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SPO12 and *SIT4* suppress mutations in *DBF2*, which encodes a cell cycle protein kinase that is periodically expressed

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ABSTRACT

To help clarify the role of *DBF2*, a previously described cell cycle protein kinase, high copy number suppressors of the *dbf2* mutation were isolated. Three open reading frames (ORF) have been identified. One ORF encodes a protein which has homology to a human small nuclear riboprotein, while the remaining two are genes which have been identified previously, *SIT4* and *SPO12*. *SIT4* is known to have a role in the cell cycle but the nature of the interaction between *SIT4* and *dbf2* is unclear. *SPO12* has until now been implicated exclusively in meiosis. However, we show that *SPO12* is expressed during vegetative growth, moreover it is expressed under cell cycle control coordinately with *DBF2*. *SPO12* is a nonessential gene, but it becomes essential in a *DBF2* delete genetic background. Furthermore, detailed analysis of the cell cycle of *SPO12* delete cells revealed a small but significant delay in mitosis. Therefore, *SPO12* does have a role during vegetative growth and it probably functions in mitosis in association with DBF2.

INTRODUCTION

It is now clear that much of the information within eukaryotic cells is transmitted by means of the phosphorylation status of key proteins. This includes processes as diverse as gene regulation as well as the G1/S and G2/M transitions in the cell cycle. In the case of budding yeast over 30 protein kinases have been discovered and a number of these have been shown specifically to regulate cell cycle progression *e.g.* *CDC28*, *CDC7*, and *DBF2* (for reviews see [1–3]).

Entry into the cell cycle is controlled by *CDC28* at START. However, in the original searches of Hartwell and his colleagues only two genes were found which functioned downstream of *CDC28* in late G1, namely *CDC4* and *CDC7*. Given the complexity of DNA replication it seemed likely that there should be additional essential genes acting in this part of the cell cycle. A new screen for mutants defective in DNA synthesis was therefore carried out and four new genes were discovered, *DBF1–4* [4,5]. Subsequent cloning and sequencing has revealed that one of these, *DBF2*, encoded a protein containing all the eleven conserved domains found in protein kinases, suggesting very strongly that the *DBF2* protein is itself a protein kinase [6]. Despite being isolated on the basis of being a DNA synthesis mutant, further analysis revealed that rather than blocking DNA synthesis, *dbf2* caused only a delay in initiation of some 40 min at the restrictive temperature. DNA synthesis then continues and finally the cells arrest as swollen pairs of cells, 'dumbbells', with an approximately 2C complement of DNA. This might be taken as indicating a subtle defect in DNA synthesis. However, experiments with *rad9 dbf2* double mutants show that *dbf2* does not block at the *RAD9* checkpoint suggesting that the DNA is undamaged (unpublished observation). Therefore, it seems more likely that the *DBF2* point of action is after S phase. Indeed, determination of the execution point and RNA hybridisation analysis of cells blocked late in the cell cycle suggested that *DBF2* may in fact act late in mitosis [6]. The situation is further complicated by the discovery of a homologue of *DBF2*, termed *DBF20* [7]. *DBF20* is more than 80% identical to *DBF2* at the amino acid level over some 490 residues, and the two genes share at least one essential function [7]. Individually, they can be deleted and are therefore non essential genes, however, deletion of both genes is lethal. Surprisingly, the expression of these genes in the cell cycle differs from one another. The *DBF20* transcript level remains constant throughout the cell cycle [7] whereas *DBF2* expression is tightly linked to the cell cycle [6].

To help determine the role of *DBF2*, we set out to isolate high copy number, dosage-dependent, suppressors of the *dbf2* mutation. Such suppressors frequently reveal gene products that interact with the principal gene of interest and three that suppressed *dbf2* were obtained. These suppressors were named SDB21 to SDB23 (Suppressor of dbf2 number 1 to 3). Two of these suppressors have turned out to be known genes, *SPO12* and *SIT4*, and these form the subject of this paper. The *spo12* mutant was originally isolated from a natural variant which carried out only a single meiotic division leading to the formation

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of dyads [8]. Further analysis revealed that approximately 95% of the dyads resulted from the occurrence of only the second meiotic division, the remaining few percent resulting from only the first meiotic division taking place. The level of meiotic recombination was found to be similar if not the same as wild type levels [9]. The gene encoding *SPO12* has been cloned and sequenced [10] but it did not reveal any homology to known protein sequences or known motifs. However, it was fortuitously discovered that the few acidic residues at the C-terminus are required for activity.

The *sit4* mutation was originally discovered amongst a group of second site mutations which suppressed the requirement of *HIS4* expression for the trans-activating proteins encoded by the genes *GCN4*, *BAS1* and *BAS2*. *SIT1* and *2* were identified as the two largest subunits of RNA polymerase II (*RBPI* and *RBPII*), while *SIT4* was found to be 56% identical to mammalian type 2A and 43% identical to mammalian type 1 serine/threonine protein phosphatases [11]. A model was proposed which suggested that *RBPI* and *RBPII* interacted directly with *SIT4*. Furthermore, it was proposed that mutations in *SIT4* led to an alteration in the specificity and/or the level of activity of RNA polymerase. This was supported by the fact that the level of transcription of several genes was affected by the *sit4* mutation.

The physiological basis for the suppression of *dbf2* by *SIT4* and *SPO12* is not yet clear. Deletants of *SPO12* are known to be viable [10] but we show here that in the absence of *DBF2*, *SPO12* deleted strains are no longer viable. Also, cells deleted for *SPO12* are delayed in mitosis. Consistent with a mitotic role for *SPO12*, the transcript is expressed under cell cycle control in late M phase coordinately with *DBF2*. A mitotic role for *SPO12* supports the notion that *DBF2* itself has a mitotic role [6]. The third gene, *SDB23*, has significant homology to a human small nuclear riboprotein and forms the subject of another paper.

MATERIALS AND METHODS

Yeast strains

The *dbf2* mutant strains used were L119-7D (*MATα* dbf2-1 ura3-52 trp1/2 ade1), L181-6B (*MATα* dbf2-2 ura3-52 leu2-3,112 trp1/2) and L182-7A (*MATa dbf2-3 ura3-52 leu2-3,112 trp1 ade1/5*). All are clean temperature-sensitive strains that show no growth at 37°C. Other strains were CG378 (*MATa ade5 leu2-3,112 trp1-289 ura3-52*) and CG379 (*MATα* his7-2 leu2-3,112 trp1-289 ura3-52) and the cell cycle mutants *cdc5, cdc9, cdc14 and cdc15* were all *MATa ade1 ade2 ura1 his7 lys2 tyr1* and were obtained from L.H.Hartwell.

Media and general methods

YPD and YNB media have been described previously [12]. Cell numbers were determined by use of a particle counter (Coulter Electronics, Dunstable, England). Yeast transformation were performed by using a modification [13] of the lithium acetate method [14].

Identification of suppressor genes

Restriction analysis of clones containing *SDB21* revealed a 0.8kb *Eco*RI fragment which was common to all of the clones. Northern hybridisation analysis using this 0.8kb *Eco*RI fragment indicated a single transcript of approximately 600 nucleotides (data not shown), therefore further subcloning was not necessary. This 0.8kb *Eco*RI fragment was then transferred to the Bluescript sequencing vector and the entire sequence determined. The whole of the DNA sequence obtained matched exactly that of *SPO12* [10], including those portions of the upstream and downstream regions which had also been sequenced.

SDB22 was isolated as a single clone with an insert of approximately 22kb. To locate the suppressor gene within this the plasmid was subjected to Tn*1000* mutagenesis in *E.coli* [16] and a library of randomly disrupted plasmids was transformed into L119-7D. Subsequent replica-plating of the transformants onto YPD at 37°C and 25°C indicated those plasmids where suppressor function had been disrupted. The plasmids were retrieved [17], transformed into DH5α and the DNA examined after small scale preparation following alkaline lysis of the cells [18]. The transposon insertion sites were located by restriction analysis and DNA sequencing was carried out using primers specific to the γ and δ sequences of Tn*1000*. The sequence obtained from five different transposon insertion sites (Fig.1), showed a 100% homology to sequence from five different locations in the *SIT4* open reading frame and upstream region [11].

Deletion of *SPO12*

A 1.7kb *Pvu*II fragment containing *SPO12* was cloned into the large *Pvu*II fragment of Bluescript (Bluescript with the multiple cloning site removed). This clone was digested with *Spe*I and *Cla*I, removing 112 bp of upstream sequence and all but 8 amino acids from the *SPO12* open reading frame to give vector pBSSPO12Δ. The *TRP1* gene was cut out of YRp12 as an *Eco*RI-*Pst*I fragment and was inserted into the multiple cloning site of Bluescript and subsequently removed as a *Spe*I-*Cla*I fragment. This fragment bearing the *TRP1* gene was then inserted into pBSSPO12Δ. The *SPO12::TRP1* DNA was removed from Bluescript as a *Pvu*II fragment and used to transform both CG378 and CG379. Transformants were grown up and genomic DNA was prepared [17]. The deletion of *SPO12* was confirmed by probing a Southern blot of genomic DNA with the *Spe*I-*Cla*I DNA fragment containing *SPO12*. Those transformants which had lost *SPO12* were further tested by sporulating a CG378(*SPO12::TRP1*)/CG379(*SPO12::TRP1*) diploid. Diploids missing *SPO12* give rise to asci containing only two diploid spores.

Synchronisation of yeast cells

The feed-starve and α-factor synchronised cells used here are derived from cultures that have been described previously [6]. Synchronisation using the *cdc14* mutant was achieved by incubating the mutant cells at 37°C for two hours, a period of time sufficient to allow all the cells to accumulate at the *cdc14* block point. To achieve an immediate release from the temperature induced block, an appropriate volume of cooled fresh medium was added to the culture so that the temperature was immediately brought to 25°C. This allowed one reasonably synchronous round of division.

Northern hybridization

Total RNA was extracted from yeast cells as previously described [12]. A 5μg sample of total RNA, denatured with glyoxal, was separated by agarose gel electrophoresis and transferred to a Gene Screen hybridization membrane (Dupont, NEN Research Products, Boston, Massachusetts) as described previously [12,19]. Probes for RNA-DNA hybridization were internal fragments from the genes concerned. For *SPO12*, the probe used was the internal *Spe*I-*Cla*I DNA fragment.

Preparation of cells for flow fluorometric analysis (FACS)

The cells were grown to mid-log phase, washed in 50mM sodium citrate then suspended in 70% ethanol. After incubation for 1h at room temperature the cells were washed in 50mM sodium citrate and suspended in 50mM sodium citrate with 1mg/ml RNase. After incubation for 1 hour at 37°C the cells were washed in 50mM sodium citrate, then suspended in 50mM sodium citrate with 50ng/ml propidium iodide and incubated overnight in the dark before analysis in a FACStar (Becton Dickinson) flow fluorometer.

RESULTS

Isolation and identification of *SDB21–SDB23*

DNA fragments able to complement the temperature-sensitive growth phenotype of a *dbf2* mutation were isolated from a yeast genomic library constructed in a multicopy yeast–*Escherichia coli* shuttle vector as. Originally 32 individual clones were found to have the ability to suppress *dbf2*. From this number three unique clones were identified by restriction and Southern hybridisation analysis. As described in Materials and Methods, *SDB21* has been found to be *SPO12* and *SDB22* is *SIT4*. The third suppressor *SDB23* is homologous to a human small nuclear riboprotein and will be described elsewhere.

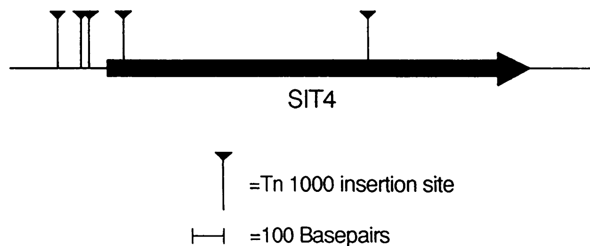

Figure 1. Shown above are the sites of transposon insertions which inactivated the ability of pSDB22 to suppress *dbf2* (see Materials and Methods).

Table 1. Allele-specificity of the high copy number suppressors of *dbf2*.

Allele	Suppressor *SDB21*	*SDB22*	*SDB23*	Controls *DBF2*	Vector*
dbf2-1	+++	+++	+++	+++	–
dbf2-2	+++	+	+	+++	–
dbf2-3	++	–	–	+++	–

+ = Growth at 37°C; the number of symbols indicating the extent of growth.
– = No growth at 37°C.
* = YEp24.

Allele-specificity of the suppressors

Each of the suppressors and *DBF2* itself were inserted into vector Yep24 and transformed into *dbf2-1, dbf2-2* and *dbf2-3*. The transformants were replica-plated onto YPD media at 25°C and 37°C. The ability of the suppressors to rescue growth at 37°C was scored by comparison with the control plasmid, Yep24-*DBF2* (Table 1). *SDB22* and *SDB23* show weak allele-specificity but *SDB21* is not allele-specific.

SPO12* is expressed periodically in the cell cycle unlike *SIT4

DBF2 is expressed under cell cycle control in late M phase (see Introduction). Since suppressors of *dbf2* are likely to be involved in the same physiological process as *DBF2* itself their genes may well be regulated in the same way. The expression patterns in the cell cycle of *SPO12* and *SIT4* were therefore examined.

Cells were initially synchronised by using a feed-starve protocol [20]. To reduce the possibility of an artifactual result, which is more likely in the first cycle of a synchronous culture, more than one cycle was monitored to ensure that the normal pattern of expression was observed. Total RNA was extracted from samples taken at intervals throughout the experiment, and transcript levels were assessed by Northern hybridization. As a loading control the level of a 2.5kb transcript from a gene adjacent to *POLI* was examined which is known to be constant during the cell cycle [19]. The level of the histone H2A transcript was also examined as a molecular measure of the synchrony achieved (Fig.2). As expected, the *DBF2* transcript was strongly periodic [6]. When the same blot was probed with *SPO12* DNA, it was immediately obvious that this gene was also periodically expressed in the cell cycle (Fig.2). Whereas the first cycle is very weak, but still discernable, subsequent cycles show a very clear periodic transcript. Furthermore this expression coincides precisely (within the resolution of the method) with the expression of *DBF2*. The weak expression in the first cycle was examined further by measuring the messenger RNA levels of a culture during emergence from G0 using the method of Nasmyth [21]. Strong expression of *SPO12* occurred early during the first cell cycle (data not shown). We therefore assume the weak first cycle expression of *SPO12* seen in the feed-starve experiment was an artifact of that method of synchrony. Despite the low level of

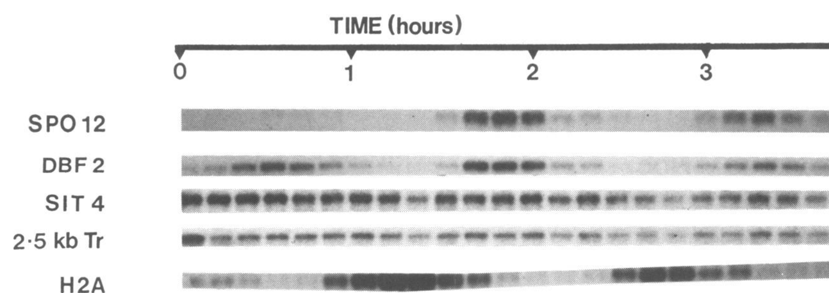

Figure 2. The cell cycle regulation of the *SPO12* and *SIT4* transcripts in a culture synchronised by feed-starve. The culture details have been described previously [6] and just over two synchronous cell cycles were monitored. Total RNA was extracted from samples and a Northern blot was prepared. This was probed with ^{32}P-labelled DNA fragments from the genes shown on the left and a suitably exposed autoradiograph is presented above.

expression of *SPO12* during the first cycle, it seems likely that it is expressed at the same time in the cell cycle as *DBF2*.

Two other methods of synchronization were also used to confirm this coincidence of expression. First α-pheromone was used (Fig.3). This arrests *MATa* cells at or near START in late G1; on release from the block, synchronous rounds of cell division take place. The controls, which are the same as in Fig.2, showed that good synchrony was obtained over the two cycles examined. Expression of *SPO12* was clearly periodic, and again its pattern of expression was identical to that of *DBF2* over two cycles of expression. Unusually, both transcripts increase in amount during the incubation with α-factor for reasons that are not understood (see also [6]).

The third method of synchronising the cells was achieved using the *cdc14* mutation (Fig.4). A *cdc14* culture was grown to mid

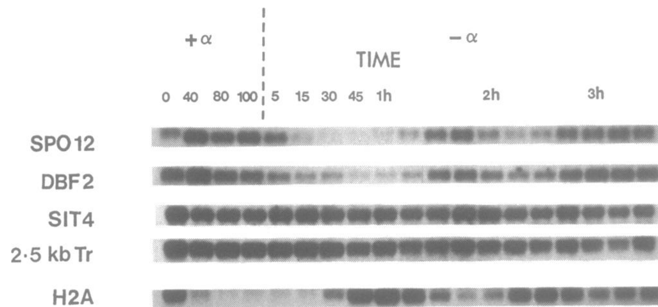

Figure 3. The cell cycle regulation of the *SPO12* and *SIT4* transcripts in a culture synchronised by α-factor. The culture details have been described previously [6] and just over two synchronous cell cycles were monitored. See the legend to Fig. 2 for further details.

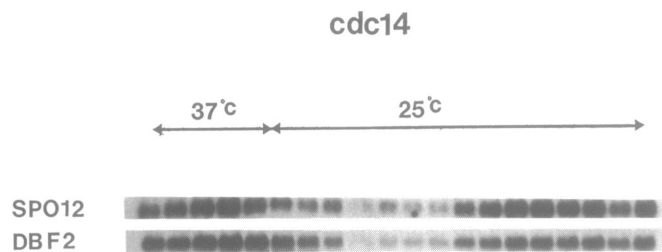

Figure 4. The cell cycle regulation of the *SPO12* transcript in a culture synchronised by *cdc14* block and release. During the incubation at 37°C samples were taken every 30min and after return to 25°C, sampling was every 15min. See the legend to Fig. 2 for further details.

log at 25°C and then transferred to 37°C for the equivalent of one generation time so that cells accumulate at the *cdc14* block. Returning the cells to 25°C reverses the *cdc14* blockage and cells undergo a reasonably synchronous cell cycle. The *cdc14* block point is in the late nuclear division phase of the cell cycle when it is likely that *DBF2* is expressed [6] and, indeed, both *SPO12* and *DBF2* transcripts increase in amount during the 37°C incubation. On return to 25°C both genes show a single coincident cycle of expression. This method of synchrony offers further proof that *DBF2* and *SPO12* are regulated at the same point in the cell cycle.

To further compare the regulation of *DBF2* and *SPO12*, the pattern of expression of these genes was examined under various conditions that lead to a cell cycle block. It had been previously shown that *DBF2* is expressed in cells blocked late in the cell cycle by holding either *cdc14*, *cdc5* or *cdc15* mutants at 37°C as well as during inhibition by nocadazole. Conversely, *DBF2* is not expressed in cells blocked in S phase by hydroxyurea or in *cdc9* mutants at 37°C [6]. The pattern of *DBF2* expression obtained here is the same as that described previously [6] and, moreover, we found that *SPO12* expression is similar to that of *DBF2* (Fig.5). Thus *SPO12* appears to be coordinately regulated with *DBF2*, possibly around late nuclear division.

In sharp contrast to *SPO12*, the transcript level of *SIT4* does not demonstrably change during the cell cycle (Figs. 2 and 3), which is in accord with the finding that *SIT4* protein levels do not fluctuate during the cell cycle [11].

FACS analysis of *SPO12* delete strains

Previous workers failed to demonstrate any perturbation of the growth rate of strains with *SPO12* deleted and consequently inferred that the *SPO12* gene product has no important role in mitotic growth [10]. Furthermore, they concluded that *SPO12* functions exclusively in meiosis. Because *SPO12* suppresses *dbf2*, a probable mitotic mutant, we decided to examine the distribution of cells, deleted for *SPO12*, within the cell cycle using flow fluorometric analysis.

Figure 6 shows the distribution of logarithmicaly growing haploid wild type cells, and an isogenic strain which has *SPO12* deleted. Like Malavasic and Elder [10] we find that *SPO12* deletes grow at the same rate as a wild type but FACS analysis shows that the strain which is missing *SPO12* has a reduced peak of cells in G1 with a 1C DNA complement and an increased peak of cells with a 2C complement of DNA in G2. A similar result was obtained using a diploid strain (Fig.6). The G1 peak is reduced by approximately 30% and the G2 peak is increased accordingly. Although not a dramatic result it does show clearly that strains deleted for *SPO12* have a mitotic phenotype, which may reflect a delay in completion of mitosis. Consistent with this mitotic delay, the FACS forward scatter data (an indication of

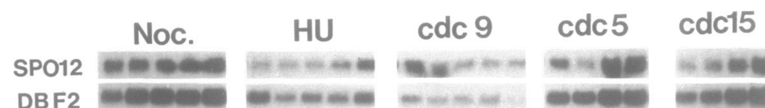

Figure 5. Comparison of *SPO12* and *DBF2* transcript levels in G2-blocked cells. The three *cdc* mutants were grown at 25°C, sampled, and transferred to 37°C, and further samples were taken at 40min intervals to 2h. For the nocodazole (Noc.)- and hydroxyurea (HU)-treated cultures, the drugs were added to mid-log cells of CG378 to final concentrations of 25μg/ml and 100mM, respectively. Each culture was sampled immediately; the nocadazole culture was then sampled at 45min intervals to 3 hours, and the hydroxyurea culture was sampled hourly to 3 h and then again at 5 h. Northern blots prepared from the samples were probed with DNA from *SPO12* and *DBF2*, and the resulting autoradiographs are presented. A constitutively expressed transcript that acts as a loading control can be found in Fig.8 of [6].

the size of the particles being measured) showed quite clearly that part of the *spo12* population is larger than any of the wild type cells.

Deletion of both *SPO12* and *DBF2* is lethal

It has been shown previously that *SPO12* is not essential to the cell for mitotic growth [10]. Also, *DBF2* was shown to be a non-essential gene for mitotic growth [6]. However, strains with *DBF2* deleted and replaced with the *LEU2* gene (*DBF2::LEU2*) do have a distinctly aberrant cell morphology and whilst the cells can survive without *DBF2*, it is clearly not without cost to the cell. Since *DBF2* and *SPO12* may interact with one another we examined the possibility of a synthetic lethality occurring when both were deleted. Table 2 summarises the results of a tetrad analysis of a cross of a *SPO12::TRP1* strain with a *DBF2::LEU2* strain.

All of the spores which received a copy of both *SPO12* and *DBF2* germinated as expected. Similarly, all but one of the spores deleted for *SPO12* germinated. The loss of *DBF2* alone does have a significant effect on germination, just under half (42%) of the spores missing the *DBF2* gene having germinated. However, the loss of *SPO12* as well as *DBF2* has a dramatic effect on spore viability. Indeed, no spores which by inference were *SPO12::TRP1* and *DBF2::LEU2* grew. Microscopic examination of these double deletes, which had failed to grow, revealed that germination had occurred but the cells had arrested as dumbbells. Therefore cells missing both *DBF2* and *SPO12* are not viable.

Table 2. Deletion of both *DBF2* and *SPO12*.

	DBF2::LEU2	*DBF2*
SPO12::TRP1	0/28	25/26
SPO12	11/26	28/28

The figures show the viability of spores inheriting combinations of wild type and mutant forms of *DBF2* and *SPO12*. The number of inviable spores inheriting delete versions of the genes was inferred from the distribution of markers in individual tetrads in the cross.

DISCUSSION

Analysis of high copy number suppressors of *dbf2* has revealed *SDB21* to be *SPO12* and *SDB22* to be *SIT4*. In light of the phenotype of the various *SIT* mutations, as outlined in the Introduction, and the fact that *SIT4* is not a phosphoprotein [22] and therefore not a direct substrate of *DBF2*, it seems likely that *SIT4*'s suppression of *dbf2* will be indirect, perhaps through the elevation or reduction of transcription of some other gene(s). If a protein encoded by one of these other genes interacted with *dbf2* it might account for the suppression. Whilst neither the levels of the *DBF2*, *DBF20* nor *SPO12* transcripts were affected by the presence of *SIT4* in high copy number (data not shown), subsequent genetic analysis of *dbf2* has revealed other genes which suppress its temperature-sensitive phenotype (J.Toyn, unpublished observation) and these may be found to have their transcription modulated by *SIT4*.

The second previously identified gene which acts as a suppressor of *dbf2*, which we have termed *SDB21* is *SPO12*.

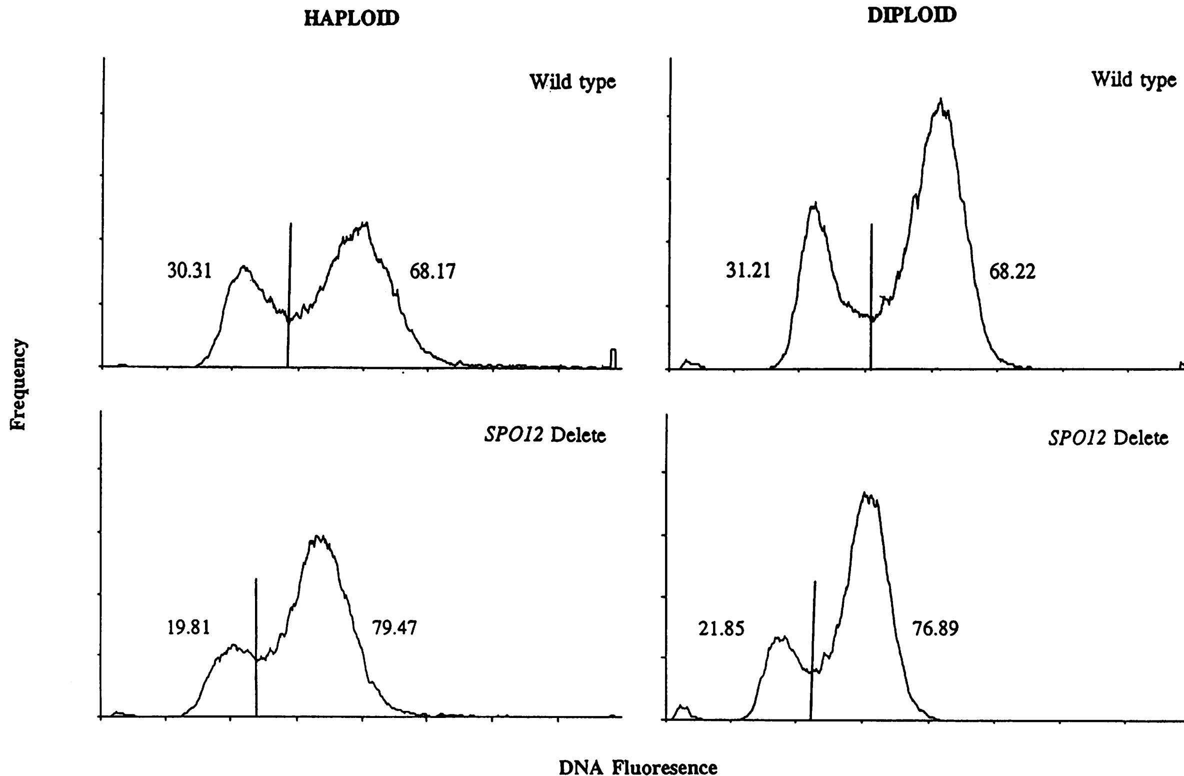

Figure 6. FACS analysis of *SPO12* delete strains. Cultures were grown to mid log phase and the cells were harvested and stained for FACS analysis. 20 000 cells were measured in each culture. Each plot has a vertical line dividing the two peaks, the numbers to the left indicate the percentage of cells found in the peak on that side and *vice versa*.

SPO12, until now, has been implicated solely in meiosis. Mutation of *SPO12* leads to aberrant ascus formation, the asci formed being almost exclusively dyads (two spored) containing diploid spores [10]. Previously it was reported that *SPO12* is not an essential gene for growth [10]; we too found that deletion of *SPO12* gave rise to viable cells. However, *SPO12* was found to become an essential gene when *DBF2* was also deleted from the genome. This synthetic lethality, as well as the suppression of *dbf2* by *SPO12*, demonstrated that *SPO12* does indeed have a role in vegetative growth and that *SPO12* and *DBF2* are involved in the same pathway of cellular events. *DBF20* is also involved in this pathway, however, the relationship between *SPO12*, *DBF2* and *DBF20*, is not symmetrical. Any one of these can individually be deleted, but only certain pair-wise combinations are lethal. So, deletion of both *DBF2* and *DBF20* is lethal [7], as is deletion of *SPO12* and *DBF2*, however, deletion of *SPO12* and *DBF20* was found to be viable (J.Toyn *et al.*, in preparation). This suggests that *SPO12* is functioning with *DBF20* to cover the vital function missing in a *DBF2* delete strain.

The pattern of expression of *SPO12* is consistent with it interacting with *DBF2*. The expression of these two genes was found to be remarkably similar under a variety of different conditions. The similarity between the two genes was so striking that almost certainly they are coordinately regulated. The only exception to this can be seen in the first cycle of expression of *SPO12* in the feed-starve synchronisation experiment. Here, the level of *SPO12* expression is much reduced when compared to subsequent rounds of expression, whereas *DBF2* shows a similar level of expression in its first cycle as it does in subsequent ones. However, in a feed-starve culture the cells are in an uncertain physiological state during the first cycle as the emerge from stationary phase and using a different method to examine cells emerging from G0 [21] it was found that *SPO12* gave a strong first cycle level of expression (data not shown). Therefore it is probable that the difference seen between *SPO12* and *DBF2* in the feed-starve culture are an artifact of the synchronisation method.

The precise point in the cell cycle at which *DBF2* and *SPO12* are expressed is difficult to establish unambiguously. We previously concluded that *DBF2* was expressed either in late M phase or early G1 [6], points that are, of course, temporally very close in an exponential culture. The expression of *SPO12* and *DBF2* in *cdc14* and *cdc15* mutants held at 37°C [6] (Figs. 4 and 5), as well as their expression in elutriation synchronised cells [6] (unpublished observations) suggests strongly that *SPO12* and *DBF2* are in fact expressed in late M phase. The major objection to this is the expression of *DBF2* and *SPO12* early in the feed-starve synchronous culture [6](Fig.2) and also the early expression of *SPO12* in cells coming out of G0 (see above). A possible explanation for this is that cells coming out of G0 or stationary phase may be in an unusual physiological state with respect to cycling cells. For instance, various gene products may have decayed and require resynthesis early in the first cell cycle. Thus *DBF2* and *SPO12*, and perhaps certain other genes as well, may have to be expressed ectopicaly in this initial cell cycle.

The coordinate expression of *SPO12* and *DBF2* strongly supports the notion that they are both involved in the same process within the cell cycle. The precise nature of this process is still not clear. *DBF2* has been shown to be involved in the maintenance of chromosomes, mutant *dbf2* strains exhibiting high levels of chromosome loss (J.Toyn and L.H.Johnston, in preparation). However, no effect on chromosome loss could be detected in strains which had *SPO12* deleted as compared to isogenic controls. The frequency of chromosome loss detected in both *SPO12* delete and wild type strains being consistent with the reported normal levels of chromosome loss for *S.cerevisiae* (data not shown).

A further difference between the function of *SPO12* and *dbf2* lies in their effect on meiosis. The discovery that one of the suppressors of *dbf2* was a sporulation protein led us to examine if *DBF2* was itself involved in meiosis. However, preliminary experiments failed to demonstrate any temperature-dependent effects of a *dbf2-1/dbf2-1* diploid on sporulation at 34°C (a temperature which prevented mitotic growth of the strain but not sporulation) as compared to the same strain at 25°C. We also did not see any abnormalities during sporulation of diploids with both copies of *DBF2* deleted (data not shown) thus the association of *DBF2* and *SPO12* appears to be specific for the mitotic cell cycle.

Since *DBF2* and *SPO12* are associated in the same cellular pathway during the mitotic growth cycle, we examined the growth characteristics of a *SPO12* delete strain. It has been previously reported that deletion of *SPO12* did not affect the growth rate as compared to isogenic wild type control levels [10]. Whilst our data is in agreement with this, we find that flow cytometric analysis of logarithmicaly growing cells revealed a difference between isogenic strains differing only at the *SPO12* locus. In a *SPO12* delete strain the G1 and G2 peaks were reduced and increased, respectively, by a significant proportion. One interpretation of this finding is that the cells are unable to complete some function in G2 at the normal rate; this delay would account for the increase in the G2 peak. Cells would then emerge from M phase at a slightly increased size, since they continue to grow and accumulate mass during the G2 delay, and arrive in G1 slightly larger than normal. Thus less time is required to achieve the critical size to traverse START, so tending to reduce the G1 peak. Presumably the G2 delay and the rapid traverse of START act to roughly balance each other out and give rise to a wild type growth rate.

Malavasic and Elder [10] concluded that *SPO12* was involved 'exclusively' in meiosis. However, we have presented data proving that *SPO12* has a function in mitosis as well as meiosis. This is not very surprising in view of the fact that most *cdc* mutants have been found to be defective in meiosis as well as mitosis [23], it seems likely that many of these gene products participate in the central processes of duplication and separation of chromosomes in both processes. Our data supports the theory that *DBF2*, *DBF20* and *SPO12* have an associated function during mitosis, whilst one of them, *SPO12*, also has a role in meiosis.

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