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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**A B S T R A C T**

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.

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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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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...
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 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 0.1 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 ±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 in 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) in yeast 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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References

[1] S. Bandyopadhyay, I. Dey, M. Suresh, G. Sundaram, The basic leucine zipper domain transcription factor Atf1 directly controls Cdc13 expression and regulates mitotic entry independently of Wee1 and Cdc25 in Schizosaccharomyces pombe, Eukaryot. Cell 13 (6) (2014) 813–821 (2014 Jun.).
[2] K. Shiomiaki, P. Russel, Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast, Genes Dev. 10 (1996) 2276–2288.
[3] S. Olliferenko, M.K. Balasubramanian, Astral microtubules monitor metaphase spindle alignment in fission yeast, Nat. Cell Biol. 4 (2002) 816–820.
[4] A. Orsi, M. Girmanli, Y. Kinata, C.R. Wilkinson, N. Jones, H. Yamano, The transcription factor Atf1 binds and activates the APC/C ubiquitin ligase in fission yeast, J. Biol. Chem. 284 (2009) 23,989–23,994.
[5] Y. Kawaiaki, K. Nagao, T. Nakamura, M. Yanagida, Fission yeast MAP kinase is required for the increased securnin–separate interaction that rescues separate mutants under stresses, Cell Cycle 5 (2006) 1831–1839.
[6] M. Sansó, M. Gogol, J. Ayé, C. Seidel, E. Hidalgo, Transcription Factors Per1 and Atf1 have distinct roles in stress- and Styl-dependent gene regulation, Eukaryot. Cell 7 (2008) 826–835.
[7] W. Dongrong Chen, M. Toone, J. Mata, R. Lyne, G. Burns, K. Kiviven, A. Brazma, N. Jones, J. Bähler, Global transcriptional responses of fission yeast to environmental stress, Mol. Biol. Cell 14 (2003) 214–229.
[8] S. Moreno, A. Klar, P. Nurse, Molecular genetic analysis of fission yeast Schizosaccharomyces pombe, Methods Enzymol. 194 (1991) 795–823.
[9] D.W. Huang, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (1) (2009) 44–57.
[10] Oliva A, Rosebrock A, Ferrezuelo F, Pyne S, Chen H, Skiena S, Fitcher B, Leatherwood J. The cell cycle-regulated genes of Schizosaccharomyces pombe. PLoS ONE Biol. 37 e225.
[11] X. Peng, R.K.M. Karuturi, L.D. Miller, K. Lin, Y. Jia, P. Kondo, L. Wang, L.S. Wong, E.T. Liu, M.K. Balasubramanian, J. Liu, Identification of cell cycle-regulated genes in fission yeast, Mol. Biol. Cell 16 (March 2005) 1026–1042.
[12] McInerny CJ. 2004. Cell cycle-regulated transcription in fission yeast, Biochem. Soc. Trans. Volume 32, part 6.
[13] B. Baum, J. Wuarin, P. Nurse, Control of S-phase periodic transcription in the fission yeast mitotic cyclin, EMBO J. 16 (15) (1997) 4675–4688.
[14] M. Agarwal, K. Papadopoulou, A. Mayevus, V. Yaylara, D.M. Quintana, A. Paoletti, C.J. McInerny, Mid1p-dependent regulation of the M–G1 transcription wave in fission yeast, J. Cell Sci. 123 (2010) 4366–4373.