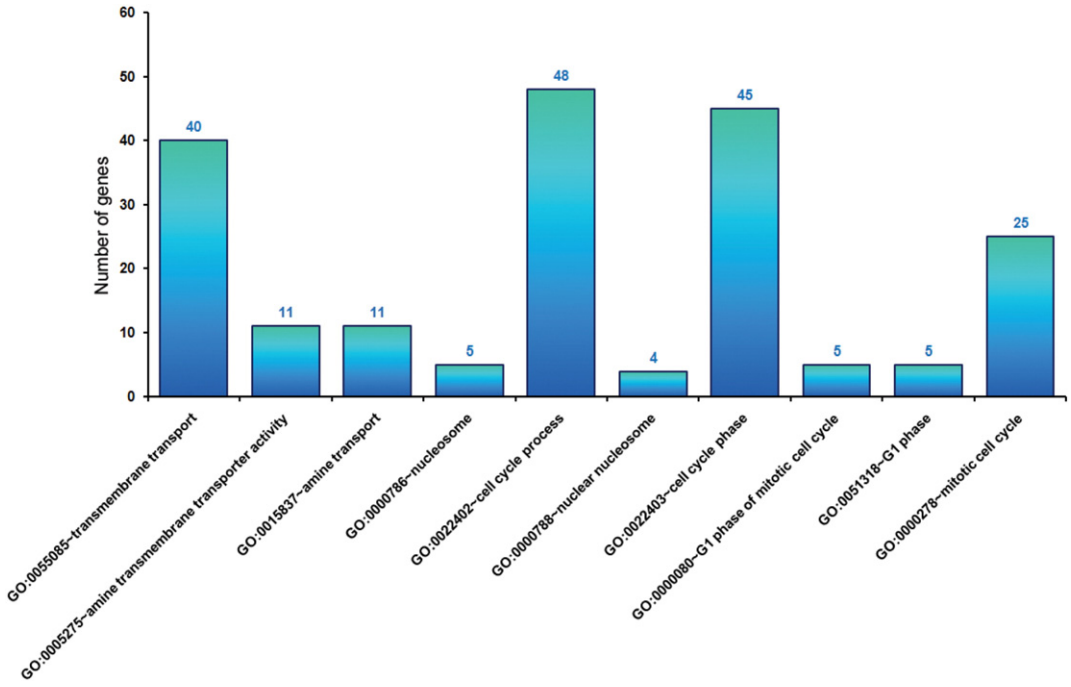

Fig. 1. Distribution of the genes induced during Atf1 overexpression in *S. pombe* cells. The number of genes corresponding to the top 10 categories of upregulated genes is shown.

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 Moreno et al. (8). All cells were grown at 30 °C in Edinburgh's Minimal Medium (EMM)-Leucine.

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 microlitres of denatured salmon sperm DNA (10 mg/ml) was added

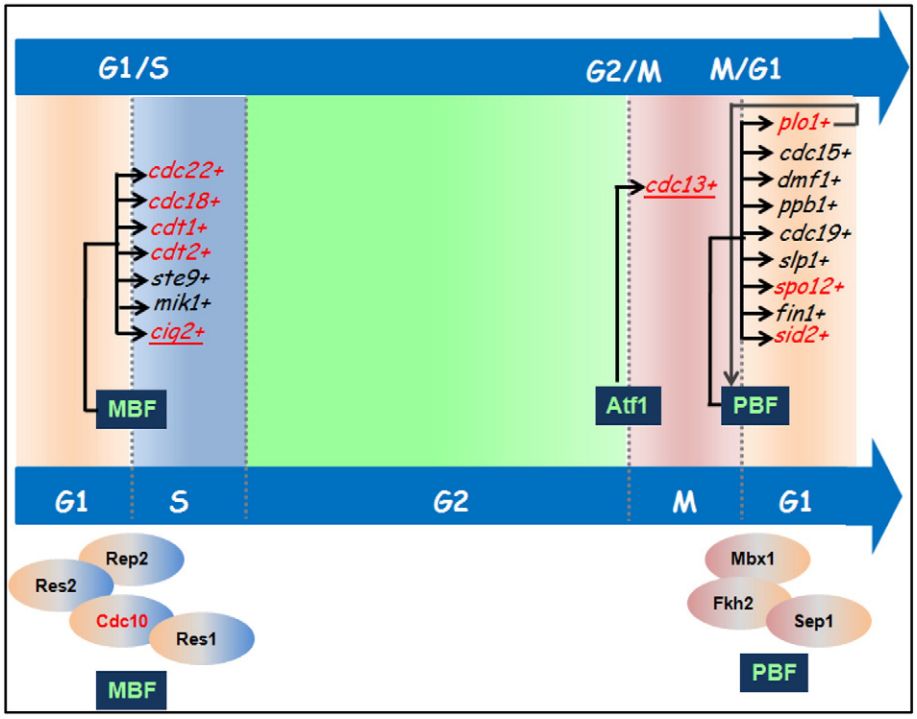

Fig. 2. Transcriptional program of *S. pombe* cell cycle: Important genes related to a particular cell cycle transition event are shown. The transcription factors regulating their expression are shown in '*blue*' boxes. Subunit composition of both MBF and PBF transcription factors are shown. Genes whose expression can be additionally regulated by Atf1 are indicated in '*red*' font.

Table 1
Functional Annotation of upregulated genes (10 most statistically significant categories).

	Term	P-Value
1	GO:0055085–transmembrane transport	8.14E-08
2	GO:0005275–amine transmembrane transporter activity	2.82E-05
3	GO:0015837–amine transport	1.76E-04
4	GO:0000786–nucleosome	0.002251
5	GO:0022402–cell cycle process	0.002924
6	GO:0000788–nuclear nucleosome	0.00299
7	GO:0022403–cell cycle phase	0.003045
8	GO:0000080–G1 phase of mitotic cell cycle	0.00394
9	GO:0051318–G1 phase	0.00394
10	GO:0000278–mitotic cell cycle	0.004082

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 ul YES and spread onto appropriate selection plates.

2.4. Overexpression of Atf1

Wild type *S. pombe* cells were transformed separately with the plasmids pGS017 (empty vector pREP41; control) or pGS018 (pREP41 + Atf1; for Atf1 overexpression, a gift from Elizabeth Veal). pGS018 contains the full length Atf1 gene 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 Atf1.

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 100midi. This array contained probes for both *Schizosaccharomyces 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.

Table 2
List of Cell Division and DNA replication genes dependent on Atf1 for expression.

Gene symbol	Representative public ID	Gene title	Fold change
cdc18	SPBC14C8.07c.S1	MCM loader	4.51
SPBC1604.19c	SPBC1604.19c.S1	TRAPP complex subunit Trs85 (predicted)	4.32
cig2	SPAPB2B4.03.S1	G1/S-specific B-type cyclin Cig2	4.02
yox1	SPBC21B10.13c.S1	Transcription factor, homeobox type Yox1 (predicted)	3.81
alp7	SPAC890.02c.S1	Microtubule protein	3.52
tas3	SPBC83.03c.S1	RITS complex subunit 3	3.43
ste7	SPAC23E2.03c.S1	Meiotic suppressor protein Ste7	3.24
mcm3	SPCC1682.02c.S1	MCM complex subunit Mcm3	3.03
pds5	SPAC110.02.S1	Cohesin-associated protein Pds5	2.96
psm3	SPAC10F6.09c.S1	Mitotic cohesin complex subunit Psm3	2.75
mid1	SPCC4B3.15.S1	Medial ring protein Mid1	2.7
plo1	SPAC23C11.16.S1	Polo kinase Plo1	2.66
cut4	SPBC106.09.S1	Anaphase-promoting complex subunit Apc1	2.62
cut7	SPAC25G10.07c.S1	Kinesin-like protein Cut7	2.57
cdc22	SPAC1F7.05.S1	Ribonucleoside reductase large subunit Cdc22	2.51
spo12	SPAC3F10.15c.S1	Spo12 family protein	2.51
ppk24	SPBC21.07c.S1	serine/threonine protein kinase Ppk24	2.49
apc2	SPBP23A10.04.S1	Anaphase-promoting complex subunit Apc2	2.42
SPCC553.01c	SPCC553.01c.S1	Meiotic chromosome segregation protein	2.41
fnx1	SPBC12C2.13c.S1	Vacuolar membrane amino acid uptake transporter Fnx1	2.38
cdc10	SPBC336.12c.S1	MBF transcription factor complex subunit Cdc10	2.37
cdt1	SPBC428.18.S1	Replication licensing factor Cdt1	2.37
isp3	SPAC1F8.05.S1	Sequence orphan	2.37
sid2	SPAC24B11.11c.S1	Sid2p-Mob1p kinase complex protein kinase subunit Sid2	2.36
cut2	SPBC14C8.01c.S1	Securin	2.34
SPAC18G6.09c	SPAC18G6.09c.S1	Sequence orphan	2.29
meu29	SPAC25H1.05.S1	Sequence orphan	2.28
swi6	SPAC664.01c.S1	Chromodomain protein Swi6	2.27
mug80	SPBC1D7.03.S1	Cyclin Clg1 (predicted)	2.24
hop1	SPBC1718.02.S1	Linear element associated protein Hop1	2.23
myp2	SPAC4A8.05c.S1	Myosin II heavy chain Myo3	2.22
cdc25	SPAC24H6.05.S1	Serine/threonine protein phosphatase Cdc25	2.22
mug123	SPCC16C4.17.S1	Meiotically upregulated gene Mug123	2.21
cdt2	SPAC17H9.19c.S1	WD repeat protein Cdt2	2.18
cdr2	SPAC57A10.02.S1	GIN4 family protein kinase Cdr2	2.17
rad8	SPAC13G6.01c.S1	Ubiquitin-protein ligase E3 (predicted)	2.15
chp1	SPAC18G6.02c.S1	Chromodomain protein Chp1	2.14
nap1	SPCC364.06.S1	Nucleosome assembly protein Nap1	2.13
mug143	SPAC167.06c.S1	Sequence orphan	2.1
SPBC660.08	SPBC660.08.S1	Sequence orphan	2.09
cdc23	SPBC1347.10.S1	MCM-associated protein Mcm10	2.06
smc5	SPAC14C4.02c.S1	Smc5–6 complex SMC subunit Smc5	2.06
SPCC594.06c	SPCC594.06c.S1	Vacuolar SNARE Vam7 (predicted)	2.05
sap1	SPCC1672.02c.S1	Switch-activating protein Sap1	2.04
but1	SPAC27D7.12.S1	Neddylation pathway protein But1	2.04
spc24	SPBC336.08.S1	Spindle pole body protein Spc24	2.04
mal2	SPAC25B8.14.S1	Kinetochore protein Mal2	2.01

3. Results and discussion

Differential gene expression was observed for genes corresponding to 3445 probes. This data was further refined by setting a ≥2.0 fold

change cut-off for differential gene expression. 372 genes satisfied this cut-off criterion. 200 of them represented genes upregulated as a result of Atf1 overexpression while the rest 73 represented genes downregulated under the experimental conditions. The Yeast Genome2.0 array contains probes for both *S. pombe* as well *as S. cerevisiae*. Given the high degree of homology of the genome sequence of both these organisms, positive hybridization results were also obviously observed for probes designed against *S. cerevisiae* genes. For functional annotation analysis, these data were excluded from the list. Functional annotation and clustering of the upregulated genes identified as mentioned above was done using DAVID functional annotation tool (9).

Functional annotation on the basis of Gene ontology classified the upregulated genes into 107 GO categories. Of these, the top 10 categories with the most significant P-values are listed in Table 1 (See Supplementary Table S1 for the complete list). 5 out of these 10 categories were found to be directly related to cell cycle.

Fig. 1 shows the distribution of the number of genes classified under each of these 10 categories. Here again it can be clearly seen that the highest number of genes were identified in the "GO: 0022402–cell cycle process" category. Thus our analysis clearly shows that Atf1 overexpression significantly alters the pattern of cell cycle related transcription in *S. pombe* cells.

Functional Annotation clustering (high stringency) of the upregulated genes was also done using DAVID, based on enrichment algorithm with annotation content coverage including Gene Ontology terms, protein–protein interactions, protein functional domains, sequence general features, homologies, gene functional summaries, etc. This analysis classified the upregulated genes into 67 clusters (See Supplementary Table S2 for complete list). The cluster with the highest enrichment score mainly consisted of genes coding for membrane proteins while the second most enriched cluster contained genes related to cell cycle processes. Again 6 of these 67 clusters represented genes directly related to cell cycle.

The results of our experiment implicate Atf1 as a general enhancer of transcription of multiple cell cycle genes in *S. pombe*. A list of the cell division and DNA replication related genes identified to be expressed in an Atf1 dependent manner in our experiment is given in Table 2. A closer scrutiny of the genes in this list revealed that Atf1 could influence multiple important stages of the *S. pombe* cell cycle. These included the G1-S as well as the M-G1 transition events.

In *S. pombe* about 747 genes with cell cycle dependent expression patterns have been identified (10–12). The transcription program during S phase entry and progression is regulated by the MBF/DSC1 transcription factor complex (similar to the mammalian E2F/DP) composed of Cdc10, Res1 and Res2 (13). Expression of genes that regulate the transition from M–G1 is controlled by the PBF transcription factor (14). Very little is known about the transcription factors that drive G2/M progression. Our lab was the first to identify that the transcription factor Atf1 controls the expression of the mitotic cyclin *cdc13*$^{+}$, related to G2/M transition in *S. pombe* (1). Fig. 2 provides a schematic illustration of genes that are known to be important for G2–M; M–G1 and G1–S transitions in the *S. pombe* cell cycle (12–14). Red font has been used to highlight the genes that were identified in our screen.

It can be easily seen from the above figure that Atf1 exerts a large influence on the transcriptional program of the *S. pombe* cell cycle. Not only can it control the levels of individual genes involved the transition events; it can also regulate the expression of their transcription factors. Hence through Plo1, Atf1 can indirectly influence the transcriptional requirements of M–G1 transition, and through Cdc10 (MBF subunit) and Yox1 (MBF regulator) it does the same for G1/S transition. Our observations implicate Atf1 at the core of the transcriptional program of cell cycle progression, with a potential to support activation of all of the phase specific transcriptional programs.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.09.014.

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