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Increase in Fru-2,6-P_2 levels results in altered cell division in *Schizosaccharomyces pombe*

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Abstract

Mitogenic response to growth factors is concomitant with the modulation they exert on the levels of Fructose 2,6-bisphosphate (Fru-2,6-P_2), an essential activator of the glycolytic flux. In mammalian cells, decreased Fru-2,6-P_2 concentration causes cell cycle delay, whereas high levels of Fru-2,6-P_2 sensitize cells to apoptosis. In order to analyze the cell cycle consequences due to changes in Fru-2,6-P_2 levels, the bisphosphatase-dead mutant (H258A) of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase enzyme was over-expressed in *Schizosaccharomyces pombe* cells and the variation in cell phenotype was studied. The results obtained demonstrate that the increase in Fru-2,6-P_2 levels results in a defective division of *S. pombe*, as revealed by an altered multisepted phenotype. The H258A-expressing cells showed impairment of cytokinesis, but normal nuclear division. In order to identify cellular mediators responsible for this effect, we transformed different *S. pombe* strains and observed that the cytokinetic defect was absent in cells defective for Wee1 kinase function. Therefore, in *S. pombe*, Wee1 integrates the metabolic signal emerging from changes in Fru-2,6-P_2 content, thus coupling metabolism with cell proliferation. As the key regulators of the cell cycle checkpoints are conserved throughout evolution, these results may help to understand the experimental evidences obtained by manipulation of Fru-2,6-P_2 levels in mammalian cells.
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Keywords: Glycolysis; Fru-2,6-P_2; Proliferation; Cell cycle control; Fission yeast

1. Introduction

One of the essential processes for cell cycle progression is the activation of the glycolytic flux. This metabolic change is essential for providing the energy and the biosynthetic precursors that cells require for entering the cell cycle. Glycolysis is stimulated when growth-promoting agents are added to quiescent cells and in many types of transformed cells. The major regulatory step in the glycolytic pathway is regulated by 6-phosphofructo-1-kinase activity. Activation of glycolysis involves the increase in Fructose 2,6-bisphosphate (Fru-2,6-P_2) levels, a potent allosteric stimulator of 6-phosphofructo-1-kinase [1]. Interestingly, the growth factors and mitogens leading to cell proliferation increase Fru-2,6-P_2 levels, which triggers the activation of the glycolytic flux as part of the cell division process [2].

Fru-2,6-P_2 content is regulated by the bi-functional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PF2KFB), which catalyzes both its synthesis and degradation (reviewed in [3]). Four different genes encoding isoenzymes (PF2KFB1–4) have been found in mammals. In accordance with the role of Fru-2,6-P_2 in cell proliferation, growth factors and transforming

Abbreviations: Fru-2,6-P_2, Fructose 2,6-bisphosphate; PF2KFB, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; TIGAR, TP53-induced glycolysis and apoptosis regulator; HA, hemagglutinin; DAPI, 4′,6-Diamidino-2-phenylindole

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oncogenes modulate the activity and expression of several PF2KFB isoforms. F-type and PF2KFB3 isoenzymes are constitutively expressed in proliferating tissues, in transformed cell lines and various tumours [4,5]. Mitogenic signals regulate F-type expression through MAPK and PI3K/Akt cascades [6,7], and AP1 and E2F oncogenic proteins are important for this effect [8,9]. Furthermore, the PF2KFB3 isoenzyme is mainly regulated by hypoxia [10], and has been recently proven to be an essential downstream metabolic mediator of oncogenic Ras transformation [11].

The links between Fru-2,6-P_2 cell content and cell cycle state raise the question whether changes in the concentration of this metabolite would alter the cell division process. Different reports have demonstrated that Fru-2,6-P_2 levels increase in different proliferative states. This increase occurs during G1/S transition and levels remain high throughout the cell cycle [6]. How alteration of these levels could affect the cell cycle progression is still unclear. Rat-1 fibroblasts overproducing Fru-2,6-P_2 maintain similar proliferative rates to control cells, although this increase in Fru-2,6-P_2 levels causes apoptosis in growth-factor withdrawal conditions [12]. In the Mv1Lu epithelial cell line, the decrease in Fru-2,6-P_2 levels by over-expression of the bisphosphatase domain of PF2KFB induces a delay in cell cycle progression [13]. Moreover, decrease of Fru-2,6-P_2 levels by depletion of PF2KFB3 in HeLa cells leads to reduced cell viability and an increase in the apoptotic rate [14]. Interestingly, a recent report demonstrates that the decrease in Fru-2,6-P_2 levels correlates with the protection of cells from ROS (reactive oxygen species)-induced apoptosis. The enzyme TIGAR (TP53-induced glycolysis and apoptosis regulator), a p53-induced gene, possesses a functional Fru-2,6-BPase domain and can lower Fru-2,6-P_2 levels in cells, resulting in the inhibition of glycolysis and an overall decrease in ROS levels [15].

In order to analyze cell cycle consequences due to changes in Fru-2,6-P_2 levels, the liver bi-functional enzyme 6PF2KFB1 and the bisphosphatase dead mutant (H258A) [16] were over-expressed in *Schizosaccharomyces pombe* (*S. pombe*) cells and the variation in cell phenotype was studied. Fission yeast proved to be an excellent model organism to study regulation of the cell cycle [17], as the effect of any over-expressed protein was easily detected by changes in the cellular phenotype.

The results obtained in this work demonstrate that an increase in Fru-2,6-P_2 levels results in a defect in the normal cell division of *S. pombe*, as revealed by an altered multi-septed phenotype that is dependent on the presence of the Wee1 kinase. Thus, Wee1 may be a relevant cellular target of the metabolic signals emerging from changes in Fru-2,6-P_2 content. To our knowledge, this is the first report linking alterations in Fru-2,6-P_2 levels with key regulators of the cell cycle process, such as the Wee1 kinase.

2. Materials and methods

2.1. Fission yeast strains, media and growth conditions

Strains are listed in Table 1. Rich medium was YES, selective medium was EMM2 supplemented with 225 mg/l of the required amino acids [18]. Standard techniques for fission yeast genetics were followed [18]. Fission yeast constructions were transformed by lithium acetate as described in [18]. Micrographs of *S. pombe* cells were obtained using a Zeiss Axioplan microscope with an MC80 photographic camera incorporated. DAPI staining was as described in [18].

Table 1
S. pombe strains used

Strain	Genotype	Source
wt 117 h^-	leu1-36 ura4-D18	Lab stock
wee 1–50	leu1–36 ura4-D18 wee1–50	Lab stock
Δmik1	leu1–36 ura4-D18 mik1::$ura4^+$	Lab stock
cdc2-1w	leu1–36 ura4-D18 cdc2-1w	Lab stock
cdc2-3w	leu1–36 ura4-D18 cdc2-3w	Lab stock
cdc2-3w Δcdc25	leu1–36 ura4-D18 cdc2-3w cdc25::$ura4^+$	Lab stock
adh::cdc25	leu1-36 ura4-D18 adh :$cdc25^+$ cdc25::$ura4^+$	Lab stock

2.2. DNA manipulation and plasmids construction

H258A cDNA was previously cloned in pET bacterial expression plasmid as described in [19]. Plasmid pREP1-H258A was constructed as follows: the cDNA of H258A was amplified from the pET vector with the following oligonucleotides: FOR, GTTTAACTTTAAGAAGGAGATATAC and REV (countering the *Not*I restriction at 3′ of the cDNA) ATAATTGCGGCCGCGGTAATGGG-CAGGTAC. The resulting PCR product was digested with *Nde*I and *Not*I and ligated into a pREP1 plasmid [20,21] digested with the same enzymes. The resulting plasmids containing the entire H258A cDNA under the inducible promoter *nmt1* fused to two hemagglutinin (HA) epitopes and a hexahistidine tag at the COOH-terminal region was transformed to *S. pombe* strains (Table 1), as episomal plasmids.

2.3. Fluorescence-activated Cell Sorting (FACS) Analysis

To analyze DNA content, cells were collected by brief centrifugation and fixed by re-suspension in ice-cold 70% ethanol. Cells were then re-suspended in 0.5 ml of 50 mM sodium citrate plus 0.1 mg/ml RNAse A, and incubated at 37 °C for at least 2 h. Just before processing, 0.5 ml of 50 mM sodium citrate plus 4 μg/ml propidium iodide was added. The fluorescence intensities of individual cells were measured by flow cytometry, using a Beckton Dickinson FACScan, and DNA content was measured through the FL2 channel. Results were analyzed by *Cell Quest* software.

2.4. Protein extracts and Western blotting

Twenty optical densities (ODs) of exponential growth cultures (OD600 = 0.5) were used to prepare boiled protein extracts for Western blotting as described in Forsburg's web page (http://www-rcf.usc.edu/~forsburg/plasmids.html). Primary antibodies: 1/1000 of monoclonal antibody anti-HA (12CA5) from ascites fluid was used and detected with horseradish peroxidase-conjugated antibodies mouse or rabbit (Bio-Rad). Membranes were visualized by enhanced chemoluminescence (ECL kit, Amersham-Pharmacia).

2.5. Fructose-2,6-bisphosphate content determination

Twenty optical densities (ODs) of exponential growth cultures (OD600 = 0.5) were used to prepare extracts. Cells were briefly harvested, washed in 50 mM NaOH, and re-suspended in 250 μl of 50 mM NaOH. Glass beads were added to the tubes, cells were vortexed for 30 min, and spun. The supernatants were incubated at 80 °C for 5 min to inactivate Fructose 1,6-bisphosphate, then samples were spun again (13000 rpm, 30 min, 4 °C), and the supernatants were used for Fru-2,6-P_2 determination. The method used is based on the degree of activation of the enzyme Pyrophosphate: D-fructose 6-phosphate 1-phosphotransferase (PP_i-PFK) from potato tuber by Fru-2,6-P_2 [22]. The concentration of Fru 2,6-P_2 was spectrophotometrically determined by monitoring the rate of NAD^+ formation concomitant to the breakdown of Fru 1,6-P_2. A discrete sample automatic analyzer (Cobas Mira S analyzer, Roche) was used to perform the

procedure and to monitor the spectrophotometric changes. The assay reaction was performed at 30 °C in a reaction vessel of a multicuvette segment, automatically fed by the Cobas system. The course of the reaction was automatically monitored at 340 nm by the formation of NAD^+, after the automatic addition of the starter reagent PPi-PFK to the assay reaction mixture. The concentration of the samples was calculated from the defined mathematical function from the standard curve. The values for the samples and controls were automatically provided as μmol/l.

3. Results

3.1. Effect of over-expression of PF2KFB constructs in S. pombe wild-type cells

The *S. pombe* wild type strain *117h*$^-$ was transformed with pREP1 expression plasmids coding for the full-length PF2KFB enzyme (pREP1-Full) and for the H258A-mutated protein (pREP1–H258A). The H258A mutation abolished the bisphosphatase activity while retaining the kinase activity [16]. The expression of these proteins was controlled by the strong *nmt1*$^+$ promoter, which was induced by thiamine (B1) removal. The pREP1 empty vector was used to control the transformation effects. Transformed cells were grown in selective minimal medium in the presence or in the absence of thiamine, and the resulting phenotype was observed under the microscope and photographed. As shown in Fig. 1, transformation of cells with a non-codifying vector (pREP1 panels) or over-expression of the full PF2KFB isoenzyme (Full panels) did not affect the *S. pombe* phenotype. The cellular morphology of these two strains was the same in non-induced and induced conditions (+B1 and −B1, respectively), with no significant differences with a wild type culture. In contrast, over-expression of H258A mutant (H258A panels) caused a multiple septation phenotype at 24 h, only present in the stimulated (−B1) condition.

To determine whether the transformed fusion proteins were properly induced and expressed, PF2KFB protein levels were measured by Western blot analysis. As shown in Fig. 2A, both pREP1-Full and pREP1–H258A strains expressed a 56-kDa protein, HA-tagged, induced upon thiamine withdrawal from the growth medium. As expected, transformation of cells with the empty vector showed no expression of tagged proteins. To confirm that these proteins were functional in the *S. pombe* cellular context, we determined the content of Fru-2,6-P_2 (Fig. 2B). Fru-2,6-P_2 levels increased from 12- to 16-fold in the pREP1–H258A plasmid-bearing strain after thiamine withdrawal (−B1). This occurred in the early stages (16 h) and was maintained for up to 42 h. The timing of Fru-2,6-P_2 increase was in accordance with the previously observed time of PF2KFB protein expression. It is highly probable that the lack of Fru-2,6-BPase activity together with an active kinase domain

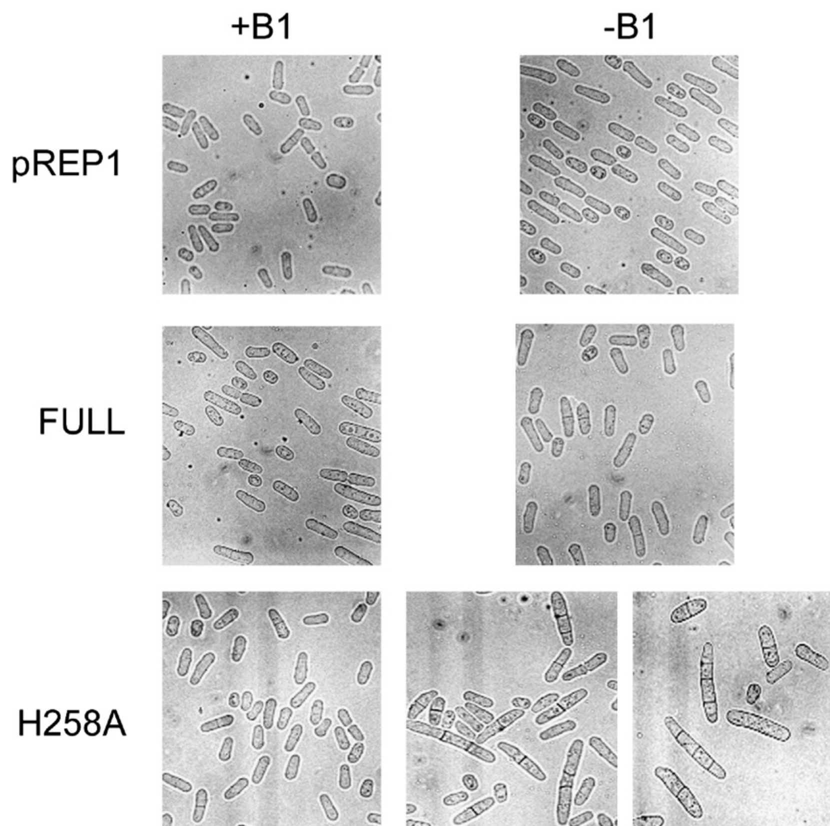


Fig. 1. 6PF2K/Fru-2,6-BPase plasmids overexpression in wild type *S. pombe*: Wild type *S. pombe* strains were transformed with the above-indicated plasmids. Induction of the *nmt1*$^+$ promoter was carried out by incubating the cells in minimal medium without thiamine (−B1, right panels) for 24 h. Left panels are non-induced cultures (+B1). Transformed cells were observed under microscope and photographed.

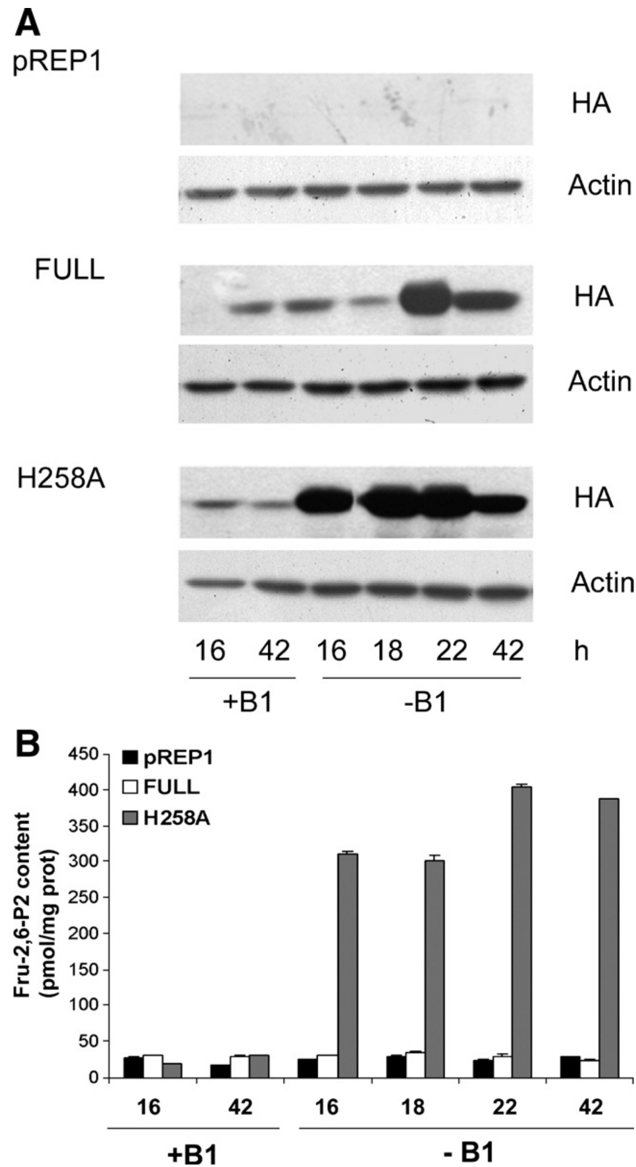


Fig. 2. Analysis of 6PF2K/Fru-2,6-BPase transformed *S. pombe* strains. (A) Western blot analysis: *S. pombe* strains bearing the indicated plasmids were grown in minimal medium, and induced in the absence of thiamine for the indicated hours. Extracts were obtained and run on 10% SDS-PAGE gel, transferred to PVDF membranes and analyzed for the presence of the fusion protein by Western blot with HA antibody, as described in Materials and methods. Also shown is a blot for Actin as a loading control. (B) Fructose-2,6-bisphosphate levels determination. *S. pombe* wild-type and transformed strains were grown in the appropriate medium and induced in the absence of thiamine (−B1), where indicated. At the indicated incubation times, extracts for Fru-2,6-P_2 quantification were obtained and analyzed for the presence of the metabolite, as described in Materials and methods. Values were corrected for the amount of total protein in each extract. Data are means ± S.E.M. from three independent determinations.

are responsible for this change. In contrast, no significant variation was detected after thiamine removal in the pREP1-Full transformed strain. The content of Fru-2,6-P_2 remained close to the basal reference concentration (20–30 pmol/mg protein) suggesting an equal balance between the rate of Fru-2,6-P_2 synthesis and degradation. As expected, Fru-2,6-P_2 levels were not modified in the pREP1 empty vector strain.

The results described above suggest that in *S. pombe*, an increase in Fru-2,6-P_2 levels interferes with the cell cycle machinery and produces a multiple septation phenotype. The cells show a defect in cytokinesis, re-entering a new cycle of division without completing the previous cytokinetic step.

3.2. Analysis of nuclear content and cell cycle profile of the pREP1–H258A strain

In order to analyze whether the H258A-induced strain impaired cytokinesis by conserving a normal nuclear division, or whether septation occurred in the absence of nuclear division, we then analyzed the nuclear content of the pREP1–H258A strain by DAPI (4′,6-Diamidino-2-phenylindole) staining method. As shown in Fig. 3A, each multisepted cell presented the appropriate number of nuclei at all times of analysis. Thus, the impairment of cytokinesis was not accompanied by a previous defect in nuclear division. The non-induced (+B1) H258A-transformed strain showed a normal phenotype, as expected.

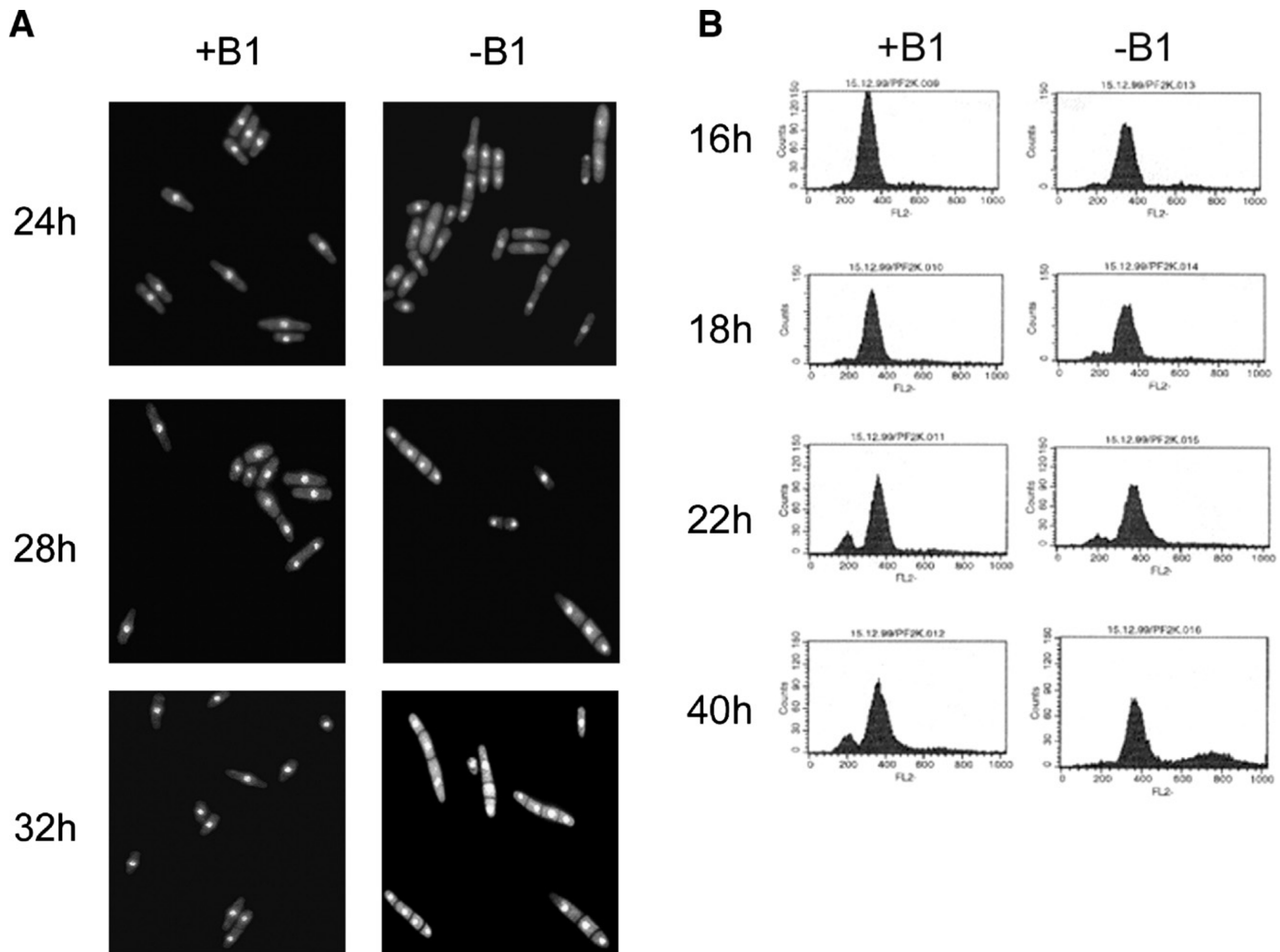


Fig. 3. (A) Nucleus analysis of pREP1–H258A strain by DAPI staining. Wild-type *S. pombe* cells transformed with the pREP1–H258A plasmid were grown and induced in the absence of thiamine (−B1). At the indicated time points, cells were fixed with 70% ethanol and their nuclei stained with DAPI as described in Materials and methods. Stained nuclei were observed under microscope and photographed. (B) Cell cycle analysis of pREP1–H258A strain. *S. pombe* pREP1–H258A strain induced by incubation in minimal medium without thiamine, was fixed in 70% ethanol at the indicated time points, and processed for FACS analysis as described in Materials and methods.

The multiseptation effect suggests some failure in the division cycle of the cell. To ascertain whether this was the case, the cell cycle profile of the H258A-transformed cells was analyzed by FACS analysis. As demonstrated in Fig. 3B, H258A-transformed non-induced cells (+B1, left panels) showed a normal cell cycle profile, as 80 to 90% of cells showed a 2n DNA content characteristic of the G_2 phase of the cell cycle, and only 2–10% of cells were in the G_1 phase (n content). In the induced culture (−B1, right panels), the population of cells in each phase of the cycle was normal at earlier times of induction, however the 2n DNA content decayed up to 65% at 40 h after thiamine removal. Interestingly, the population of cells with a DNA content >2n increased up to 32.5%, concurrently to the presence of multisepted and multi-nucleated cells as observed in Figs. 1 and 3A.

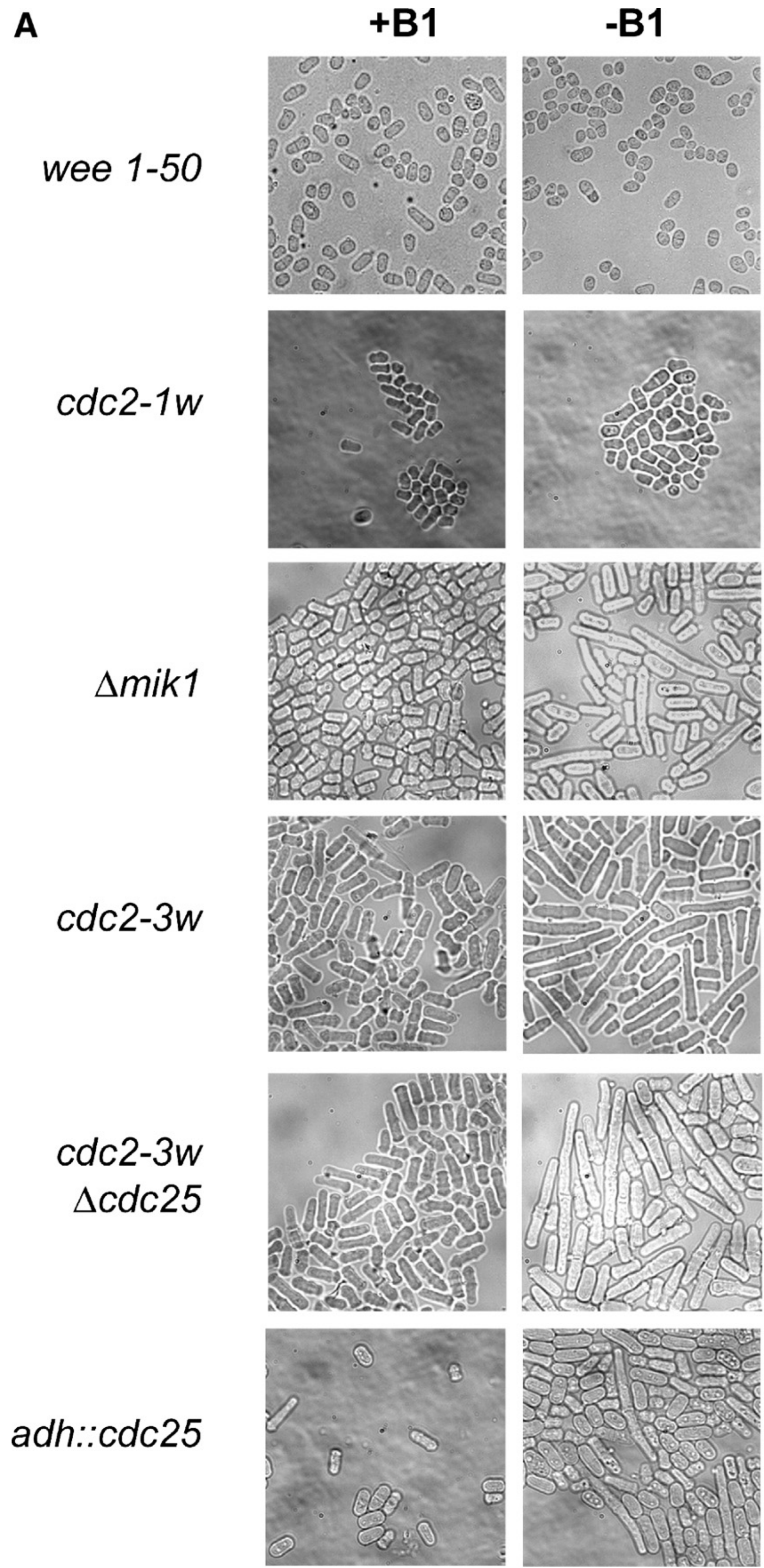
3.3. *Effect of over-expression of H258A mutant in S. pombe cell cycle mutants*

One of the main regulatory steps in *S. pombe* cell cycle is governed by the master Cdc2 cyclin-dependent kinase, which is mainly inhibited through phosphorylation in its Tyr-15 by the Wee1 and Mik1 kinases, and activated through dephosphorylation by the Cdc25 phosphatase [23–25]. To understand the mechanism by which the increase in Fru-2,6-P_2 levels account for the observed altered phenotype, the H258A construct was over-expressed in several *S. pombe* cell cycle mutants, which carry an alteration in one of the proteins involved in the above-mentioned key regulatory step. The resulting phenotype was observed under the microscope and photographed (Fig. 4A). The cell cycle *S. pombe* mutants used are described in Table 1.

The multisepted phenotype characteristic of the induced H258-transformed strain was observed in the cell cycle mutants lacking the Cdc25 regulatory step (*cdc2-3w* and *cdc2-3w Δcdc25* strains), cells over-expressing the Cdc25 regulatory phosphatase (*adh::cdc25*) or cells without the inhibitory kinase Mik1 (*Δmik1* strain) (Fig. 4A,-B1, right panels). In contrast, the multisepted phenotype was no longer present in the absence of Wee1 kinase (*wee 1–50*) or in cells with a Cdc2 cyclin-dependent kinase independent of Wee1 phosphorylation (*cdc2-1w)*. Thus, Wee1 could be the cell cycle

protein that integrates the metabolic signal emerging from changes in Fru-2,6-P_2 content, coupling metabolism with cell proliferation.

To confirm that over-expression of the H258A mutant version of the bi-functional enzyme altered the Fru-2,6-P_2 content of the *wee 1–50* thermosensitive strain at the restrictive



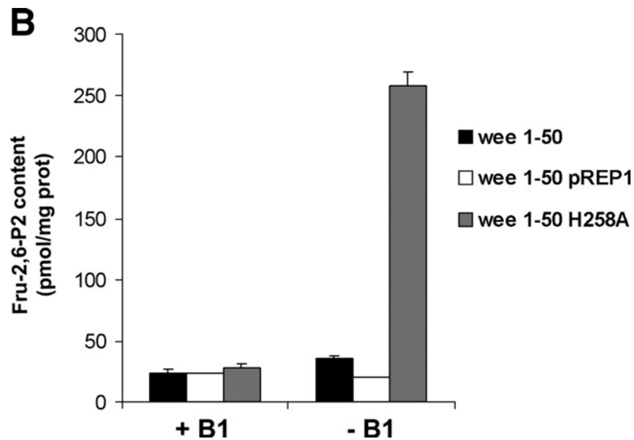


Fig. 4. (A) pREP1–H258A plasmid overexpression in *S. pombe* cell cycle mutants. The *S. pombe* cell cycle mutants listed in Table 1 were transformed with the pREP1–H258A plasmid. Induction of the *nmt1* promoter was carried out by incubating the cells in minimal medium without thiamine (−B1, right panels), for 24 h. Left panels are non-induced cultures (+B1). Transformed cells were observed under microscope and photographed. (B) Fructose-2,6-P2 levels determination in the wee 1–50 strain. *S. pombe wee 1–50* transformed and non-transformed strains were grown in the appropriate medium and induced in the absence of thiamine (−B1), where indicated. Extracts for Fructose-2,6-P_2 quantification were obtained 24 h after thiamine removal, and analyzed for the presence of the metabolite as described in Materials and methods. Values were corrected for the amount of total protein in each extract. Data are means ± S.E.M. from three independent determinations.

temperature of 30 °C, we determined Fru-2,6-P_2 levels. As shown in Fig. 4B, the removal of thiamine from the growing media increased Fru-2,6-P_2 levels more than 10 fold, confirming the expression and correct activity of H258A. Therefore the lack of a phenotype effect observed in the H258A-transformed cells in the *wee 1–50* genomic context (Fig. 4A) was not due to a defect in the expression nor in the activity of the enhanced-kinase version of PF2KFB.

4. Discussion

Although several studies have investigated the role of Fru-2,6-P_2 on the cell cycle process, the question still remains unanswered. In mammalian cells, decrease in Fru-2,6-P_2 levels due to over-expression of Fructose-2,6-bisphosphatase causes cell cycle delay [13], whereas high levels sensitize cells to apoptosis [12]. In contrast, in *S. cerevisiae*, changes in Fru-2,6-P_2 due to alteration of PFK1, PFK26 or PFK27 activities [26,27] have a modest effect and do not cause any relevant change in the cellular phenotype. To further investigate this phenomenon, we have analyzed the effect of over-overproduction of Fru-2,6-P_2 in *S. pombe*, an excellent model for studying basic cellular processes. Interestingly, genetic studies performed in this organism have shown that overexpression of PF1K (Pfk1) causes a cell cycle arrest phenotype [28].

The full-length and H258A cDNAs were over-expressed in *S. pombe* cells, and the phenotype revealed that cells transformed with the H258A mutant of PF2KFB showed an alteration of the cytokinetic step, which resulted in an altered multisepted phenotype. The multisepted phenotype is probably due to the increase in Fru-2,6-P_2 levels as a consequence of the lack of Fru-2,6-bisphosphatase activity and the high kinase activity of the H258A-overexpressed protein. Expression of full-length PF2KFB did not produce changes in Fru-2,6-P_2 concentration and the multisepted phenotype was not observed. How can the increase in the concentration of this metabolite account for this effect? Most probably, the effect of Fru-2,6-P_2 levels on *S. pombe* phenotype occurs through modulation of PF1K activity. Kinetic studies of *S. pombe* Pfk1 have revealed that, as reported in others species, Fru-2,6-P_2 is also an allosteric activator of this enzyme [29]. Interestingly, overexpression of Pf1k in *S. pombe* causes also an abnormal septation pattern [28]. Very likely, changes on PF1K activity or indirectly in other glycolytic enzymes, or compounds acting up or downstream Fru-2,6-P_2, may help to generate the metabolic signal initiated by Fru-2,6-P_2 increase. Our results suggest that a metabolic product of the glycolytic flux could be a metabolic sensor of cell cycle progression. In accordance with this hypothesis ATP, a major product of glycolysis, is used in eukaryotic cells as a sensor to control cell growth associated with cell proliferation [30]. Other possibilities are not excluded. It has also been reported that, although sugar is considered a growth-promoting agent in yeast cells, it can induce apoptosis in the absence of additional nutrients [31]. Therefore, changes in the content of carbohydrates due to the high levels of Fru-2,6-P_2 could be causing the multisepted phenotype.

Several surveillance mechanisms referred to as *checkpoints*, ensure the progression and completion of critical events in the cell division process of eukaryotic cells, blocking subsequent cell cycle transitions if earlier ones are incomplete. The appearance of septation in H258A-transformed strain with no defect in nuclear division indicated that cells had initiated a second mitosis without completing cell separation. This phenotype suggests that a checkpoint that controls the correct cytokinesis has been overcome, as cells re-enter a new division cycle without completing the cell cleavage that forms two individual cells. Interestingly, PF1K and the majority of glycolytic enzymes are actin-binding proteins as has been shown in *S. cerevisiae* [32]; modification of their activity by Fru-2,6-P2 could therefore cause a defect in septation. A morphogenetic checkpoint has been described in *S. cerevisiae* [33]; this checkpoint is a link between cell cycle and the cytoskeleton that is activated when actin is depolarized. The final response elements are the cell cycle machinery, through the Swe1p kinase, the homologue of *S. pombe* Wee1. Likewise, other reports suggest that, in *S. pombe*, an F-actin and Wee1-dependent checkpoint blocks G_2/M transition until the previous cytokinesis has been completed [34]. A recent report has shown that *S. pombe* cells lacking Mpg1 (mannose-1-phosphatase guanyl-transferase) show an aberrant septum structure, and a G2/M arrest that is dependent on Wee1 [35]. A plausible explanation for our results could be that high concentration of Fru-2,6-P_2 is affecting the glycosylation of cell-wall proteins.

It is worthy of note that over-expression of the H258A-mutated PF2KFB in the *wee 1–50* and *cdc2-1w* strains did not lead to cellular multiseptation. The increase in Fru-2,6-P_2 may alter the inhibitory function of the protein Wee1. This alteration

may prevent the normal function of Wee1, which delays the cell cycle if cytokinesis has not been completed. The fact that Mik1 or Cdc25 defective mutants did not abolish the multiseptation phenotype of H258A overexpression implies that Wee1 kinase is the metabolic sensor, and not Cdc2 activity. It is well known that Wee1 is regulated at multiple levels [23]. Changes in Fru-2,6-P_2 levels and activation of the glycolytic flux could interfere in this regulation, allowing cells to overcome the cytokinetic checkpoint.

Yeast is a unicellular organism, and has no restrictions of organ/body shape or body mass. Alteration of growth and cell shape can occur more easily than in a multi-cellular system, and new defects caused by alteration of Fru-2,6-P_2 levels can be observed. As most yeast components of the checkpoints are conserved in higher organisms, the results obtained in this work can shed new light on the consequences of changes in Fru-2,6-P_2 levels. The multisepted phenotype caused by high levels of Fru-2,6-P_2 may reflect a dysfunction in cell cycle progression. This can be linked to the cell cycle delay induced in mammalian cells by lowered levels of Fru-2,6-P_2 [13]. Interestingly, two different studies connect Fru-2,6-P_2 levels with the cellular response to stress. In *S. cerevisiae*, this metabolite has been proved to be essential to maintain adequate glycolysis when cells are exposed to weak acid stress [36]. In higher eukaryotes, the protective function of the TIGAR enzyme against oxidative stress-induced apoptosis has been linked to its ability to reduce Fru-2,6-P_2 levels [15].

In summary, the results obtained in this work enhance our understanding of the role of Fru-2,6-P_2 in the context of cell division, and highlight its significance for cell proliferation. To the best of our knowledge this is the first experimental evidence linking the alteration of a glycolytic activity with a defect in a cytokinesis checkpoint.

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