FEMS Microbiology Letters, 364, 2017, fnx132

doi: 10.1093/femsle/fnx132
Advance Access Publication Date: 22 June 2017
Research Letter

RESEARCH LETTER – Physiology & Biochemistry

Antagonistic regulation of cyclin expression by the bZIP transcription factors Pcr1 and Atf1 during G2/M transition

Sushobhana Bandyopadhyay[†], Protiti Maiti Ghosh[#], Sohini Basu[#], Madhurima Paul, Syed Benazir Alam, Elizabeth Das and Geetanjali Sundaram[*]

Department of Biochemistry and Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata-700019, West Bengal, India

[*]**Corresponding author:** Department of Biochemistry and Centre for Research in Nanoscience and Nanotechnology, University of Calcutta, 35, Ballygunje Ballygunge Circular Road, Kolkata-700019, West Bengal, India. Tel: +(91)9433191982; E-mail: geetanjali.sundaram@gmail.com
One sentence summary: The antagonistic effects of the bZIP transcription factors for expression of the mitotic cyclin cdc13 can regulate mitotic entry decisions in fission yeast.
[#]These two authors contributed equally to this work.
[†]**Present address:** Department of Biochemistry & Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605.
Editor: Jana Jass

ABSTRACT

The transcription factor Atf1 is known to promote cell survival during various stress conditions in *Schizosaccharomyces pombe* by activating the expression of appropriate genes. It can also activate transcription of other important genes responsible for cell cycle progression. An Atf1-dependent increase in the expression of cell division promoting genes will oppose activation of checkpoints necessary to ensure repairs and cell survival during stress. Hence, selective inhibition of the cell cycle-related functions of Atf1 would be indispensable for cellular survival during stress. Here we present evidence in favour of selective inhibition of Atf1's ability to activate *cdc13*$^{+}$ transcription. We show that the transcription factor Pcr1 can specifically inhibit the recruitment of Atf1 on *cdc13* promoter and thereby prevent Atf1-mediated mitotic acceleration. We also show that this opposition of Atf1 functions by Pcr1 extends to the G1-S transition event as well. Altogether these results suggest a previously unknown antagonistic function of Atf1 and Pcr1 in regulating Cdc13 expression during cell cycle progression.

Keywords: *S. pombe*; Atf1; Pcr1; Cdc13 bZIP; Mitosis

INTRODUCTION

In *Schizosaccharomyces pombe*, six bZIP transcription factors, namely, Atf1, Pcr1, Pap1, Zip1, Atf21 and Atf31, have been reported (Takeda *et al.* 1995; Watanabe and Yamamoto 1996; Ohmiya *et al.* 1999; Fujii *et al.* 2000). Atf1 regulates the transcriptional response of *S. pombe* to changes in the external environment (Shiozaki and Russell 1996; Wilkinson *et al.* 1996; Sansó *et al.* 2008). It can bind to DNA either as a homodimer or as a heterodimer with another bZIP transcription factor Pcr1 (Wahls and Smith 1994; Watanabe and Yamamoto 1996; Kanoh *et al.* 1996) and also controls meiotic recombination, sexual differentiation and heterochromatin formation (Wahls and

Received: 27 September 2016; **Accepted:** 20 June 2017

Table 1. List of strains and plasmids used in this study.

Strain/plasmid number	Genotype/description	Source
GSY001	*h- leu1–32 ura4-D18*	Paul Russell
GSY014	*h- wee1::ura4+leu1–32ura4-D18*	Yeast Genetic Resource Centre
GSY027	*h- atf1::ura4+leu1–32ura4-D18*	Kazuhiro Shiozaki
GSY048	*h- leu1–32 ura4-D18 cdc25–22*	Paul Russell
GSY117	*h- leu1–32 ura4-D18 atf1::ura4+cdc25–22*	Paul Russell
GSY436	*h- leu1–32 ura4-D18 atf1HA6His:ura4+*	Kazuhiro Shiozaki
GSY344	*h- pcr1::ura4+leu1–32*	Makoto Kawamukai
pGS017	pREP41	Yeast Genetic Resource Centre
pGS044	pREP41+Pcr1	This study
pGS083	pREP4k+Atf1	This study
pGS064	pREP4k	Iain Hagan
pGS018	pREP41+Atf1	Elizabeth A. Veal

Smith 1994; Wu and Lichten 1994; Kon *et al.* 1997; Yamada *et al.* 2004; Gao, Davidson and Wahls 2008). It is also necessary for the accumulation of the cells in G1 during nitrogen starvation (Shiozaki and Russell 1996) and also associated with the spindle orientation checkpoint (Kawasaki *et al.* 2006). It also regulates the anaphase-promoting complex (Ors *et al.* 2009). We have established Atf1 as a regulator of the transcriptional program of the cell cycle in *S. pombe* (Bandyopadhyay *et al.* 2015). It controls the expression of the mitotic cyclin Cdc13 in *S. pombe* and thereby determines mitotic timing. In fact, enhanced Atf1 levels can override the G2/M checkpoint (Bandyopadhyay *et al.* 2014). Our reports indicate that enhanced Atf1 activity can potentially accelerate phase transitions during the cell cycle. Interestingly, during stress responses, Atf1 activity is known to increase but there is no acceleration of the cell cycle. Survival during these adverse conditions is, therefore, determined by the cell's ability to check the cell cycle accelerating function of Atf1 without compromising the ability of Atf1 to target stress response genes. This would obviously require a selective discrimination between Atf1's target DNA binding sites during stress conditions. In this study, we illuminate the potential mechanism allowing selective participation of Atf1 in the stress response program, without engaging in cell cycle functions. Here we show that Pcr1 can perform this very important regulatory function. Our findings, therefore, unveil a novel mechanism of eukaryotic cell cycle regulation via the antagonistic interactions between bZIP transcription factors.

MATERIALS AND METHODS

Strains, media and growth conditions

Table 1 lists the strains used in this study. Growth conditions were as described by Moreno, Klar and Nurse (1991). Media used was YES and temperature was 30°C or as mentioned. For overexpression, experiments cells were grown overnight in Edinburgh Minimal Medium (EMM) without leucine supplemented with 20 μM thiamine. Subsequently, they were washed and resuspended at 1:6 dilution into media containing no thiamine and grown for another 24 h.

Microscopy

Seventy per cent ethanol was used for fixing the cells. Imaging was done using the Olympus BX51 Fluorescence Microscope at $\times$100 magnification. ImageJ was used for cell length quantification (Schneider, Rasband and Eliceiri 2012).

Viability assays

Tenfold serial dilutions of cultures of exponentially growing cells were made. These dilutions (5 μl) were spotted onto suitable media plates. Photography of these plates was performed after incubation for 4 days at a particular temperature.

Schizosaccharomyces pombe transformations

Transformations were done as described previously (Bandyopadhyay *et al.* 2014).

Cloning of Pcr1 and Atf1

The full-length *pcr1*$^+$ gene was amplified from genomic DNA of wild-type *S. pombe* cells. The gene was then inserted between the Sal1 and Sma1 sites of pREP41 vector.

Forward: ATATGTCGACCAAGATGACTGCCAAA
Reverse: TAACCCGGGTTCTTTTTCAGATGGGC

The full-length *atf1*$^+$ gene was amplified from genomic DNA of wild-type *S. pombe* cells. The gene was then inserted between the Nde1 and Sma1 sites of pREP4K vector.

Forward: TATTGGTTTCATATGTCCCCGTCTCCC
Reverse: ATCCCGGGGGCTAGTACCCTAAATTG

RNA isolation and real-time PCR

RNA isolation and qPCR was done as described previously (Bandyopadhyay *et al.* 2014).

Primers used for real-time PCR are as follows:
Cdc13 Forward: GGATGACTACCCGTCGTTTAAC
Cdc13 Reverse: TGGAAGACACACAGTGGCTTTCTT
Atf1Forward: GTACTCAGCAACAGCCTATGT
Atf1 Reverse: CGTGGTTTGGCGTAATGTTAAT
Pcr1 Forward: CTCACAATGCTCTCCACTCTC
Pcr1Reverse: TTGAGTAGGGCTAACGGAAAC
18S rRNA Forward: TGTACTGTGAAACTGCGAATGGCTC
18S rRNA Reverse: GCAAGGCCATGCGATTCGAA

Preparation of denatured cell extracts and immunoblotting

Denatured cell extracts were prepared as described previously (Bandyopadhyay *et al.* 2014).

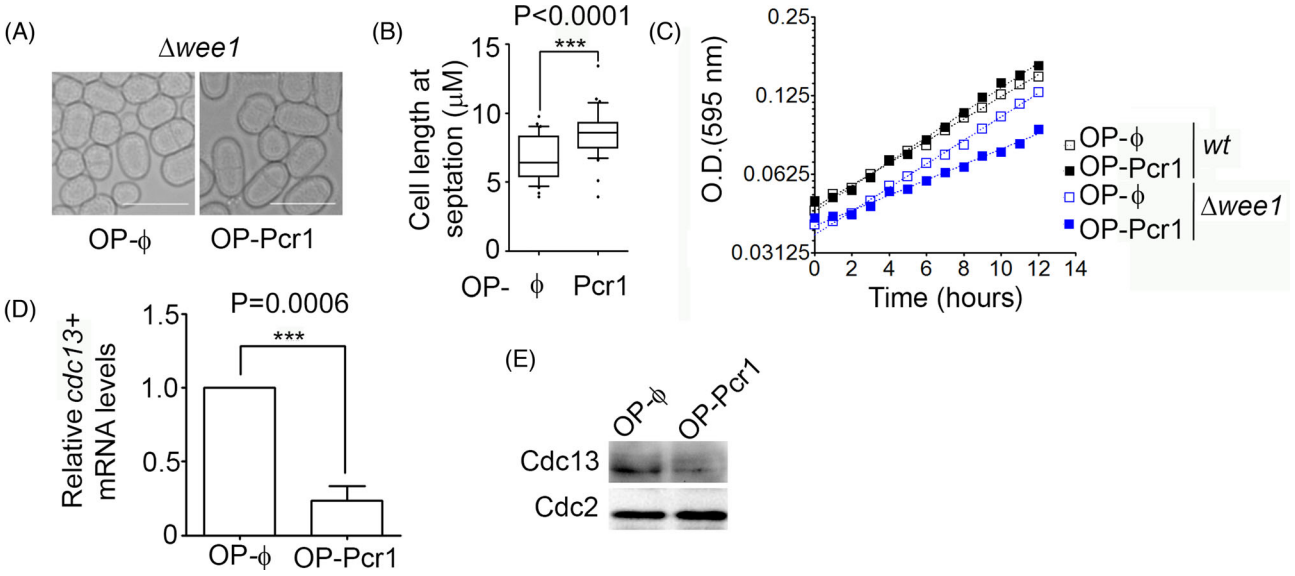

Figure 1. Pcr1 overexpression delays mitotic initiation in *S. pombe* cells. (**A**) Bright field images of Δ*wee1* cells transformed with pREP41 (φ) or pREP41 + Pcr1. For overexpression, cells were grown in EMMleucine–thiamine for 24 h at 30°C, fixed and then processed for imaging. Bar 10 μm. (**B**) Quantification of the length of the cells shown in using ImageJ software. Statistical analysis was done using the Graph Pad Prism application, *** indicates $P < 0.001$. (**C**) Growth rates of *wt* and Δ*wee1* cells overexpressing Pcr1 were determined at 30°C. Data represent means of three independent experiments. (**D**) qPCR analysis of *cdc13*$^+$ expression in wild-type cells overexpressing Pcr1. 18s rRNA expression was used for normalisation. Data represent mean of three independent experiments. Statistical analysis was done using Graph Pad Prism application. *** indicates, $P < 0.001$. (**E**) Pcr1 was overexpressed in wild-type *S. pombe* cells, and the levels of the Cdc13 protein were detected by immunoblotting. All data are representative of three independent experiments.

Chromatin immunoprecipitation analysis

ChIP assays for testing the binding of Atf1 were done as described before (Bandyopadhyay *et al.* 2014) with some modifications. Mock IgG was used for immunoprecipitations to normalise the background (18S ORF was used to determine the validity of the results).

DNA content analysis by flow cytometry

Cells were fixed in 70% ethanol. The fixed cells were washed and rehydrated in 50 mM sodium citrate buffer. The cells were resuspended in 500 μl of 50 mM sodium citrate buffer containing 0.1 mg/ml of RNase A and incubated at 37°C for 4 h. Propidium iodide (2 μg/ml) was used for DNA staining.

RESULTS AND DISCUSSION

Pcr1 overexpression delays mitotic initiation in *Schizosaccharomyces pombe*

Pcr1 is a well-characterised binding partner for Atf1, and the heterodimeric complex of these two bZIP domain containing proteins is a known activator of expression of stress response genes in *S. pombe*. Recently, in a screen for transcription factor overexpression phenotypes Pcr1 was found to cause an increase in cell length of *S. pombe* cells (Vachon *et al.* 2013). This indicated that enhanced Pcr1 levels may lead to mitotic delay. To further substantiate this specific role of Pcr1 in promoting mitotic delay, we overexpressed Pcr1 in Δ*wee1 S. pombe* cells. Wee1 is responsible for adding the inhibitory phosphorylation on Cdc2 (CDK) and is, therefore, an inhibitor of mitotic entry. In absence of Wee1, cells are relieved of this inhibition and enter prematurely into mitosis resulting in shorter cell lengths. We found that Pcr1 overexpression rescued the cells from this premature mitosis and increased the length of Δ*wee1* cells from 6.9 to 8.9 μm (Fig. 1A and B). We also overexpressed Pcr1 in *wt* and Δ*wee1* cells and found that Pcr1 overexpression decreased the growth rate of Δ*wee1* cells significantly (Fig. 1C). Thus, in contrast to Atf1, overexpression of Pcr1 has an inhibitory effect on G2/M progression in *S. pombe* cells. Pcr1 can, therefore, delay mitotic entry in *S. pombe*. [NOTE: In wild-type cells, however, no such decrease in growth rate was seen. Possibly, wild-type cells can resist the Pcr1 overexpression-mediated delay via backup mechanisms that need further investigation]. We have reported earlier that increase in Cdc13 expression is responsible for the mitosis accelerating ability of Atf1 (Bandyopadhyay *et al.* 2014). So we then checked whether the mitotic delay caused by Pcr1 also occurs via modulation of *cdc13*$^+$ expression. Indeed, we found that Pcr1 overexpression led to decrease in the *cdc13*$^+$ transcript (Fig. 1D) and Cdc13 protein levels (Fig. 1E). Hence, with respect to Cdc13 expression in *S. pombe* cells, Atf1 and Pcr1 clearly have antagonistic functions.

Pcr1 overexpression inhibits the recruitment of Atf1 on the *cdc13* promoter

The above-mentioned results implicate Pcr1 as promising potential mediator of a regulatory mechanism for checking the cell cycle accelerating functions of Atf1. If Pcr1 does have that ability, then overexpression of Pcr1 should rescue the *cdc25–22* cells from the Atf1-dependent checkpoint override reported earlier (Bandyopadhyay *et al.* 2014). Indeed, we found that *cdc25–22* mutants expressing high levels of Pcr1 along with Atf1 got arrested at the G2/M boundary with an elongated cell morphology that is characteristic of these mutants (Fig. 2A). This result confirms that Pcr1 can oppose the ability of Atf1 to accelerate mitotic entry. This antagonistic effect of Pcr1 and Atf1 on mitotic entry potentially indicates two possibilities. Either Pcr1 may inhibit the binding of Atf1 to the *cdc13* promoter or it may compete with Atf1 to do so and function directly as a repressor of *cdc13*$^+$ transcription. The former possibility will require Pcr1 to function in

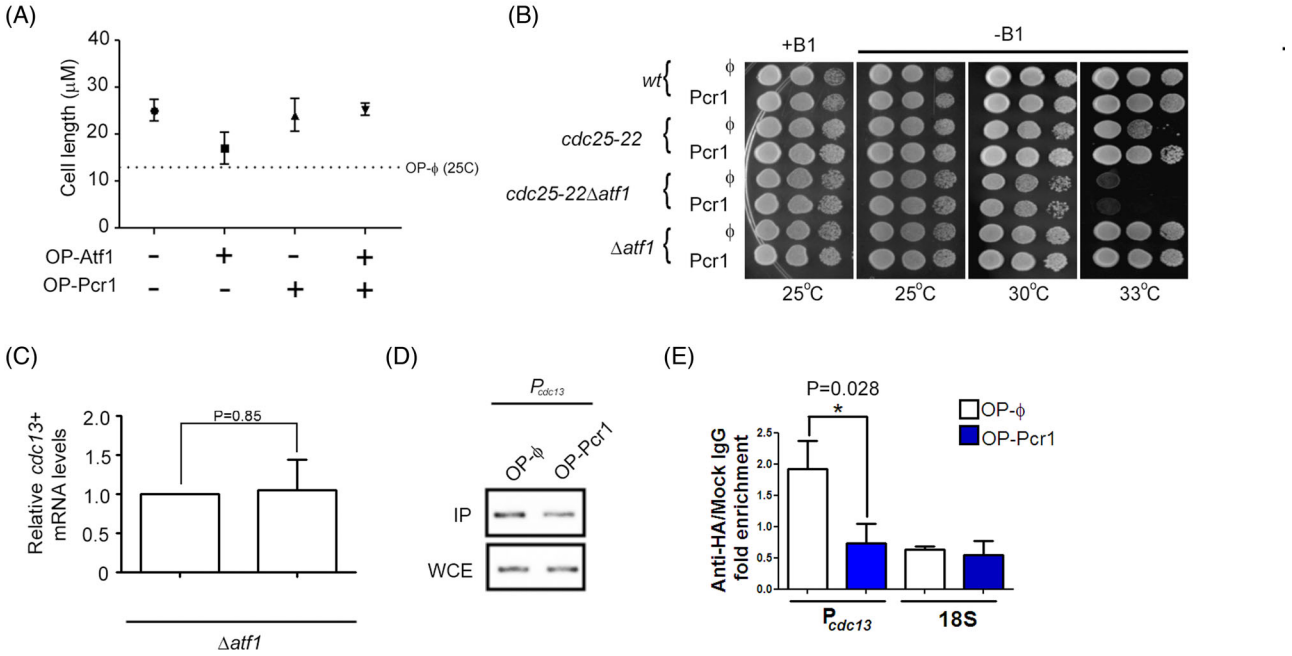

Figure 2. Pcr1 inhibits recruitment of Atf1 on *cdc13* promoter. (**A**) Quantification of the cell length due to simultaneous overexpression of Atf1 and Pcr1 in *cdc25–22* cells. For overexpression, cells were grown in EMM–leucine–thiamine for 24 h at 25°C, shifted to 37°C for 4 h, fixed and then processed for imaging. Data represent mean (±s.d.) of median cell lengths measured from three independent experiments. (**B**) *wt*, *cdc25–22*, *cdc25–22Δatf1* and *Δatf1 cells* transformed with pREP41 (*φ*) or pREP41 + Pcr1 were grown to log phase, and serial dilutions were then spotted onto EMM–leucine±thiamine plates. The plates were incubated at the indicated temperatures for 4 days before being photographed. Data are representative of three independent experiments. (**C**) qPCR analysis of *cdc13*$^+$expression in *Δatf1* cells overexpressing pREP41 (*φ*) or pREP41 + Pcr1 at 30°C. 18s rRNA expression was used for normalisation. Data represent mean of three independent experiments. Statistical analysis was done using Graph Pad Prism application. (**D**) *Schizosaccharomyces pombe* cells carrying an HA-6His-tagged genomic copy of Atf1 were transformed with pREP41 (*φ*) or pREP41 + Pcr1 and incubated for 24 h at 30°C in EMM–leucine– thiamine media. Cells were harvested and lysed after formaldehyde cross-linking, and chromatin bound to Atf1-HA was obtained after immunoprecipitation with anti–HA antibodies (IP-anti-HA). Recovered DNA was analysed by PCR amplification with primers designed against *cdc13* promoter (P_{cdc13}). WCE, whole-cell extracts before immunoprecipitation. (**E**) qPCR analysis of the P_{cdc13} levels in immunoprecipitated samples (IP-anti-HA) used in (D). Non-specific target (18S: internal region of 18S rRNA gene ORF) was used for normalisation. Statistical analysis was done using Graph Pad Prism application. * indicates, $P < 0.05$. Data represent mean of three independent experiments. All data are representative of three independent experiments.

an Atf1-dependent manner. We argued that if Pcr1 functions independently of Atf1, overexpression of Pcr1 should be able to rescue the enhanced *ts* phenotype of the *Δatf1cdc25–22* mutants. However, we found that Pcr1 overexpression did not change the viability of the *Δatf1cdc25–22* cells (Fig. 2B), indicating that Pcr1 inhibits Atf1 and thereby represses *cdc13*$^+$. Our observation that Pcr1 overexpression fails to repress *cdc13*$^+$ transcription in *Δatf1* cells (Fig. 2C) also supported this possibility. This was further confirmed by our observation that Pcr1 overexpression led to a decrease in Atf1 recruitment on the *cdc13* promoter (Fig. 2D and E). Thus, Pcr1 overexpression delays mitosis by inhibiting recognition of *cdc13* promoter by Atf1 and thereby reducing the *cdc13*$^+$ expression in *S. pombe* cells.

Interestingly, we found that Pcr1 overexpression rescues the *ts* phenotype of the *cdc25–22* mutants (Fig. 2B). Further investigation is however necessary to understand the reason for this observation.

Pcr1 is important for inhibition of Atf1-dependent activation of *cdc13*$^+$ expression during oxidative stress

We then investigated the physiological relevance of our observations. Atf1 and Pcr1 together are known to be important for transcription of genes important for survival during oxidative stress (H_2O_2 treatment). Atf1 expression is high under such conditions (Chen *et al.* 2003; Sansó *et al.* 2008). Therefore, Atf1-dependent targeting of *cdc13* promoter requires selective inhibition under such conditions in order to support cell survival. Data from genome-wide experiments show that *cdc13*$^+$ expression is, in fact, less in cells treated with H_2O_2 and that both Atf1 and Pcr1 expression are high during these conditions (Chen *et al.* 2003). We validated these results using qPCR and confirmed that *cdc13*$^+$ expression is repressed after treatment with 0.6 mM H_2O_2 for 30 min (Fig. 3A). Hence, a mechanism for selective inhibition of Atf1-dependent increase in expression of cell cycle-related genes does also operate in *S. pombe* cells under physiological conditions. Under these conditions, *cdc13*$^+$ expression in *Δpcr1* cells actually increases, in contrast to the repression observed in *wt* cells (Fig. 3B). Thus, in absence of Pcr1, the Atf1-dependent increase in *cdc13*$^+$ expression cannot be prevented.

All these results implicate Pcr1 as an important negative regulator of Atf1-dependent expression of cell cycle genes during oxidative stress. The fact that Pcr1 expression levels are slightly higher than Atf1 during oxidative stress would also ensure that the above-mentioned inhibition would prevail. All earlier reports of functional dependency between Atf1 and Pcr1 have shown the latter to assist Atf1 in its transcription-related functions. This is the first report of Pcr1 inhibiting Atf1 functions.

We then checked whether such a regulatory interaction between Atf1 and Pcr1 extends beyond the G2-M transition. In *S. pombe*, hydroxyurea (HU) treatment is known to cause S phase arrest and activates a G1/S checkpoint. We found that Atf1 overexpression delayed the G1/S checkpoint activation after 20 mM HU treatment. In fact, upon 10 mM HU treatment, no change in DNA replication kinetics was seen in Atf1-overexpressing cells

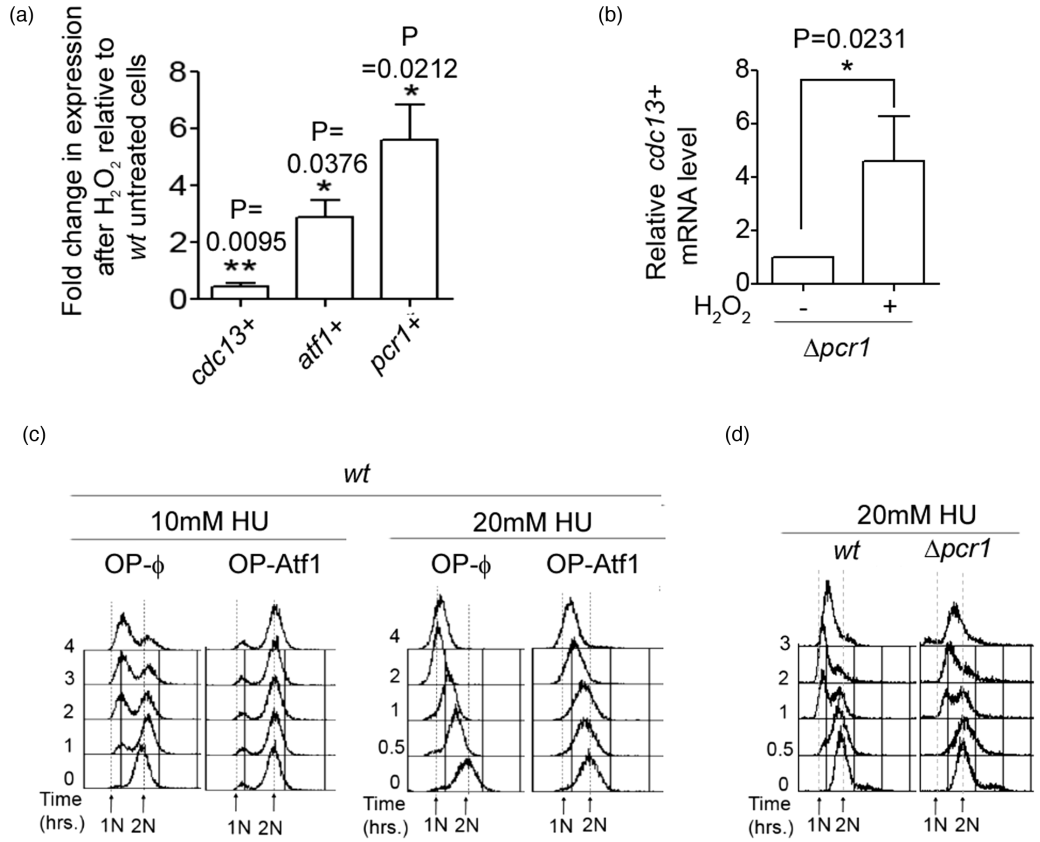

Figure 3. Pcr1 is important for *cdc13*$^+$ repression during oxidative stress. (**A**) qPCR analysis of the expressions of *cdc13*$^+$, *atf1*$^+$ and *pcr1*$^+$ in wild-type cells treated with 0.6 mM hydrogen peroxide for 30 min. 18S rRNA expression was used for normalisation. Bars represent fold change in expression after H_2O_2 treatment relative to *wt* untreated cells. Data represent mean of three independent experiments. Statistical analysis was done using Graph Pad Prism application. * indicates, $P < 0.05$; ** indicates $P < 0.01$. (**B**) qPCR analysis of *cdc13*$^+$ expression in Δ*pcr1* cells treated with 0.6 mM hydrogen peroxide for 30 min. 18S rRNA expression was used for normalisation. Data represent mean of three independent experiments. Statistical analysis was done using Graph Pad Prism application. * indicates, $P < 0.05$. (**C**) Flow cytometric analysis of DNA content of exponentially *S. pombe* cells overexpressing Atf1 after treatment with 10 or 20 mM hydroxyurea, for the indicated time periods. (**D**) Flow cytometric analysis of DNA content of exponentially growing cultures of wild-type and Δ*pcr1* cells after being treated with 20 mM hydroxyurea for the indicated time periods. All data are representative of three independent experiments. The differences with respect to the profile in Fig. 3C are due to the fact that cells are grown on YES.

while the *wt* control cells got arrested in S phase (Fig. 3C). Thus, Atf1 can also inhibit G1/S checkpoint activation. HU is a known inhibitor of ribunucleotide reducatase (RNR). Atf1 overexpression has already been shown to result in increase of expression of *cdc22+* which codes for RNR large subunit (Bandyopadhyay *et al.* 2015). Our observations are therefore consistent with the report that Atf1 can increase transcription of many genes essential for G1-S transition including *cdc22*$^+$ (Bandyopadhyay *et al.* 2015). We further observed that deletion of Pcr1 (like Atf1 overexpression) also led to a delay in G1/S checkpoint activation (Fig. 3D). Thus, Atf1 and Pcr1 have contrasting effects on both G2/M and G1/S transitions. Atf1 can accelerate these transitions while the latter delays them. Our results implicate Pcr1 to be a major player in the cellular mechanism responsible for selectively inhibiting Atf1's ability to control transcription in a manner fine-tuned to the transcriptional requirements of a specific cell cycle stage or changes in external environment. Cell cycle or stress-dependent changes in the balance of Atf1 and Pcr1 activities in the cells may, therefore, be expected to contribute significantly towards controlling phase transition decisions in *S. pombe*. Our observations thus bring to light the presence of a novel regulatory mechanism that can control cell cycle progression in *S. pombe*.

ACKNOWLEDGEMENT

SB, PG and SB received fellowship from the Department of Biotechnology, Govt of India–Interdisciplinary Program in Life Sciences–University of Calcutta (DBT-CU IPLS) program, Department of Science and Technology–Woman Scientist–A (DST-WOS-A) fellowship and Department of Science and Technology–Science and Engineering Research Board (DST-SERB) fellowship respectively. MP received fellowship from University Grants Commission–Research Fellowship for Meritorious Students (UGC-RFSMS). We thank Department of Special Assistance–University Grants Commission (DSA-UGC), DST–Funds for Improvement of S&T Infrastructure (DST-FIST), Centre for Advanced Studies (CAS) and DBT-IPLS, Department of Biochemistry, CU, for infrastructural support. We thank Paul Russell (Scripps Research Institute), Kazuhiro Shiozaki (NIST, Japan), Iain Hagan (University of Manchester, UK) and Makoto Kawamukai (Shimane University) for gift of strains and plasmids.

Conflict of interest. None declared.

REFERENCES

Bandyopadhyay S, Sundaram G. Genome wide transcription profiling reveals a major role for the transcription factor Atf1 in regulation of cell division in *Schizosaccharomyces pombe*. *Genomics Data* 2015;**6**:184–7.

Bandyopadhyay S, Dey I, Suresh M *et al*. 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**;2014;813–21.

Chen D, Toone WM, Mata J *et al*. Global transcriptional responses of fission yeast to environmental stress. *Mol Biol Cell* 2003;**14**:214–29.

Fujii Y, Shimizu T, Toda T *et al*. Structural basis for the diversity of DNA recognition by bZIP transcription factors. *Nat Struct Biol* 2000;**7**:889–93.

Gao J, Davidson MK, Wahls WP. Distinct regions of ATF/CREB proteins Atf1 and Pcr1 control recombination hotspot *ade6–M26* and the osmotic stress response. *Nucleic Acids Res* 2008;**36**:2838–51.

Kanoh J, Watanabe Y, Ohsugi M *et al*. *Schizosaccharomyces pombe* gad7_ encodes a phosphoprotein with a bZIP domain, which is required for proper G1 arrest and gene expression under nitrogen starvation. *Genes Cells* 1996;**1**:391–408.

Kawasaki Y, Nagao K, Nakamura T *et al*. Fission yeast MAP kinase is required for the increased securin-separase interaction that rescues separase mutants under stresses. *Cell Cycle* 2006;**5**:1831–9.

Kon N, Krawchuk MD, Warren BG *et al*. Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the M26 meiotic recombination hotspot in *Schizosaccharomyces pombe*. *P Natl Acad Sci USA* 1997;**94**:13765–70.

Moreno S, Klar A, Nurse P. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* 1991;**194**:795–823.

Ohmiya R, Kato C, Yamada H *et al*. Isolation of multicopy suppressors of the calcium sensitivity of a mutant lacking the bZIP transcription factor Atf1 in fission yeast. *Mol Gen Genet* 1999;**261**:297–306.

Ors A, Grimaldi M, Kimata Y *et al*. The transcription factor Atf1 binds and activates the APC/C ubiquitin ligase in fission yeast. *J Biol Chem* 2009;**284**:23989–94.

Sansó M, Gogol M, Ayté J *et al*. Transcription factors Pcr1 and Atf1 have distinct roles in stress- and StyI-dependent gene regulation. *Eukaryot Cell* 2008;**7**:826–35.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;**9**:671–5.

Shiozaki K, Russell P. Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1transcription factor in fission yeast. *Gene Dev* 1996;**10**:2276–88.

Takeda T, Toda T, Kominami K *et al*. *Schizosaccharomyces pombe* atf1_ encodes a transcription factor required for sexual development and entry into stationary phase. *EMBO J* 1995;**14**:6193–208

Vachon L, Wood J, Kwon EJG *et al*. Functional characterization of fission yeast transcription factors by overexpression analysis. *Genetics* 2013;**194**:873–84.

Wahls WP, Smith GR. A heteromeric protein that binds to a meiotic homologous recombination hot spot: correlation of binding and hot spot activity. *Gene Dev* 1994;**8**:1693–702.

Watanabe Y, Yamamoto M. *Schizosaccharomyces pombe pcr1*$^{+}$ encodes a CREB/ATF protein involved in regulation of gene expression for sexual development. *Mol Cell Biol* 1996;**16**:704–11.

Wilkinson MG, Samuels M, Takeda T *et al*. The Atf1 transcription factor is a target for the Sty1 stress- activated MAP kinase pathway in fission yeast. *Gene Dev* 1996;**10**:2289–301.

Wu TC, Lichten M. Meiosis-induced double-strand break sites determined by yeast chromatin structure. *Science* 1994;**263**:515–8.

Yamada T, Mizuno K, Hirota K *et al*. Roles of histone acetylation and chromatin remodeling factor in a meiotic recombination hotspot. *EMBO J* 2004;**23**:1792–803.