Biochemical and Biophysical Research Communications 424 (2012) 579–585

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Context dependent splicing functions of Bud31/Ycr063w define its role in budding and cell cycle progression

Debjani Saha, Shataparna Banerjee, Samirul Bashir, Usha Vijayraghavan *

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India

ARTICLE INFO

Article history:
Received 21 June 2012
Available online 9 July 2012

Keywords:
Bud31
Yeast
G1–S transition
Budding
Pre-mRNA splicing

ABSTRACT

The yeast Bud31 protein, a Prp19 complex (NTC) member, aids spliceosome assembly and thus promotes efficient pre-mRNA splicing. The *bud31* null cells show mild budding abnormalities at optimal growth temperatures and, at higher temperatures, have growth defects with aberrant budding. Here we have assessed cell cycle transitions which require Bud31. We find Bud31 facilitates passage through G1–S regulatory point (Start) but is not needed for G2-M transition or for exit from mitosis. To co-relate Bud31 functions in cell division with splicing, we studied the splicing status of transcripts that encode proteins involved in budding. We find Bud31 promotes efficient splicing of only some of these pre-mRNAs, for example, *ARP2* and *SRC1*. Wild type cells have a long and a short isoform of *SRC1* mRNA and protein, out of which the shorter mRNA splice variant is predominant. *bud31Δ* cells show inefficient *SRC1* splicing and entirely lack the shorter *SRC1* spliced mRNA isoform. Yeast *PRP17*, another NTC sub-complex member, is also required for G1–S and G2-M cell cycle transitions. We examined genetic interactions between *BUD31* and *PRP17*. While both factors were needed for efficient cell cycle dependent gene expression, our data indicate that distinct pre-mRNAs depend on each of these non-essential splicing factors.

1. Introduction

In *Saccharomyces cerevisiae*, a number of splicing factors have auxiliary effects on other cellular processes like cell cycle [1], ribosome biogenesis [2] and DNA repair [3]. The additional functions for splicing factors can arise from one of two reasons. Firstly, some factors could be directly involved in two different cellular processes. Alternatively, their role in splicing can, in turn, have indirect effects on gene expression required for an unrelated cellular pathway. Mutants in some *S. cerevisiae* splicing factors affect cell cycle progression. Prp17 is required for G1–S transition as well as for progression through G2-M phase [4,5]. Other splicing factor mutants *prp8*, *prp16*, *prp22* and various *syf* alleles show extensive genetic interactions with *prp17Δ*. Many of these mutants also affect the cell division cycle [6]. Alleles in *prp8* and *prp22* are delayed for S phase progression and arrest in late G2 [6,7]. A connection between splicing and cell cycle regulation has been established in *Schizosaccharomyces pombe* also, wherein a number of temperature-sensitive *prp* mutants showed cell cycle defects at restrictive temperatures [8]. This leads to the nomenclature of the CSC complex (Cell cycle and Splicing complex) [1]. The G2 cell cycle arrest in budding yeast *prp17Δ* mutant and in the *isy1Δ syf2Δ* double mutant derives from inefficient splicing of *TUB1* and *TUB3* pre-mRNAs that code for spindle microtubules. This defect can be rescued by tubulin expression from intronless *TUB1* and *TUB3* cDNAs. This indicates that the inability to produce tubulin is a major reason for the G2 arrest of *prp17Δ* and *isy1Δ syf2Δ* cells [1,5]. However, other double mutants in genes for splicing factors with cell cycle functions like *prp17Δsyf1Δ*, *prp17Δisy1Δ* are inviable [1,6]. This indicates extensive interactions and overlap between these factors for their roles in cell division and pre-mRNA splicing.

Bud31 is non-essential for the viability of budding yeast [9]. It was identified as a member of NTC-associated complex in a mass spectrometry-based proteomic study [10]. Its splicing functions, suggested by its proteomic associations, were confirmed in a preliminary study where splicing defects were observed for two transcripts [10,11]. The report by Masciadri et al. [11] described cytoskeletal abnormalities and budding defects in *bud31* null cells. An *in vitro* biochemical analysis, showed Bud31 aids both catalytic steps of pre-mRNA splicing and its association with assembling spliceosomes was demonstrated [12]. Here, we assess the requirement of Bud31 for various cell division transitions and address the possibility that Bud31 splicing functions are linked to the cellular defects seen in null mutants. Our data show Bud31 is required for the splicing of some, though not all pre-mRNAs, encoding proteins required for budding. We also examine if *PRP17* and *BUD31*

Abbreviations: NTC, nineteen complex; CSC, cell cycle and splicing complex.

* Corresponding author.
E-mail addresses: uvr@mcbl.iisc.ernet.in, uvr123@gmail.com (U. Vijayraghavan).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.156

are functionally redundant, as their mutants share some cell cycle phenotypes and both factors are members of the same spliceosomal sub-complex. Our complementation studies suggest no overlap in their cellular functions, pointing to their roles in splicing of distinct pre-mRNAs.

2. Materials and methods

2.1. Strains and plasmids

The diploid *bud31::KANMX4* (*bud31::kanMX4*) and *BY4743* strains, from EUROSCARF, were sporulated to obtain the corresponding haploids. The temperature sensitive *prp2-1* mutant in the essential splicing factor *PRP2* was used as a control for splicing status assays. Full length *BUD31* ORF was PCR amplified using *BUD31*/*Bud31* FP (5′CGGGATCCCTCACCTCACAAAACGAC3′) and RP (5′CGGGATCCCAGAACAATACTGCAACG3′), *Bam*HI digested and cloned into pGBDUC1 for complementation studies.

2.2. Cell synchronization protocols and flow cytometry analysis

Early log phase cultures, grown at permissive temperature (23 °C), were taken for synchronization. Then they were released from arrest and aliquots were grown at 23 °C and 37 °C [5]. Synchronization in late G1, prior to Start, was obtained with alpha-factor treatment [5]. Early S phase synchronization was achieved with 0.3 M hydroxyurea treatment for 3 h. Arrest in metaphase/ anaphase was obtained with 15 μg nocodazole for 3 h. Flow cytometry to determine DNA content was performed as in [13].

2.3. Reverse transcription PCR for splicing status analysis

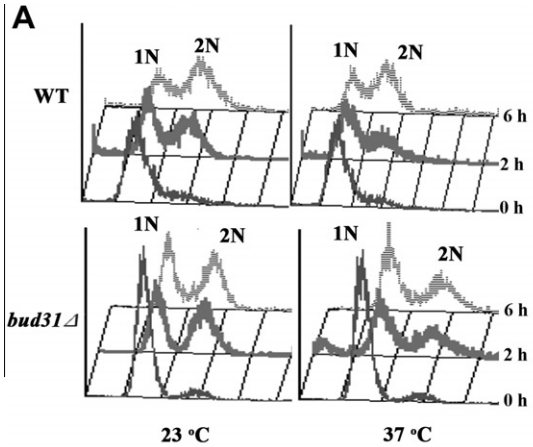
bud31 null strain was grown at 23 °C, to an $O.D_{595}$ of 0.5 in YPD supplemented with G418 (200 μg/ml). The culture was then shifted to 37 °C and aliquots corresponding to ~10 O.D of cells were withdrawn at 0, 3 and 5 h time points. Cell pellets were stored in −80 °C till further use. The *prp2-1* temperature sensitive mutant was grown at 23 °C to $O.D_{595}$ of 0.5 and then shifted to 37 °C; cells in culture aliquots taken at 0 and 2 h were pelleted. Total RNA was extracted from all cell pellets using trizol and 5 μg of each DNaseI treated RNA was used for cDNA synthesis as in [14,15]. Sequencing of *SRC1* cDNAs, representing mRNA, from WT and *bud31Δ* cells grown at 23 and 37 °C was done as follows. A pool of ten clones was made, all of which contained the cDNA amplicon from mRNA of WT cells grown at 37 °C. Another pool of ten clones was made with cDNA amplicon from mRNA of *bud31Δ* cells grown at 37 °C. These two pooled cDNA recombinants were sequenced.

Primers used for the reverse transcription and PCR of cDNAs were: ARP2E1FP5′ATAATGGACCCACATAATCC3′, ARP2E2RP5′ACCAGTACCCTGATCAAGGA3′; SRC1E1FP5′ACCTGTGGATCCAAGTTATC3′, SRC1E2FP5′ATGTCTCTTCTGTCTCGGAA3′; YSC84E1FP5′TCCAATTCCTCGAAGCTTGA3′, YSC84E2FP5′ACTCCTTAGAACTTTCGCGG3′; PFY1E1FP5′ACCCAACTACGATCGCAAAT3′, PFY1E2RP5′TCGAGTAGAT GACAGCTTTG3′; SAC6E1FP5′AGCCCTAAGGAGTACACCAA3′, SAC6E2RP5′CCTCTTGAGTCAAAATTGGA3′ SNR7FP5′GCAGCTTTACAGATCAATGG3′, SNR7RP5′ACGCCCTCCTTACTCATT3′.

3. Results

3.1. Effects of Bud31 on G1–S cell cycle transition

A preliminary study by Masciadri et al. [11] reported budding and cell cycle defects in a *bud31* null mutant, at 25 °C. We examined the role of Bud31 for cell cycle progression at permissive (23 °C) and non-permissive (37 °C) growth temperatures. *bud31Δ* and isogenic wild type (WT *BY4743*) cultures grown at 23 °C were first synchronized at different points of the cell cycle and



B

	G1 (1N DNA)		S/G2 (2N DNA)	
	23 °C	37 °C	23 °C	37 °C
BY4743	25 %	28 %	53 %	54 %
bud31Δ	47%	55%	33%	30%

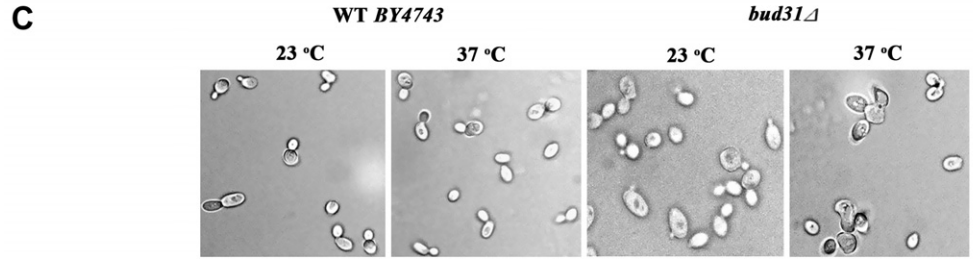


Fig. 1. Delayed G1–S transition in absence of Bud31. (A) G1 arrested WT *BY4743* and *bud31Δ* cultures were released into YPD broth at 23 and 37 °C. The DNA content (1N and 2N) in culture samples is shown. (B) WT *BY4743* and *bud31Δ* synchronized cultures, grown for 6 h, at 23 and 37 °C were quantitated to determine percent distribution of cells in G1 and S–G2 stages. (C) Cellular morphology of WT and *bud31Δ* after 6 h of growth at 23 and 37 °C.

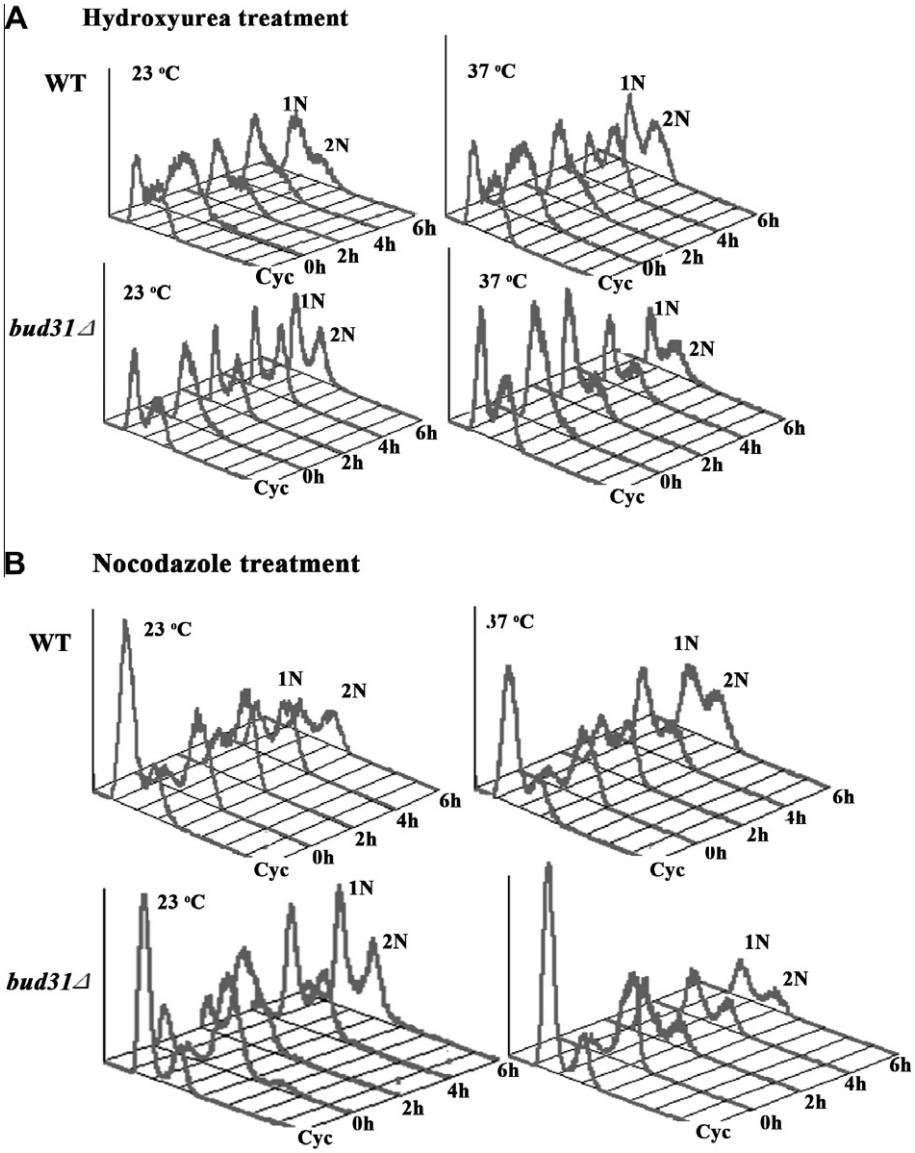


Fig. 2. Bud31 is not required for G2-M transition and exit from mitosis. Hydroxyurea treated, early S phase arrested (A) and nocodazole treated metaphase/ anaphase arrested (B) WT *BY4743* and *bud31Δ* cultures were released to YPD broth at 23 and 37 °C. FACS analysis of cellular DNA content is shown.

then released for growth at 23 or 37 °C. The resumption of cell division cycle was assessed in these cultures. WT G1 synchronized cells resumed growth at 23 and at 37 °C and progressed through G1–S by 2 h. By 6 h, ~55% were in the G2-M phase (Fig. 1A, top panel, Fig. 1B). At 23 °C, *bud31Δ* cultures were delayed for transition from late G1 to S phase and only ~30% of cells attained 2N DNA content even 6 h after release from pheromone arrest. This cell cycle progression defect persisted in cultures grown at 37 °C (Fig. 1A, bottom panel, Fig. 1B). Further the *bud31Δ* cells, even at permissive temperatures, were enlarged with a single bud, a phenotype that also indicates a greater time spent in G1. At 37 °C, an exaggerated phenotype of multiple budding is evident (Fig. 1C).

3.2. G2-M cell cycle transition can occur in the absence of Bud31

We examined whether the execution of other cell cycle transitions for example entry to mitosis (G2-M transition) and exit from mitosis required Bud31 activity. Early S phase arrest was achieved with hydroxyurea treatment which ensured that cells (*bud31Δ* and WT) had passed through the G1–S execution point. These synchronized cultures after release to media at 23 and 37 °C, were monitored for cell cycle progression. WT cells, at both growth temperatures, resumed DNA replication and completed G2 and M phases between 2 and 6 h (Fig. 2A, top panel). Interestingly, *bud31Δ* cells also proceeded through cell cycle at 23 and 37 °C (Fig. 2A, bottom panel) and completed mitosis between 2 to 4 h. These data demonstrate the dispensability of Bud31 for the G2-M cell cycle transition. The last cell cycle regulatory point is in late anaphase/telophase, after which cells undergo cytokinesis to exit mitosis. Using nocodazole treatment we arrested WT and *bud31Δ* cultures in metaphase/ anaphase. On release from this arrest, *bud31Δ* cells completed mitosis by 2 h as cells with 1N DNA content were detected. This phenotype was seen in cultures grown at both temperatures. Thus, our data showed that progression through the G1–S transition of the cell cycle requires Bud31 functions while passage through other regulatory windows can occur in its absence.

3.3. Molecular analysis of budding defects observed in absence of Bud31

We investigated the link between Bud31 splicing functions and the cellular defects seen in null mutants. By screening the Ares Lab Yeast Intron Database (http://metarray.ucsc.edu/cgi-bin/intron/yirIntrondb) we identified pre-mRNAs that encode products with functions in budding. While majority of *S. cerevisiae* introns have consensus *cis*-intronic elements, the spacing between these elements is unusual in some cases. In selecting candidate pre-mRNA targets of Bud31 we took this point into account. Another deviant intron feature is the presence of an intron towards the 3' end of the transcript and this too is seen in minor proportion of the genome's introns. After scrutiny of introns for all these features we chose *ARP2*, *PFY1*, *SRC1*, *SAC6* and *YSC84* (Table 1) for analysis of their splicing status. *ARP2*, *PFY1*, *SAC6* and *YSC84* have a single intron closer to the 5′ end of the transcript while *SRC1* is an example of an atypical transcript with an intron closer to it's 3′ end. Another unusual feature of *SRC1* is the presence of two non-canonical, closely

Table 1
Intron characteristics of various transcripts selected for RT-PCR analyses.

Transcript	Intron position in the transcript	Intron length (nts)	Brp – 3′ss distance (nts)
SRC1	3′ end	125	25
ARP2	5′ end	122	14
PFY1	5′ end	208	72
YSC84	5′ end	167	40
SAC6	5′ end	110	18

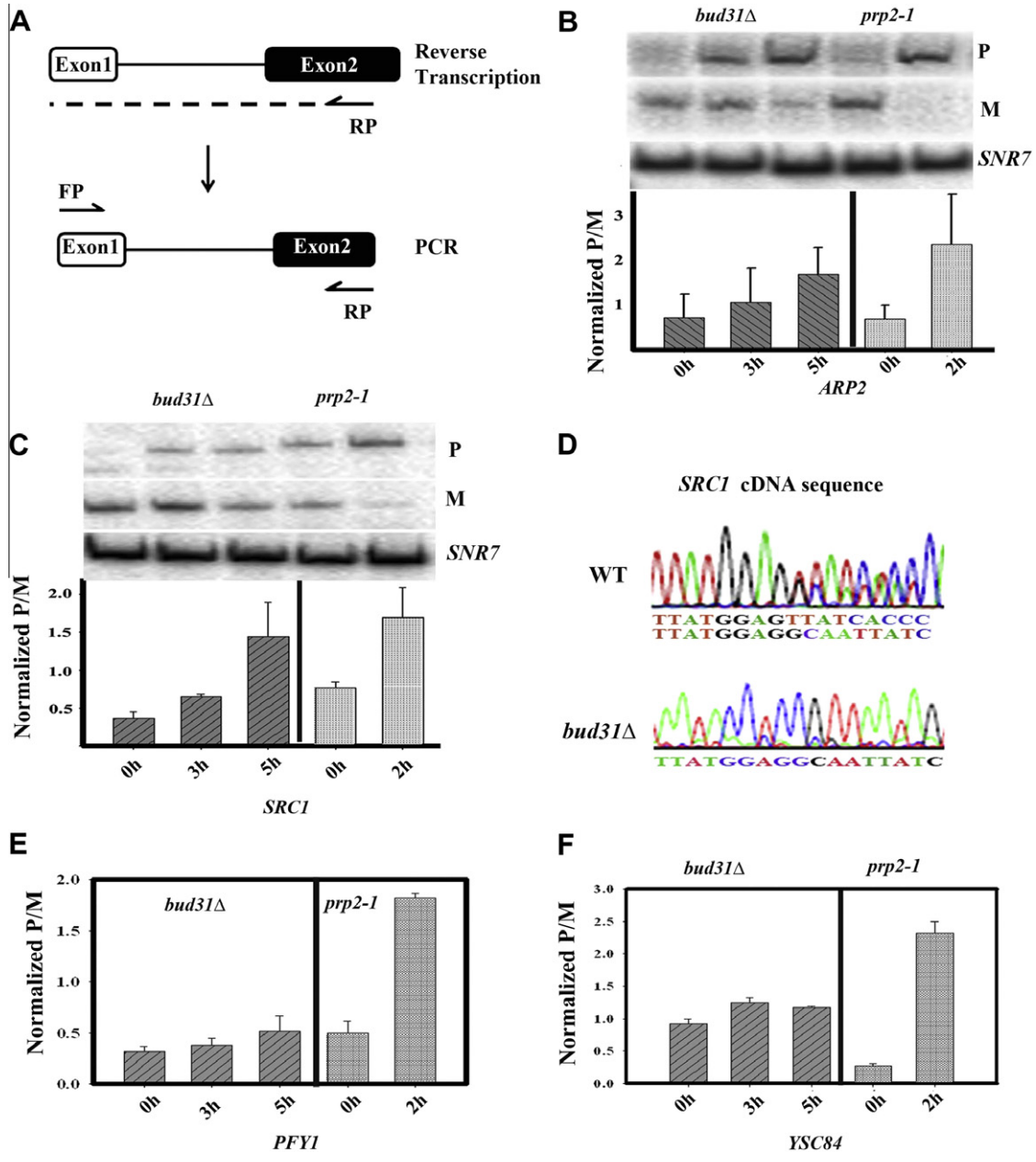


Fig. 3. Splicing status of cellular transcripts coding for proteins involved in budding. (A) Schematic diagram of the RT-PCR assay with gene-specific reverse primers used for cDNA synthesis and the primer pair used for limited number of PCR cycles. *bud31Δ* cells grown at 23 °C were shifted to 37 °C. Cellular RNA from culture aliquots harvested at 0, 3 and 5 h were used for RT-PCR analysis of *ARP2* (B), *SRC1* (C, D), *PFY1* (E) and *YSC84* (F). All cDNAs were labeled with trace ∝32P dATP. Representative native PAGE gels separating cDNAs produced from pre-mRNA and mRNA of *SRC1* and *ARP2* are shown in (B, C) respectively. The pre-mRNA (P) and mRNA (M) levels were normalized to that of *SNR7* (intronless transcript) from three biological RNA replicates. The P/M ratio was then plotted for all transcripts. (D) Sequences of *SRC1* cDNAs obtained from mRNAs in WT and *bud31Δ* cells grown at 37 °C are shown.

spaced 5′ splice-sites that can be alternatively spliced to give two protein isoforms. The 208 nts *PFY1* intron is unusual as its intron length differs from two global averages (100 nts and 400 nts) known for yeast intron lengths [15,16]. Further this intron has a long distance between the branch point to 3′ splice-site. The *YSC84* intron also has a relatively longer branch point to 3′ splice-site distance (Table 1).

bud31Δ and the WT strains grown at 23 °C were shifted to 37 °C. Culture aliquots were then collected at the point of shift (0 h), after 3 and 5 h at the non-permissive temperature. RNA from cells, in each culture sample, was reverse transcribed with a specific exon2 reverse primer for every transcript analyzed. The cDNAs generated were amplified using a specific exonic forward primer to detect pre-mRNA and mRNA for each transcript analyzed (Fig. 3A). As a control for this assay, RNA from the mutant *prp2-1* was used. This is a temperature-sensitive mutant in an essential helicase and is defective for the first step of splicing. To quantitate the mRNA and pre-mRNA levels the respective cDNA products were normalized to the cDNA levels for *SNR7*, an intronless non-coding RNA. In *bud31Δ* cells grown at high temperatures the splicing efficiency of *ARP2* (Fig. 3B) and *SRC1* (Fig. 3C) were significantly affected. Unspliced pre-mRNAs were accumulated and the cells had decreased levels of spliced mRNA, both parameters are signatures of splicing defects (Fig. 3B and C top image). In WT cells two non-canonical 5′ splice-sites are utilized to produce two *SRC1* cDNA isoforms, but the upstream 5′ splice-site is the predominant site used (Fig. 3D, upper sequence) [17]. In contrast, in *bud31Δ*, *SRC1* cDNAs were generated largely from the downstream 5′ splice-site (Fig. 3D, lower sequence). Hence in addition to reduced splicing efficiency of *SRC1* in *bud31Δ* cells, the 5′ splice-site utilization was altered. The splicing of *PFY1* and *YSC84* transcripts were also affected in *bud31Δ*, but to a lesser extent (Fig. 3E and F), while *SAC6* was efficiently spliced in *bud31Δ* cells even at 37 °C (data not shown). Taken together, these data show Bud31 functions are required for splicing of some but not all pre-mRNAs encoding proteins involved in budding.

3.4. Genetic Interactions between BUD31 and PRP17

BUD31 and *PRP17* are factors of the NTC-associated complex [10] and null alleles at either locus confer temperature-sensitivity [4,18,19]. Further *prp17* mutants like *bud31Δ*, are defective for G1–S cell cycle transition [5,20,21]. We investigated genetic redundancy between these two factors, due to their overlap in cell cycle execution point and their association in the same spliceosomal sub-complex. We examined if *BUD31* over-expression rescues the temperature sensitivity of *prp17Δ* and vice versa. A haploid *prp17Δ* strain was transformed with the plasmid pGBDUC1*BUD31* to over-express a functional Bud31 protein. Serial culture dilutions of this transformed strain, and control strains, were tested for growth at various temperatures (23, 34 and 37 °C). Growth was compared with *prp17Δ* cells transformed with a plasmid overexpressing Prp17. While over-expression of *BUD31* did not have any deleterious effects on *prp17Δ* cells, it could not rescue the growth defect at 34 or 37 °C. As expected *PRP17* itself when over-expressed entirely complemented the growth defect of *prp17Δ* (Fig. 4A). Whether *PRP17* can complement loss of Bud31 function was tested by *PRP17* over-expression in *bud31Δ* cells grown at different temperatures. We observe that the growth defects of *bud31Δ* persists (Fig. 4B). These data show no genetic redundancy between these two factors of the NTC associated Cef1p complex.

4. Discussion

An earlier study of the *bud31Δ* strain described its slow growth, prolonged G1 cell cycle phase and multiple budding phenotype [11]. Other reports investigating proteomic associations of the NTC complex implicated a splicing function for Bud31 [10]. Its role in spliceosome assembly, activation and catalysis was recently shown through *in vitro* splicing assays [12]. These biochemical analyses support the observed interactions of Bud31 with the NTC spliceosome sub-complex. Interestingly, many members of the NTC or NTC associated complex, for e.g., Cef1, Clf1, Isy1, Prp17, Prp19, Prp22 and Syf1 have multiple points of action in the cell cycle [6,22–25]. Here we investigated various cell division cycle transitions for their dependence on Bud31. These data showed that Bud31 functions are largely restricted to G1–S transition. Bud site selection and growth is one of the pathways activated on passage through G1–S regulatory point; our data which show an execution point for Bud31 are consistent with budding defects in its null mutant [26]. Our detailed investigations on the

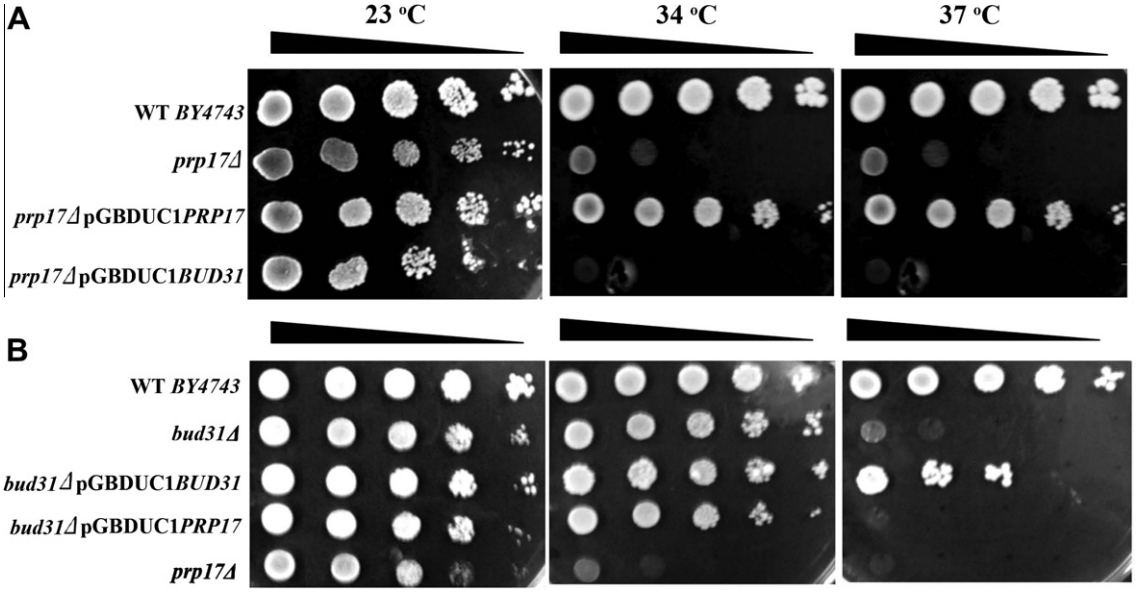


Fig. 4. *BUD31* and *PRP17* are not functionally redundant. (A) *prp17Δ* cells were transformed with the Bud31 over-expression plasmid, pGBDUC1*BUD31*, and the growth of transformants tested at indicated temperatures. (B) pGBDUC1*PRP17* transformed *bud31Δ* cells were assayed for growth at different growth temperatures. *bud31Δ*, *prp17Δ* and WT *BY4743* untransformed strains served as controls.

link between Bud31 cell cycle execution point and its pre-mRNA splicing role show that inefficient splicing of *SRC1*, *ARP2*, *PYF1* and *YSC84* transcripts in *bud31Δ* contribute to its cellular phenotypes. Further, we find Bud31 promotes the use of an alternative 5' splice-site thereby affecting the abundance of one *SRC1* isoform. Independent reports support our data showing relationships between *SRC1*, *ARP2* and *BUD31*. For instance, mutations in *HUB1*, which promotes alternative splicing of *SRC1* [17], are lethal when combined with *bud31Δ* [27]. *bud31Δ* is also synthetic lethal when combined with mutants in members of the Arp2/3 complex like, *ARC15* and *ARC35* [27]. Actin cytoskeletal organization would be affected by inefficient splicing of *ARP2* and thus can confer the abnormal morphology of *bud31Δ* noted here and reported in another study [11]. Similarly, reduced splicing of *SRC1* is likely to cause faster progression into mitosis, sister chromatid segregation defects and growth abnormalities as seen for the viable *src1* null mutant [28]. Hence the cumulative effects on the splicing of various pre-mRNA transcripts are reflected in *bud31Δ* cellular phenotype. Alternatively, the possibility that the cell cycle defect is a direct consequence of the loss of Bud31 cannot be ruled out as genetic interactions are known with factors with cell cycle related functions i.e., *TOF1*, *CDC13*, *CLK1*, *FAR8*, and *CTF8* [27]. A number of factors in the NTC associated complex display extensive genetic and direct physical interactions [10,29,30]. Although *BUD31* does not show any genetic interactions with NTC associated factors [27], synthetic lethality of the double mutant *bud31Δ ist3::KAN* is reported [31]. As a corollary, we find that despite their similarity in functionally linking efficient G1–S cell cycle progression and pre-mRNA splicing, Bud31 and Prp17 are not functionally redundant. Also, the splicing of *SRC1* transcripts is not affected in *prp17Δ* [17,28]. These data together suggest that non-essential splicing factors perform auxiliary functions for distinct transcripts. In summary, we find that Bud31 regulates budding and cell cycle by facilitating G1–S transition through its context dependent splicing functions.

Acknowledgments

This work was funded by an infrastructure grant to Division of Biological Sciences, Indian Institute of Science from the Department of Biotechnology; by scholarships from Council of Scientific and Industrial Research, Government of India to D.S. and from Indian Institute of Science to S.B. Dr. Piyush Khandelia is acknowledged for the pCHP425*BUD31* plasmid.

References

[1] O. Dahan, M. Kupiec, Mutations in genes of *Saccharomyces cerevisiae* encoding pre-mRNA splicing factors cause cell cycle arrest through activation of the spindle checkpoint, Nucleic Acids Res. 30 (2002) 4361–4370.

[2] D.J. Combs, R.J. Nagel, M. Ares Jr., S.W. Stevens, Prp43 is a DEAH-Box spliceosome disassembly factor essential for ribosome biogenesis, Mol. Cell. Biol. 26 (2006) 523–534.

[3] M. Grey, A. Dusterhoft, J.A. Henriques, M. Brendel, Allelism of PSO4 and PRP19 links pre-mRNA processing with recombination and error-prone DNA repair in *Saccharomyces cerevisiae*, Nucleic Acids Res. 15 (1996) 4009–4014.

[4] N. Vaisman, A. Tsouladze, K. Robzyk, S. Ben-Yehuda, M. Kupiec, Y. Kassir, The role of *Saccharomyces cerevisiae* Cdc40p in DNA replication and mitotic spindle formation and/or maintenance, Mol. Gen. Genet. 247 (1995) 123–136.

[5] G. Chawla, A.K. Sapra, U. Surana, U. Vijayraghavan, Dependence of pre-mRNA introns on PRP17, a non-essential splicing factor: implications for efficient progression through cell cycle transitions, Nucleic Acids Res. 31 (2003) 2333–2343.

[6] S. Ben-Yehuda, I. Dix, C.S. Russell, M. McGarvey, J.D. Beggs, M. Kupiec, Genetic and physical interactions between factors involved in both cell cycle progression and pre-mRNA splicing in *Saccharomyces cerevisiae*, Genetics 156 (2000) 1503–1517.

[7] J.E. Shea, J.H. Toyn, L.H. Johnston, The budding yeast U5 snRNP Prp8 is a highly conserved protein which links RNA splicing with cell cycle progression, Nucleic Acids Res. 22 (1994) 5555–5564.

[8] S. Urushiyama, T. Tani, Y. Ohshima, Isolation of novel pre-mRNA splicing mutants of *Schizosaccharomyces pombe*, Mol. Gen. Genet. 253 (1996) 118–127.

[9] E.A. Winzeler, D.D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, B. Andre, R. Bangham, R. Benito, J.D. Boeke, H. Bussey, A.M. Chu, C. Connelly, K. Davis, F. Dietrich, S.W. Dow, M. El Bakkoury, F. Foury, S.H. Friend, E. Gentalen, G. Giaever, J.H. Hegemann, T. Jones, M. Laub, H. Liao, N. Liebundguth, D.J. Lockhart, A. Lucau-Danila, M. Lussier, N. M'Rabet, P. Menard, M. Mittmann, C. Pai, C. Rebischung, J.L. Revuelta, L. Riles, C.J. Roberts, P. Ross-MacDonald, B. Scherens, M. Snyder, S. Sookhai-Mahadeo, R.K. Storms, S. Veronneau, M. Voet, G. Volckaert, T.R. Ward, R. Wysocki, G.S. Yen, K. Yu, K. Zimmermann, P. Philippsen, M. Johnston, R.W. Davis, Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis, Science 285 (1999) 901–906.

[10] A.C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick, A.M. Michon, C.M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M.A. Heurtier, R.R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, Functional organization of the yeast proteome by systematic analysis of protein complexes, Nature 415 (2002) 141–147.

[11] B. Masciadri, L.B. Areces, P. Carpinelli, M. Foiani, G. Draetta, F. Fiore, Characterization of the *BUD31* gene of *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 320 (2004) 1342–1350.

[12] D. Saha, P. Khandelia, R.T. O'Keefe, U. Vijayraghavan, *Saccharomyces cerevisiae* NineTeen complex (NTC)-associated factor Bud31/Ycr063w assembles on precatalytic spliceosomes and improves first and second step pre-mRNA splicing efficiency, J. Biol. Chem. 287 (2012) 5390–5399.

[13] H.H. Lim, C.J. Loy, S. Zaman, U. Surana, Dephosphorylation of threonine 169 of Cdc28 is not required for exit from mitosis but may be necessary for start in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 16 (1996) 4573–4583.

[14] U. Vijayraghavan, M. Company, J. Abelson, Isolation and characterization of pre-mRNA splicing mutants of *Saccharomyces cerevisiae*, Genes Dev. 3 (1989) 1206–1216.

[15] A.K. Sapra, Y. Arava, P. Khandelia, U. Vijayraghavan, Genome-wide analysis of pre-mRNA splicing: intron features govern the requirement for the second-step factor, Prp17 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, J. Biol. Chem. 279 (2004) 52437–52446.

[16] J.A. Pleiss, G.B. Whitworth, M. Bergkessel, C. Guthrie, Transcript specificity in yeast pre-mRNA splicing revealed by mutations in core spliceosomal components, PLoS Biol. 5 (2007) e90.

[17] S.K. Mishra, T. Ammon, G.M. Popowicz, M. Krajewski, R.J. Nagel, M. Ares Jr., T.A. Holak, S. Jentsch, Role of the ubiquitin-like protein Hub1 in splice-site usage and alternative splicing, Nature 474 (2011) 173–178.

[18] M.H. Jones, D.N. Frank, C. Guthrie, Characterization and functional ordering of Slu7p and Prp17p during the second step of pre-mRNA splicing in yeast, Proc. Natl. Acad. Sci. USA 92 (1995) 9687–9691.

[19] V. Seshadri, V.C. Vaidya, U. Vijayraghavan, Genetic studies of the PRP17 gene of *Saccharomyces cerevisiae*: a domain essential for function maps to a nonconserved region of the protein, Genetics 143 (1996) 45–55.

[20] O. Dahan, M. Kupiec, The *Saccharomyces cerevisiae* gene CDC40/PRP17 controls cell cycle progression through splicing of the ANC1 gene, Nucleic Acids Res. 32 (2004) 2529–2540.

[21] Y. Kaplan, M. Kupiec, A role for the yeast cell cycle/splicing factor Cdc40 in the G1/S transition, Curr. Genet. 51 (2007) 123–140.

[22] C.H. Chen, W.Y. Tsai, H.R. Chen, C.H. Wang, S.C. Cheng, Identification and characterization of two novel components of the Prp19p-associated complex, Ntc30p and Ntc20p, J. Biol. Chem. 276 (2001) 488–494.

[23] L.H. Hwang, A.W. Murray, A novel yeast screen for mitotic arrest mutants identifies DOC1, a new gene involved in cyclin proteolysis, Mol. Biol. Cell 8 (1997) 1877–1887.

[24] R. Ohi, A. Feoktistova, S. McCann, V. Valentine, A.T. Look, J.S. Lipsick, K.L. Gould, Myb-related *Schizosaccharomyces pombe* cdc5p is structurally and functionally conserved in eukaryotes, Mol. Cell. Biol. 18 (1998) 4097–4108.

[25] W. Zhu, I.R. Rainville, M. Ding, M. Bolus, N.H. Heintz, D.S. Pederson, Evidence that the pre-mRNA splicing factor Clf1p plays a role in DNA replication in *Saccharomyces cerevisiae*, Genetics 160 (2002) 1319–1333.

[26] L. Ni, M. Snyder, A genomic study of the bipolar bud site selection pattern in *Saccharomyces cerevisiae*, Mol. Biol. Cell. 12 (2001) 2147–2170.

[27] M. Costanzo, A. Baryshnikova, J. Bellay, Y. Kim, E.D. Spear, C.S. Sevier, H. Ding, J.L. Koh, K. Toufighi, S. Mostafavi, J. Prinz, R.P. St Onge, B. VanderSluis, T. Makhnevych, F.J. Vizeacoumar, S. Alizadeh, S. Bahr, R.L. Brost, Y. Chen, M. Cokol, R. Deshpande, Z. Li, Z.Y. Lin, W. Liang, M. Marback, J. Paw, B.J. San Luis, E. Shuteriqi, A.H. Tong, N. van Dyk, I.M. Wallace, J.A. Whitney, M.T. Weirauch, G. Zhong, H. Zhu, W.A. Houry, M. Brudno, S. Ragibizadeh, B. Papp, C. Pal, F.P. Roth, G. Giaever, C. Nislow, O.G. Troyanskaya, H. Bussey, G.D. Bader, A.C. Gingras, Q.D. Morris, P.M. Kim, C.A. Kaiser, C.L. Myers, B.J. Andrews, C. Boone, The genetic landscape of a cell, Science 327 (2010) 425–431.

[28] S. Rodriguez-Navarro, J.C. Igual, J.E. Perez-Ortin, SRC1: an intron-containing yeast gene involved in sister chromatid segregation, Yeast 19 (2002) 43–54.
[29] G.M. Wilmes, M. Bergkessel, S. Bandyopadhyay, M. Shales, H. Braberg, G. Cagney, S.R. Collins, G.B. Whitworth, T.L. Kress, J.S. Weissman, T. Ideker, C. Guthrie, N.J. Krogan, A genetic interaction map of RNA-processing factors reveals links between Sem1/Dss1-containing complexes and mRNA export and splicing, Mol. Cell 32 (2008) 735–746.
[30] M.D. Ohi, A.J. Link, L. Ren, J.L. Jennings, W.H. McDonald, K.L. Gould, Proteomics analysis reveals stable multiprotein complexes in both fission and budding yeasts containing Myb-related Cdc5p/Cef1p, novel pre-mRNA splicing factors, and snRNAs, Mol. Cell. Biol. 22 (2002) 2011–2024.
[31] Q. Wang, J. He, B. Lynn, B.C. Rymond, Interactions of the yeast SF3b splicing factor, Mol. Cell. Biol. 25 (2005) 10745–10754.