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CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 21 January 2012
Final form accepted: 11 May 2012
Published online: 19 May 2012

Volume 17 (2012) pp S1-S15
DOI: 10.2478/s11658-012-0017-4

Supplementary material

PHYLOGENETIC ORIGIN AND TRANSCRIPTIONAL REGULATION AT THE POST-DIAUXIC PHASE OF *SPI1* IN *Saccharomyces cerevisiae*

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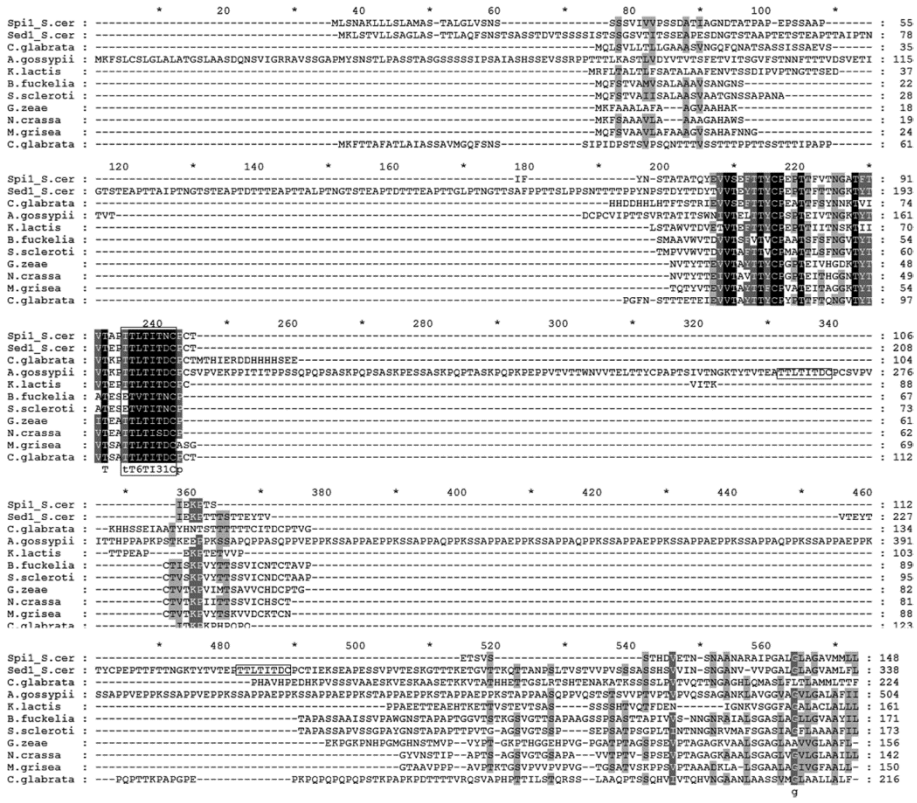
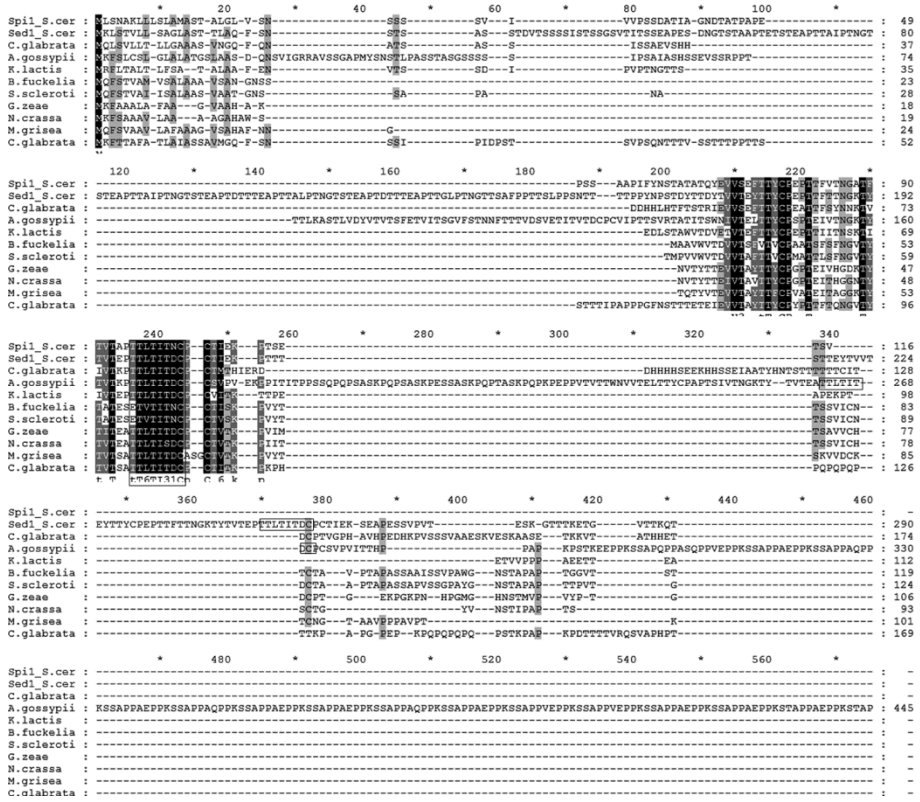
Details of the phylogenetic analysis with MrBayes

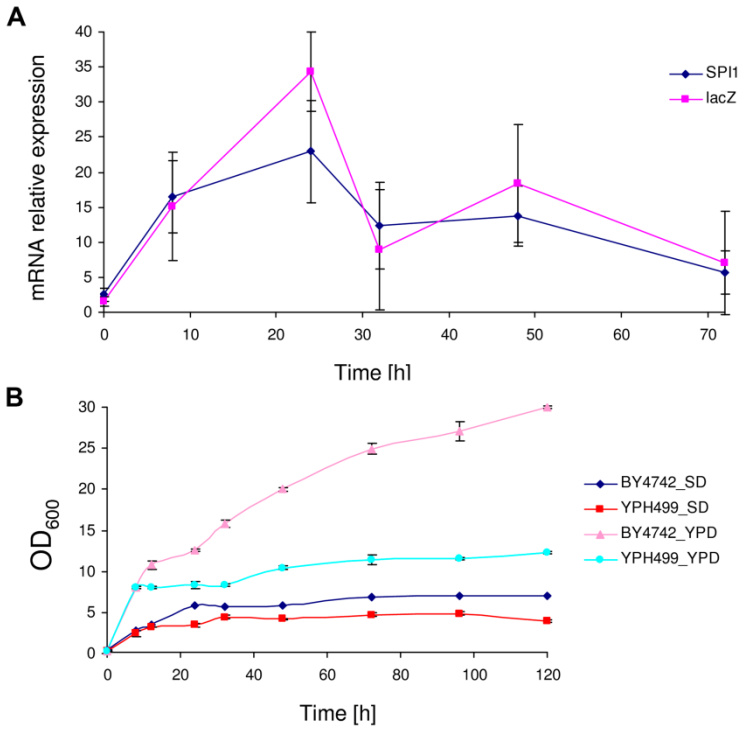
```
input
outgroup XP503675_Y.lipolytica; outgroup XP001385034_P.stipitis;lset applyto=(all) rates=Invgamma;unlink shape=(all) pinvar=(all);
prset ratepr=variable aamodelpr=mixed;mcmcp ngen=1000000 nchains=4 printf=1000 samplefreq=1000 savebrlens=yes
sumt/sump burnin=700
   Overlay plot for both runs:
   (1 = Run number 1; 2 = Run number 2; * = Both runs)
   +---------------------------------------------------+ -1673.08
   |2                     1                    1       |
   |                         1         2               |
   |1     1 1                     2 11               2 |
   |          2   12 2  *     1       1      *     2  2|
   |         1   22 1  2 2     2 2 12       1  2       |
   |   2     2 21      1      2 2     2  *       2     |
   |    2            12    11      1    1       1 11 1 |
   |    1  2 *  21 1       22* 1       1   2  1  11 1  |
   | 2 1 1 2              2   2   22    2 * 2 21       |
   | 11 2* 1            1 1          2            2 2  |
   |  2     2 1  2    12          1             2     1|
   |           1  1 2                              2   |
   |                            1             2        |
   |                             1      1              |
   |                                   2   1           |
   +-----+----+----+----+----+----+----+----+----+----+ -1682.25
   ^                                                   ^
   700000                                              1000000
   Run   Arithmetic mean   Harmonic mean
   --------------------------------------
     1      -1671.94          -1687.88
     2      -1671.98          -1687.64
   --------------------------------------
   TOTAL    -1671.96          -1687.77
   --------------------------------------
                                                    95% Cred. Interval
                                                 ----------------------
   Parameter       Mean        Variance        Lower          Upper          Median        PSRF *
   ------------------------------------------------------------------------------------------------
   TL            4.555042      0.160492      3.798000       5.349000       4.544000       1.002
   alpha         5.060125     53.277599      1.671216      13.695501       3.785095       0.998
   pinvar        0.142337      0.002270      0.060436       0.242107       0.140259       0.999
   ------------------------------------------------------------------------------------------------
   Amino acid model probabilities:
              Posterior     Standard
   Model      Probability   Deviation
   ----------------------------------
   Poisson      0.000       0.000000
   Jones        0.000       0.000000
   Dayhoff      0.000       0.000000
   Mtrev        0.000       0.000000
   Mtmam        0.000       0.000000
   Wag          0.000       0.000000
   Rtrev        0.000       0.000000
   Cprev        0.000       0.000000
   Vt           1.000       0.000000
   Blosum       0.000       0.000000
   ----------------------------------
   List of taxa in bipartitions:

      1 -- XP390301_G.zeae
      2 -- XP960686_N.crassa
      3 -- XP368507_M.grisea
      4 -- XP001557757_B.fuckeliana
      5 -- XP001588389_S.sclerotiorum
      6 -- XP503675_Y.lipolytica
      7 -- XP001385034_P.stipitis
      8 -- NP986804_A.gossypii
      9 -- XP453996_K.lactis
     10 -- NP011077_Spi1_S.cerevisiae
     11 -- NP010362_Sed1_S.cerevisiae
     12 -- XP710492_C.albicans
     13 -- XP448661_C.glabrata
     14 -- XP447538_C.glabrata
   Summary statistics for taxon bipartitions:
   ID -- Partition        #obs   Probab.   Stdev(s)   Mean(v)    Var(v)     PSRF  Nruns
   ------------------------------------------------------------------------------------
    1 -- ...**.........    602  1.000000  0.000000  0.363348  0.007403  0.999    2
    2 -- ..*...........    602  1.000000  0.000000  0.097996  0.002139  1.000    2
    3 -- ...*..........    602  1.000000  0.000000  0.077669  0.001656  0.998    2
    4 -- ....*.........    602  1.000000  0.000000  0.103415  0.001818  0.998    2
    5 -- .*............    602  1.000000  0.000000  0.076848  0.001057  0.998    2
    6 -- ............*.    602  1.000000  0.000000  0.194025  0.004552  0.999    2
    7 -- .........*....    602  1.000000  0.000000  0.255290  0.004225  0.998    2
    8 -- .*************    602  1.000000  0.000000  0.104863  0.001342  0.999    2
    9 -- .............*    602  1.000000  0.000000  0.505809  0.012382  0.998    2
   10 -- .....*........    602  1.000000  0.000000  0.153262  0.003324  1.015    2
   11 -- .......*......    602  1.000000  0.000000  0.344413  0.008404  0.998    2
   12 -- ........*.....    602  1.000000  0.000000  0.244985  0.005779  0.999    2
   13 -- ..........*...    602  1.000000  0.000000  0.071447  0.001689  1.001    2
   14 -- ...........*..    602  1.000000  0.000000  0.577952  0.014843  0.998    2
   15 -- ......*.......    602  1.000000  0.000000  0.171726  0.004546  0.999    2
   16 -- .........**...    601  0.998339  0.002349  0.191443  0.004486  0.999    2
   17 -- .....*********    598  0.993355  0.004698  0.169413  0.004070  0.999    2
   18 -- .......**.....    593  0.985050  0.002349  0.168874  0.005580  1.013    2
   19 -- .....**....*..    580  0.963455  0.009397  0.132990  0.003340  0.999    2
   20 -- ......*....*..    575  0.955150  0.007048  0.132262  0.003947  1.004    2
   21 -- .......****.**    572  0.950166  0.009397  0.137887  0.004417  1.000    2
   22 -- ..***.........    510  0.847176  0.018794  0.076813  0.001538  1.006    2
   23 -- .......****..*    446  0.740864  0.014095  0.098824  0.002728  1.002    2
   24 -- ..************    403  0.669435  0.025841  0.049331  0.001070  1.003    2
   25 -- .......**....*    295  0.490033  0.049333  0.092730  0.003673  0.999    2
   26 -- .****.........    152  0.252492  0.014095  0.029488  0.000531  1.017    2
   27 -- .........**..*    135  0.224252  0.021143  0.063249  0.002274  1.003    2
   28 -- .........**.**    100  0.166113  0.004698  0.084661  0.002397  1.013    2
   29 -- ...***********     84  0.139535  0.028190  0.069800  0.001666  1.001    2
   30 -- .........**.*.     81  0.134551  0.007048  0.078874  0.002328  0.998    2
   31 -- .......****...     80  0.132890  0.000000  0.046119  0.001359  1.014    2
   32 -- .*...*********     34  0.056478  0.009397  0.012753  0.000105  1.148    2
   ------------------------------------------------------------------------------------
   Credible sets of trees (105 trees sampled):
      50 % credible set contains 5 trees
      90 % credible set contains 45 trees
      95 % credible set contains 75 trees
      99 % credible set contains 99 trees
```



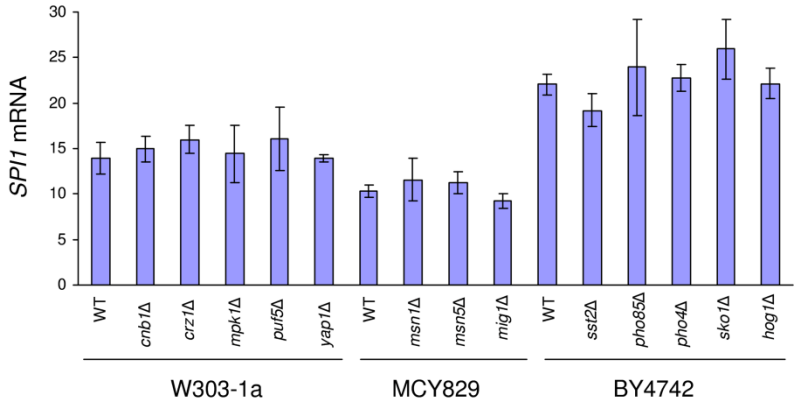
Suppl. Fig. 1. Unedited alignment of amino acid sequences used for the phylogenetic study. The alignment was made using ClustalX 2.0. The conserved motif repeated twice in *A. gossypii* protein and in Sed1p is squared.



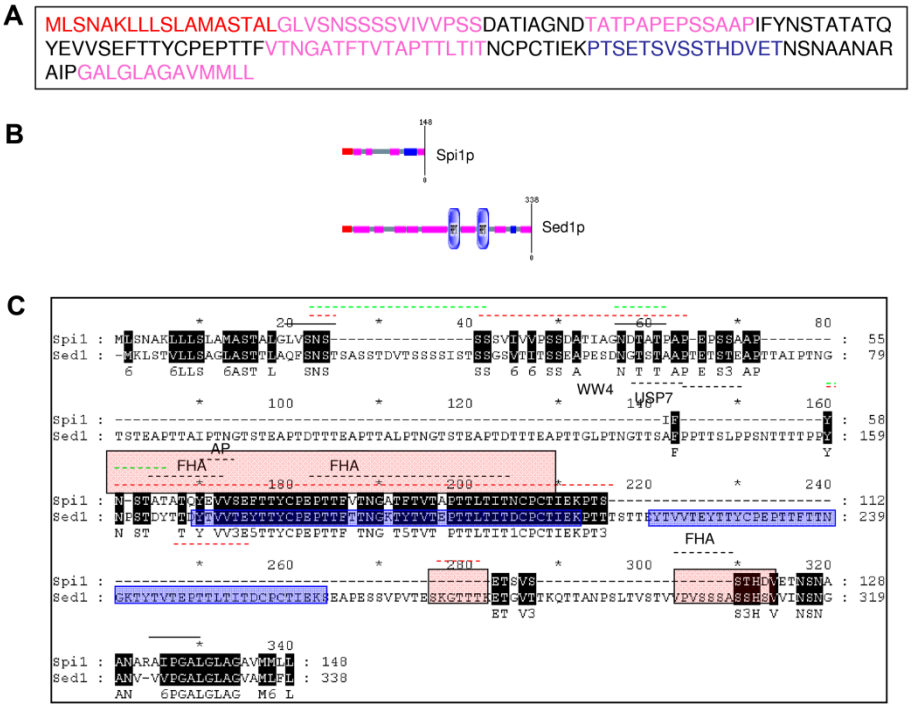
Suppl. Fig. 2. Unedited alignment of amino acid sequences using Probcons. The conserved motif is squared. Note that the results are similar to those obtained used with Clustal 2.0, at least for the sequence used for the phylogenetic analysis (Fig. 1).



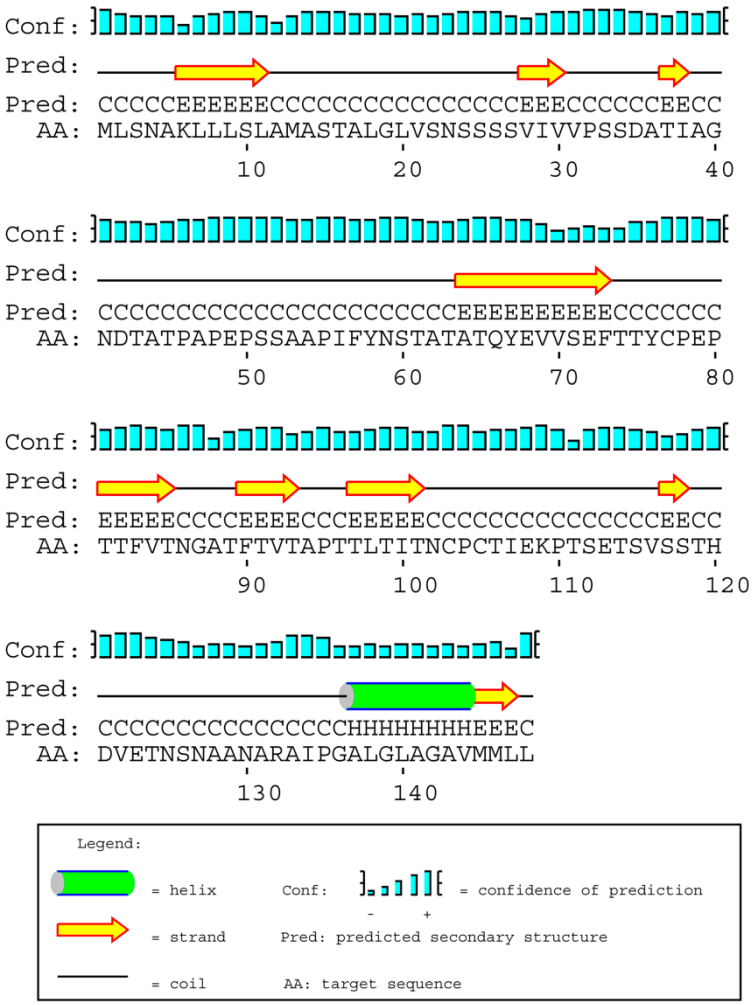
Suppl. Fig. 3. Comparison of *SPI1* and reporter expression and effect of *S. cerevisiae* strain in growth. A – Comparison of mRNA expression by Northern blot of *SPI1* and *lacZ* controlled by *SPI1* promoter in the strain YPH499. B – Comparison of the growth (measured as optical density at 600 nm) of BY4742 and YPH499 strains in rich (YPD) and minimal media (SD). Strains and media are indicated in the legend. The curve is presented in semi-logarithmic scale.



Suppl. Fig. 4. Mutant strains tested without effect in *SPI1* induction in post-diauxic conditions. *SPI1* expression analysed by Northern blot is shown after 8 h growing in YPD from OD_{600}= 0.3. The background strain of the mutants is shown at the bottom.



Suppl. Fig. 5. Motifs and domains present in Spi1p and Sed1p. A – Amino acid sequence of Spi1p and domains found by SMART. Domains are squared in the following colours: Red, signal peptide; pink, low complexity areas; blue, intrinsic disorder; RPT (blue), internal repeats. B – Distribution of domains found by SMART in Sed1p and Spi1p. The same colour code as in A) is used. C – Alignment of the amino acid sequence of Spi1p and its homologue Sed1p. Shown in pink is the serine-threonine rich domain, characteristic of these proteins and potential substrate of serine-threonine kinases. For Sed1p, shown in blue is the repeated domain, present only once in Spi1p (RPT in B). Also shown squared are the motifs recognized as domains (indicated by the name) or substrate of post-translational modifications. Black continuous: N-myristoylation; red dashed: phosphorylation; green dashed: N-glycosylation; FHA: phosphothreonine union; WW4: phosphorylation-dependent interaction domain; USP7: binding domain; AP: interaction with AP (adaptor protein; typical of the endocytic route, very common in membrane or cell wall proteins, especially those involved in transport).



Suppl. Fig. 6. Secondary structure prediction of Spi1p. α-helices are shown as green cylinders (H above the amino acid sequence) and β-sheets as yellow arrows (E above the amino acid sequence). The probability is shown as blue bars over the prediction. The results show that in Spi1p there are 9 putative regions adopting a β-sheet structure, four of them dubious due to their small size and/or their low probability. The first one corresponds to the signal peptide, the fourth includes one conserved region (VVSeFTTYCP; Fig. 1B, fifth and sixth are also within one conserved domain (TTFVT-TFTVT; Fig. 1B). The seventh is the most conserved (TTLTITNCP), and probably is important in the function of the protein. Probably these motifs, due to their evolutionary conservation, are part of the secondary structure and play a central role in the function of the protein. An α-helix is also predicted at the C-terminus of the protein, although with low probability.

Suppl. Table 1. Strains and plasmids used in this work.

A. *S. cerevisiae* strains used in this work.

Strain	Genotype	Reference
W303-1a	*MATa, ade 2-1, ura 3-1, leu 2-3, his 3-1, trp1-1*	[1]
W303-1a msn2Δmsn4Δ	*W303-1a msn2Δ3::HIS3, msn4-1::TRP1*	[2]
W303-1a cnb1Δ	*W303-1a , cnb1::LEU2*	[3]
W303-1a crz1Δ	*W303-1a , crz1::G418*	[4]
W303-1a mpk1Δ	*W303-1a mpk1::TRP1*	[5]
W303-1a pkc1^{ts}	*W303-1a pkc1^{ts}-X*	[6]
W303-1a puf5Δ	*W303-1a uth4::KanMX4*	P. Carrasco, doctoral thesis
W303-1a yak1Δ	*W303-1a yak1::KanMX4*	P. Carrasco, doctoral thesis
W303-1a yap1Δ	*W303-1a yap1::KanMX4*	F. Rández-Gil
MCY829	*MATa his3-Δ200 lys2-801 ura3-52*	[7]
MCY msn1Δ	*MCY829 msn1-Δ1::URA3*	[7]
MCY msn5Δ	*MCY829 msn5-Δ2::HIS3*	[8]
MCY mig1Δ	*MCY829 leu2::HIS3 mig1-Δ2::LEU2*	[9]
BY4741	*MAT a, his3-1, leu2-0, met15-0, ura3-0*	Euroscarf
BY4741 pho84Δ	*BY4741 pho84::KanMX4*	Euroscarf
BY4741 wsc2Δ	*BY4741 wsc2::KanMX4*	Euroscarf
BY4742	*MATα, his3-1, leu2-0, lys2-0, ura3-0*	Euroscarf
BY4742 hog1Δ	*BY4742 hog1::KanMX4*	Euroscarf
BY4742 pho85Δ	*BY4742 pho85::KanMX4*	Euroscarf
BY4742 pop2Δ	*BY4742 pop2::KanMX4*	Euroscarf
BY4742 pho4Δ	*BY4742 pho4::KanMX4*	Euroscarf
BY4742 sko1Δ	*BY4742 sko1::KanMX4*	Euroscarf
BY4742 sst2Δ	*BY4742 sst2::KanMX4*	Euroscarf
MLY40	*MATα, ura 3-52*	[10]
MLY40 phd1Δ	*MLY40 phd1::G418*	[10]
MLY40 sok2Δ	*MLY40 sok2::hygB*	[10]

B. Plasmids used in this work.

Plasmid	Description	Reference
YEp352	Multicopy shuttle vector. *URA3* marker	[11]
YEp352-PKC1	Multicopy shuttle vector. *URA3* marker. *PKC1 in PstI/BglII.*	[12]
YEp352-MPK1	Multicopy shuttle vector. *URA3* marker. *MPK1 in SphI/Ncori.*	[12]
YEp357	Multicopy shuttle vector. *URA3* marker. *lacZ.*	[13]
YEp357-SPI1p/lacZ	Multicopy shuttle vector. *URA3* marker. *lacZ* expression controlled by *SPI1* promoter.	[14]

Suppl. Table 2. Transcription factors regulating *SPI1* expression.

A. Analysis of transcription factors (TF), using YEASTRACT tool (http://www.yeastract.com/), which regulate *SPI1* expression documented by direct or indirect evidence. Abbreviations: Northern blot (NB); expression microarrays (ARR); chromatin immunoprecipitation (ChIP); ChIP-on-CHIP (ChIP-CH), mutant (mt), wild-type (WT).

TF	Reference	Evidence
Adr1p	[15]	Indirect: ARR (WT/TFmt)
Aft1p	[16]	Indirect: ARR (WT/TFmt)
Cat8p	[15]	Indirect: ARR (WT/TFmt)
Cin5p	[17]	Direct: ChIP
Crz1p	[15]	Indirect: ARR (WT/TFmt)
	[18]	Indirect: ARR (WT/TFmt)
Cst6p	[15]	Indirect: ARR (WT/TFmt)
Gat4p	[15]	Indirect: ARR (WT/TFmt)
Gcr2p	[19]	Indirect: ARR (WT/TFmt)
Gis1p	[15]	Indirect: ARR (WT/TFmt)
Gzf3p	[15]	Indirect: ARR (WT/TFmt)
Haa1p	[20]	Indirect: NB (WT/TFmt)
Hap4p	[21]	Indirect: ARR (WT/TFmt)
Hot1p	[22]	Direct: ChIP
Hsf1p	[15]	Indirect: ARR (WT/TFmt)
	[23]	Indirect: RT-PCR (WT/TFmt)
	[24]	Indirect: RT-PCR (WT/TFmt)
	[25]	Indirect: RT-PCR (WT/TFmt)
Ino2p	[26]	Indirect: ARR (WT/TFmt)
Ino4p	[26]	Indirect: ARR (WT/TFmt)
Mbp1p	[15]	Indirect: ARR (WT/TFmt)
Mcm1p	[27]	Indirect: NB (WT/TFmt)
Met31p	[15]	Indirect: ARR (WT/TFmt)
Met4p	[28]	Indirect: ARR (WT/TFmt)
Mga1p	[15]	Indirect: ARR (WT/TFmt)
Mig1p	[15]	Indirect: ARR (WT/TFmt)
Msn2p/Msn4p	[15]	Indirect: ARR (WT/TFmt)
	[29]	Indirect: ARR (WT/TFmt)
	[30]	Indirect: NB (WT/TFmt)
	[31]	Indirect: NB (WT/TFmt)
	[32]	Indirect: NB (WT/TFmt)
Pdr3p	[33]	Indirect: ARR (WT/TFmt)
Put3p	[15]	Indirect: ARR (WT/TFmt)
Rds2p	[34]	Direct: ChIP-CH
Rfx1p	[15]	Indirect: ARR (WT/TFmt)
Rgm1p	[15]	Indirect: ARR (WT/TFmt)

TF	Reference	Evidence
Rme1p	[15]	Indirect: ARR (WT/TFmt)
Rox1p	[15]	Indirect: ARR (WT/TFmt)
	[35]	Indirect: ARR (WT/TFmt)
Rpn4p	[36]	Indirect: ARR (WT/TFmt)
Sfl1p	[37]	Indirect: ARR (WT/TFmt)
Skn7p	[38]	Direct: ChIP-CH
	[15]	Indirect: ARR (WT/TFmt)
Sko1p	[22]	Direct: ChIP
	[39]	Direct: ChIP
Sok2p	[40]	Direct: ChIP-CH
	[41]	Indirect: ARR (WT/TFmt)
	[15]	Indirect: ARR (WT/TFmt)
	[42]	Indirect: ARR (WT/TFmt)
Ste12p	[41]	Direct: ChIP-CH
Stp2p	[15]	Indirect: ARR (WT/TFmt)
Tec1p	[41]	Direct: ChIP-CH
Yap1p	[15]	Indirect: ARR (WT/TFmt)
	[28]	Indirect: ARR (WT/TFmt)

Analysis of transcription factors found with Funcassociate 2.0 (http://llama.mshri.on.ca/funcassociate/) shows as enriched GO terms (adjusted p-value* < 0.05): nitrogen compound metabolic process (< 0.001), ion binding (< 0.001), nucleic acid metabolism (< 0.001), stimulus response (0.009), glucose metabolism regulation (0.03) and nutrient response (0.035).

* In Funcassociate 2.0, p-value is the result of single hypothesis one-sided of the association between attribute and query (based on Fisher's exact test) and adjusted p-value is the fraction (as a %) of 1000 null-hypothesis simulations having attributes with this single-hypothesis p-value or smaller.

B. Transcriptional factors (TF), using YEASTRACT, which presents consensus binding motif in the sequence of the *SPI1* promoter. The position in the sequence relative to the transcription start (ATG) is indicated, as well as its orientation (forward in black and reverse in red). TF which have been demonstrated as *SPI1* regulators, by direct or indirect evidence (see A), are underlined.

TF	Position and orientation
Ash1p	-296, -65, -909, -565, -14
Azf1p	-197
Cbf1p	-888
Crz1p	-358
Fkh1/2p	-561, -812, -766, -333
Gcr1p	-268, -150, -488, -212
Gis1p	-368, -260, -253
Gln3p	-303
Hac1p	-134
Hsf1p	-187, -177
Mcm1p	-311, -290, -168, -278, -372
Mot3p	-306, -978
Msn2/4p	-373, -265, -258
Nrg1p	-872, -700, -264, -257, -155, -373
Pho4p	-889
Pip2p	-366
Rph1p	-373, -265, -258
Rtg1/3p	-575
Sko1p	-708
Stb5p	-565, -497, -366, -105, -879, -547, -322
Ste12p	-993
Tec1p	-207
Xbp1p	-928, -868, -361, -184, -922
Yap1p	-9, -834
Yap3	-9
Yrr1p	-561

Analysis of transcription factors found with Funcassociate 2.0 shows as enriched GO terms (adjusted p-value < 0.05): nitrogen compound metabolic process (< 0.001), ion binding (< 0.001), nucleic acid metabolism (< 0.001), filamentous growth (0.005) and stress transcriptional response (0.008). Note that the most over-represented categories (adjusted p-value < 0.001) were found in both tables.

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