

Supplemental Data

Structure and *in vivo* requirement of the yeast Spt6 SH2 domain

Stefan Dengl, Andreas Mayer, Mai Sun, and Patrick Cramer*

¹Gene Center and Center for Integrated Protein Science Munich (CIPSM), Department of Chemistry and Biochemistry, Ludwig-Maximilians-Universität München, Feodor-Lynen-Str. 25, 81377 Munich, Germany.

*Correspondence: Phone: +49-89-2180-76965, Fax: +49-89-2180-76999, Email: cramer@LMB.uni-muenchen.de

Contents

Figures S1-S5

Table S1

[illegible]

cgSpt6	THIPITYVEDEIAIRYQNSERRAQEFPNKPPYVKYCIALARYMHSPLMHEYANLSPEELKS	900
paTex	MKLTKIMVSEAGASVYSASELAKEFPELDVSLRGAVSIARRLQDPLAELVKIEP---KS	468
	:. . * *. : * *. ** **:***: :. :	

Fig. S1: Alignment of Spt6 and Tex protein sequences

Alignment of the *C. glabrata* Spt6 (cgSpt6) and *P. aeruginosa* Tex (paTex) protein sequences by ClustalW. Secondary structural elements are indicated in red and green, for α -helices and β -strands respectively (information about secondary structure comes from the X-ray structure in the case of Tex and from secondary structure prediction (PROFsec) in the case of Spt6. Only the residues with an expected average accuracy > 82% were highlighted in the alignment). Tex-domains and the Spt6 SH2 domain are colored as in Figs. 1A and 5. Insertions in Spt6 are numbered as in Fig. 5.

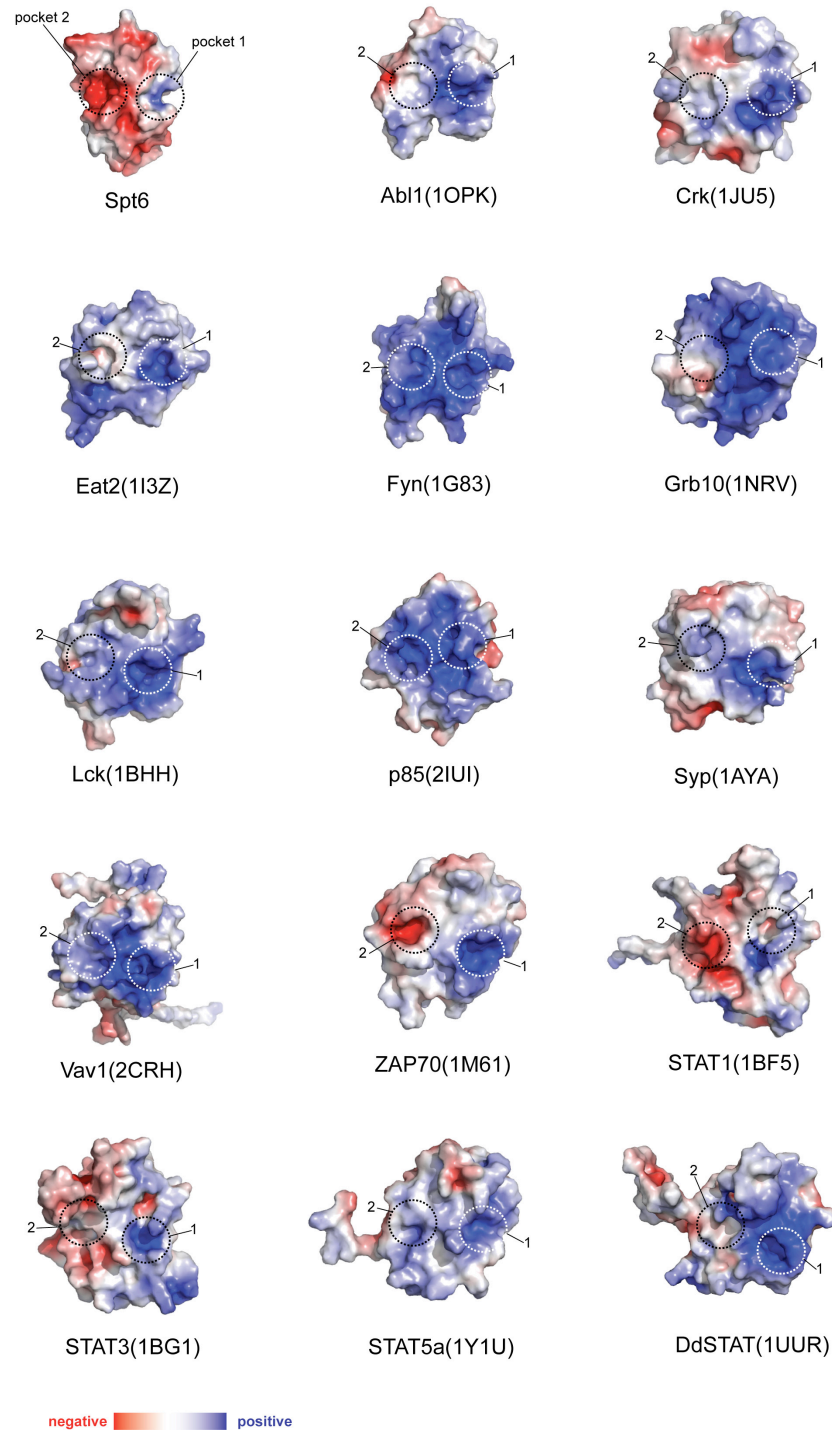


Fig. S2: Surface charge distribution of SH2-domains

Surface charge was calculated with APBS (Baker, Sept et al. 2001) using the same parameters for all the domains. Binding pockets are indicated by dashed circles

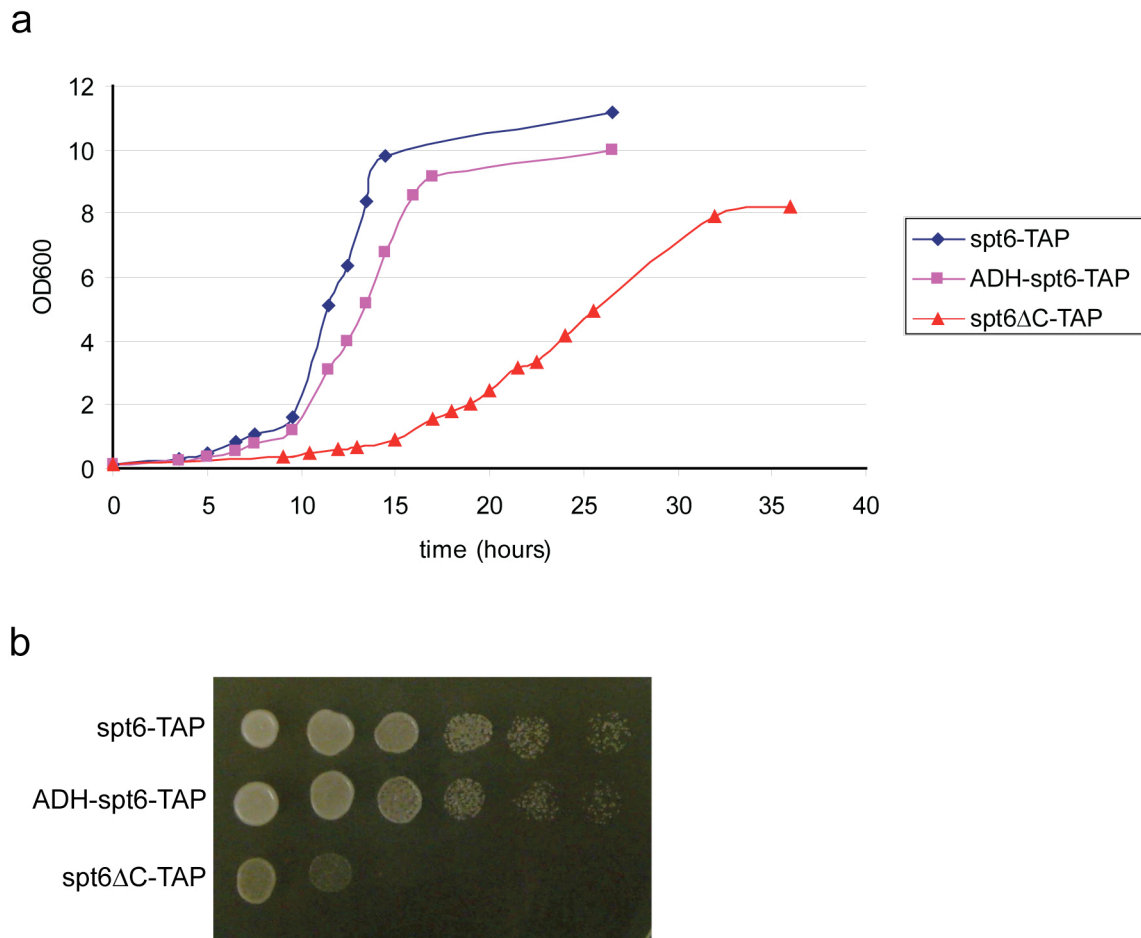


Fig. S3: Growth of yeast strains used in the gene expression analysis

(a) Growth of yeast strains (OD₆₀₀) as a function of time (hours). Yeast strains were grown in YPD liquid culture at 25°C. Growth curves were performed in biological duplicate and represent the average of two experiments.

(b) Serial dilutions grown on YPD solid medium.

Pre-cultures of the corresponding yeast strains were grown to stationary phase (YPD, 25°C) and spotted on YPD plates. The serial dilutions were grown at 25°C over two nights and were based on equal amounts of cells, so that the growth rates of the different yeast strains could be compared.

a

	wt-Tap	spt6 Δ SH2-Tap	spt6-ADH-Tap
spt6-ADH-Tap	0.99	0.97	1
spt6 Δ SH2-Tap	0.97	1	
wt-Tap	1		

b

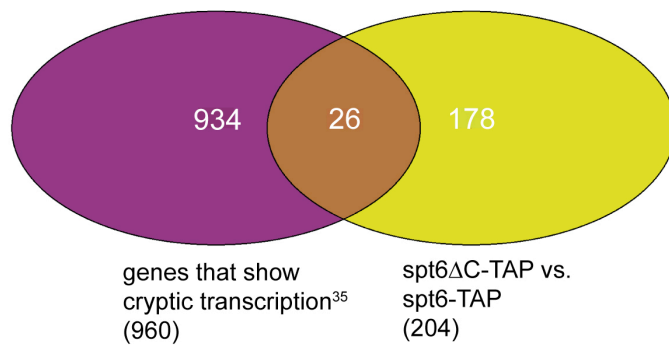
	spt6 Δ C-TAP (vs. spt6-TAP)	spt6 Δ C-TAP (vs. ADH1-spt6 -TAP)	Δ dst1	Δ spt4	Δ rft1
Δ rft1	0.06	0.08	0.03	0.44	1
Δ spt4	0.15	0.15	0.06	1	
Δ dst1	0.01	-0.01	1		
spt6 Δ C-TAP (vs. ADH1-spt6-TAP)	0.88	1			
spt6 Δ C-TAP (vs. spt6-TAP)	1				

Fig. S4. Pearson correlation matrices

(a) Pearson's correlation matrix for the gene expression profiles (see Fig. 6c). Datasets were RMA-normalised by application of PARTEK Genomics Suite 6.4 software. Replicate datasets were then averaged and the Pearson correlation coefficients calculated between the whole averaged datasets (total of 5665 ORFs).

(b) Pearson's correlation matrix for the hierarchical clustering analysis in Fig. 6b. Coefficients were calculated for genes that were significantly changed in at least one of the elongation factor mutants (total 964 ORFs).

a



b

ORF name	gene symbol
YBR083W	TEC1
YDR216W	ADR1
YDR277C	MTH1
YER028C	MIG3
YIL119C	RPI1
YMR042W	ARG80
YOR344C	TYE7
YPL177C	CUP9
YPR193C	HPA2

ORF name	gene symbol
----------	-------------

YBR203W	COS111
YBR208C	DUR1,2
YBR284W	---
YBR296C	PHO89
YEL030W	ECM10
YER103W	SSA4
YFR023W	PES4
YFR053C	HXK1
YGL156W	AMS1
YHR146W	CRP1
YIL015W	BAR1
YIL073C	SPO22
YIL169C	---
YJR119C	JHD2
YKL163W	PIR3
YKL188C	PXA2
YKR103W	NFT1
YLR214W	FRE1
YMR006C	PLB2
YMR266W	RSN1
YNL040W	---
YNR012W	URK1
YOL119C	MCH4
YOL152W	FRE7
YOL162W	---
YOR071C	NRT1

Fig. S5. Overlap of spt6ΔC-TAP-dependent genes with ORFs showing cryptic transcription in the spt6-1004 mutant and with known yeast Pol II transcription factors

(a) Venn diagram depicting the overlap of spt6ΔC-TAP-dependent genes (yellow) with those ORFs showing cryptic transcription in the spt6-1004 mutant (magenta). Of the 960 ORFs known to exhibit cryptic transcription in the Spt6-1004 mutant, the transcript levels of 26 genes are significantly altered in the spt6ΔC-TAP mutant. A complete list of the 26 ORFs is shown below. The list of the 960 ORFs is from reference 35.

(b) List of 9 known yeast transcription factor genes exhibiting significantly altered mRNA levels between spt6ΔC-TAP mutant strain and the corresponding isogenic wild-type strain. The ORF names and the corresponding gene symbols are given. The list of the known 269 yeast transcription factors is from reference 36.

Table S1: Structural elements of SH2 domains

Protein, species ^a , (PDB accession no.)	core motif ^b	βEβF	αB [Å] ^c	αL ^d	R155 ^e	K203 ^e	Z-score (Dali server) ^f
Src-type							
Spt6, Cg, (-)	GDFVIRQ	+	26.0	+	G	D	-
Abl1, Mm, (1OPK)	GSFLVRE	+	14.3	-	R	R	-
Aps, Rn (1RPY)	GLFVIRQ	-	29.0	+	R	R	8.6
Blk, Mm, (1BLJ)	GSFLIRE	+	14.8	-	R	K	8.0
PTK6/Brk, Hs, (1RJA)	GAFLIRV	+	16.7	-	R	L	8.1
Cbl, Hs, (2CBL)	GSYIFRL	-	14.2	+	Y	A	6.5
Crk, Hs, (1JU5)	GVFLVRD	+	16.5	-	R	I	8.4
CHK, Hs, (1JWO)	GLVLVRE	+	17.3	-	G	R	9.8
Fes/Fps, Hs, (1WQU)	GDFLIRE	+	17.9	-	R	I	7.2
Fyn, Hs, (1G83)	GTFLIRE	+	17.4	+	R	K	9.8
GADS, Mm, (1R1Q)	GFFIIRA	+	12.9	+	R	K	9.3
Grb10, Hs, (1NRV)	GLFLLRD	+	14.7	-	R	N	-
Grb14, Hs, (2AUG)	GVFLVRD	+	14.7	+	R	N	9.8
Grb2, Hs, (1GRI)	GAFLIRE	+	15.2	-	R	K	8.6
Grb7, Hs, (1MW4)	GLFLVRE	+	10.1	-	R	L	4.5
Hck, Hs, (3HCK)	GSFMIRD	+	15.0	-	R	K	8.5
Itk/Tsk, Mm, (1LUK)	GAFMVRD	+	14.7	-	R	K	7.7
P56-Lck, Hs, (1BHH)	GSFLIRE	+	12.2	-	R	K	9.2
Nck1, Hs, (2CI9)	GDFLIRD	+	16.9	-	R	K	9.6
Nck2, Hs, (2CIA)	GDFLIRD	+	16.5	-	R	K	10.1
P85-N, Hs, (2IUG)	GTFLVRD	+	15.8	+	R	K	9.3
PLCγ1, Bt, (2PLD)	GAFLVRK	+	14.5	-	R	R	6.7
Syp, Mm, (1AYA)	GSFLARP	+	17.0	-	G	K	9.0
SHP-1, Hs, (1X6C)	WTFLVRE	+	17.0	-	G	K	8.1
Sap, Hs, (1D1Z)	GSYLLRD	+	12.9	-	R	R	7.7
Eat2, Mm, (1I3Z)	GNFLIRD	+	11.1	-	K	L	9.6
SH3BP2, Hs, (2CR4)	GLYCI RN	+	14.8	-	S	R	6.9
Shc1, Hs, (1MIL)	GDFLIRE	+	15.3	-	R	L	-
Socs3, Mm, (2BBU)	GTFLIRD	+	15.0	+	G	R	4.2
Syk, Hs, (1CSZ)	GKFLIRA	+	13.6	-	R	R	8.0
Vav1, Hs, (2CRH)	GTFLVRQ	+	14.2	+	R	K	7.2
ZAP70-1, Hs, (1M61)	GLFLLRQ	+	16.4	-	R	P/I	10.4
ZAP70-2, Hs, (1M61)	GKFLLRP	+	14.4	+	R	L	10.4
STAT-type							
STAT1, Hs, (1BF5)	GTFLLR F	-	5.7 + 8.8	+	P/E	K	4.2
STAT3, Hs, (1BG1)	GTFLLR F	-	10.8 + 9.8	+	P/E	K	4.1
STAT5, Mm, (1Y1U)	GTFLLR F	-	8.7 + 9.9	+	K	K	5.5
STAT, Dd, (1UUR)	GTFIIR F	-	7.3 + 10.5	+	R	L	5.7

Structural data was obtained from the Protein Data Bank (PDB) and inspected for the indicated structural elements.

^a Cg=*Candida glabrata*; Hs=*Homo sapiens*; Mm=*Mus musculus*; Rn=*Rattus norvegicus*; Bt=*Bos taurus*; Dd=*Dictyostelium discoideum*

^b highly conserved SH2 core motif of consensus sequence GXF/YBBR (X for any, B for hydrophobic amino acids) containing the arginine residue (in red) binding the phosphate

^c length of helix αB in Angstroem

^d presence of an α helix resembling the αL helix in Spt6 in position relative to the SH2-fold

^e residue found at the same position as R155 and K203 in the structure of the Src domain.

^f Z-score derived from a DALI-server⁶⁷ search for related protein structures using the Spt6 SH2-domain as a query. Higher scores indicate higher similarities (similarities with a Z-score < 2 are insignificant)