New Phytologist Supporting Information

Article title: ZmpTAC12 binds single-stranded nucleic acids and is essential for accumulation of the plastid-encoded polymerase complex in maize

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The following Supporting Information is available for this article:

Supporting information Figs S1-S6

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ZmpTAC12 OspTAC12 AtpTAC12 PtpTAC12 PppTAC12	1 1 1 1	MASCYNF RIFPGMSTAVPAGPVTAPAHS TCKSS VFS LEH RGLIF GTRARIKCVKDS MASCSRTMLLPGMAPQATAQTVPRPLQSLKVFAGLEHRRRVLFSGVSSRTRGRIRSVKDDS MASISTTTWLYRGQVCTDSGKSSNCIVQRRVKCGFELKTLHAGITSRDRSLRHCIKCKKDDG MASISTTTWLYRGQVCTDSGKSSNCIVQRRVKCGFELKTLHAGITSRDRSLRHCIKCKKDDG MQTPFIGSFPAGTLEVRKAPSLPCIKCEKKD- METRGIMLQGRLSYPSSACTQGKNSVSVRSQRNVVGCGILLKSRRNVWLCGGSSGISGSLVYRRGRSVSFWSLRVGKSSG
ZmpTAC12	65	LHEPSKIEPPPYSSYFDSTSGQLEPASGARASIPCKEYWPEGTAARVRAARAPAPVGESAGMPSFGT
OspTACI2	63	
ALPTACI2	20	
PppTAC12	81	EGRGGLVVRCNESDEVDMPPEDPAYTSYVDPNTGEPSPSYGTRAPLEPAAFWGDDAKRVVRSCOAATPSDTGMKDRGGA
7moTAC12	1 3 3	
OspTAC12	131	KPGSREKGYKEOVASALAGEGTETSGDEGESVVALEASSDETLEETKDSLDE VVVEMPKEENLSEVEMDKMMGRPHP
AtpTAC12	143	NPCSRRKKNRKATEENVTVBTNDVSDSEDSSBEEDNDSSDGFVTYKNEFEREEETCFELDKKLGRPHP
PtpTAC12	100	KPGSRRKKYKASVAAPESSEASIEFIDSEALESYEEMKEEPKDESSDYVIYDTEPEEEDTGYELDKKLGRPHP
PppTAC12	161	MSSRDFVSKRKNASVASRDGQKVFNEFDEEVEBEEDDERNASGEBEDDEEDAGLVLQQSE
ZmpTAC12	213	FIDEAKAMSIGEPK <mark>ISEELWMHMR</mark> RK <mark>SOBEE</mark> M <mark>MSRWORRRE</mark> DVDTVFAKAMAETGQIKIFGDHPSRTEAALA
OspTAC12	209	FVDFQKAMSVGEPKSSEELWWNWRRKSEENEMWSRWQRRRPDVDTVFAKAMAETGQIKIFGDHPTRTEAALA
AtpTAC12	213	FIDETKKKQIDKTLISDESMWNWRKPEKEOMSRWQRRRPDVETVFLKAMAETGOVKLYGDEPTLTETSLY
PtpTAC12 PppTAC12	173 222	FIDEKVKKPIEGILPQEELWWNWRNPENEQWSRWQRRKPDVETVSMPIEKVFLKAMAETGQVKLYGKEPTLTEASLY EGLVHESDQRLENDELWWNHQKPSKDKEPWSAWQKRMGDGDSVIATAMAKAGQIELFGDKPTIAEASLA
ZmpTAC12	285	K <mark>T</mark> RRHLYKEERLEAEQRRLEEIGPIAYYSEWV <mark>EAYK</mark> NKDTSREAIQKHFEE <mark>TGEDENVQLIKMFQHQTAGE</mark> YRIMMGTDV
OspTAC12	281	KARRHLFKEERLEAEQRRLEEIGPIAYYSEWV <mark>B</mark> AYK <mark>NK</mark> DTSREAVQKHFEE <mark>T</mark> GEDENTQLIIMFQHQTAGEERIMMGTDV
AtpTAC12	283	RARRHLFKEERLQAERRLAKEGPMAHYSEWVKRWKR-DTSREAVQKHEEETGEDENTQLIEMFSHGTDREYRIMMGTDI
PtpTAC12	251	RARKHLYKEERLEAEORRLERIGPMGYYSEWVKAWK - DTSREAIOKHEETGEDENAOLIAMBCHOTOREERIMMGTD
PPPTACIZ	291	KARAKVE I DERVELDELERKABAGAVATTAEVWGAWAA-DISAVI WEEKAAS <mark>IGEGVV DOLEDDILOHOSKREIRKWOGID</mark>
ZmpTAC12	365	RIQRDPLAMRMREDQIKQIWGGDPVYPTINY QDPDEVIDYRGPEFHEPTEEVVPYLMEHGIMIIKEELYARLNEEREV
AtpTAC12	362	RIKRDPLAMRMREDQIKQIWGGDPVYPTINYIQDPNAVMDFRGPDFHEPTFNMLSYLKENGKVISREMHEAILTKEKT
PtpTAC12	330	RI <mark>RRDPLAMRMRED<mark>LIKQIWGGDPVYPTVNYIQDPNE</mark>IIDYRGPDFHEPTF<mark>NMLDYLKEHGKIISRKELEKILAKEKT</mark></mark>
PppTAC12	370	RIARDPLTLRMSDEE <mark>IIQVWGGDPVYPT</mark> KNYE <mark>QDPDAWA</mark> DYRNENLHEPTFDIVDLLQDDGILII <mark>REOHQEILDREERE</mark> -
ZmpTAC12	445	NQ ITYTEEAKDPMATAIDIGEHSYNEDSDDEDEDVDKAAAQPQSLEDSEDERDVAEV <mark>BEKW</mark> NONWSAEKSTGQAEKPK
OspTAC12	441	<u>NOELTY</u> TEEVRDPMAT <mark>AVDIGE</mark> HSYNEDSDDDEEDADKVVAQPESLEDDEDDGDDAEDAEGKVSRNWSVLKTTG <u>QA</u> ENPK
AtpTAC12	440	
PEPTACI2 PppTAC12	408 449	ERBKELTEDDE DAMUGKANDIGEN
ZmpTAC12	525	
OspTAC12	521	
AtpTAC12	494	PKPKKEGRWSLDEAVDDAENLTDFLMDFEEFTDP
PtpTAC12	462	DK <mark>P</mark> KKEGPLSLEEAIDDSENLTDFLMDFEQDE
PppTAC12	493	DSSDLLEYWGDEEDEEDSIKDVDN

Fig. S1 Genes homologous to ZmpTAC12 are found in land plants.

Multiple sequence alignment showing pTAC12 orthologs in maize (Zm), rice (Os), *Arabidopsis* (At), *Populus trichocarpa* (Pt), and *Physcomitrella patens* (Pp). Identical residues are shaded black and similar residues are shaded grey. Highly conserved region is boxed in green. The coiled coil domain was predicted using COILED-COILS

(http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_lupas.html) and is boxed in blue. Orange sequence corresponds to the predicted plastid transit peptide. Identified peptides by mass spectrometry (Table S2) are highlighted in red. The grey line above the alignment marks the peptide used for antigen production.



Fig. S2 Zmptac12 gene encodes two different protein isoforms.

A, Schematic representation of maize ZmpTAC12 gene organization. Probes used for RNA blot hybridizations (grey arrowheads) and positions of RT-PCR primers (black arrows) are indicated on the genomic structure. B, RT-PCR to characterize ZmpTAC12 transcripts. PCR was performed using cDNA synthesized from total RNA. ZmpTAC12 fulllength cDNA (1) and the middle segment (2) were generated using ptac12 f2/r2 and ptac12 f/r primers, respectively. DNA fragment was separated by 1% agarose gel electrophoresis followed by visualization with ethidium bromide. The sizes of calculated RT-PCR products are indicated with arrows and the numbers to the right of the gels indicate the size of the DNA ladder. C, Immunodetection of ZmpTAC12-HA stably introduced in Arabidopsis by agroinfection. The anti-pTAC12 antibody was used to detect ZmpTAC12 from total protein extracts (~25 µg) from Zea mays wild-type plants (Zm) and transgenic Arabidopsis (At) seedlings expressing ZmpTAC12-HA. An anti-HA antibody was used to confirm the expression of the HA-tagged ZmpTAC12 protein. At-zm12xHA-1-4 are independent transgenic Arabidopsis lines expressing ZmpTAC12-HA at different levels. D, Immunodetection of ZmpTAC12 and RpoA in crude leaf extracts (25µg) from base sections of second leaves. Samples were assayed in presence or absence of protease inhibitors. E, Recovery of ZmpTAC12 in immunoprecipitations following DNase I treatment. Stroma proteins (~ 500 µg) were used for immunoprecipitation using protein A/G magnetic beads. Eluted proteins were resolved by 12% SDS-PAGE and subjected to immunoblot analysis with pTAC12 and RpoA antibodies. The antibodies used for immunoprecipitation are indicated above. The two bands specific to ZmpTAC12 are indicated by arrows. The prominent band at ~55 kDa corresponds to the IgG heavy (IgG-H) chain. Numbers to the right represent M_r of molecular weight standards. F, DNA depletion from coimmunoprecipitation pellets following DNase I treatment. Extracted nucleic acids from coimmunoprecipitates were tested by PCR for 35 cycles using *psaAB* and *psbA* primers. Genomic DNA (5 ng) was amplified in control reactions. Numbers to the right represent the base pairs (bp) of DNA fragments. Sup, supernatant; P, pellet; a and b, sera from two different immunized rabbits.





Fig. S3 Coimmunoprecipitation assays identified chloroplast DNAs (DIP) associated with maize ZmpTAC12.

Summary of DIP-Chip data. Stromal extract was treated with the restriction enzyme AluI/EcoRI (grey line) or AluI (black line) and RNAse A prior to immunoprecipitation. Nucleic acids recovered from immunoprecipitation were subjected to alkali hydrolysis to remove residual RNA prior to analysis by microarray hybridization. The ratio of signal in the pellet versus the supernatant (F635/F532) for replicate array fragments is plotted according to chromosomal position.

Chromosomal Position



Genes with NEP promoters







Fig. S4 Transcript accumulation of plastid encoded genes and phenotypes of wild-type and *Zmptac12-2* seedlings grown under different light conditions.

A, Isolated total RNA (10 µg) was subjected to RNA gel blot analyses and hybridized to DNA probes of indicated genes. B, Comparison of mesocotyl length in wild-type (left) and *Zmptac12-2* (right) grown under different light condition. Seedlings were grown in white (200 µmol $m^{-2} s^{-1}$) light or darkness, or under red (6 µmol $m^{-2} s^{-1}$) or farred (2 µmol $m^{-2} s^{-1}$) for 7 days at 26-28°C. White arrows indicate boundary between mesocotyl and first leaf.



Fig. S5 Release of thylakoid-associated maize ZmpTAC12 fraction by sonication and analyses of PEPcomplex assembly.

A, Immunoblot analysis of RpoA in wild-type and *Zmptac2* mutants. B, Immunoblot of a total protein fraction after sonication. Proteins were isolated from total leaf tissue of WT seedlings and sonicated to release ZmpTAC12 from thylakoids. Approximately 25 µg of protein were loaded per lane. P, pellet; S, supernatant. C, PEP-complex assembly detection by BN-PAGE and subsequent immunoblotting with anti-pTAC12. Approximately 50 µg of sonicated protein fractions from WT and *Zmptac12-2* seedlings were loaded per lane. D, E Sucrose-gradient sedimentation analysis. Total leaf proteins from basal half of second leaf were solubilized in Triton-X-100 protein lysis buffer with sonication and soluble fractions subjected to sucrose gradient centrifugation for analysis of PEP-complex assembly. Fractions were collected and immunoblotted with antibodies against pTAC12, and Rubisco (RbcL). Respective fractions containing Rubisco, PEP (plastid-encoded RNA polymerase), and TAC (transcriptionally active chromosome) are indicated by bars; P, pelleted material; P*, pelleted material from WT or mutant leaf samples, respectively.

	-35 -10 → TSS1(-175	5)
ZmpsaA	TCCAGAATAACCAATG-TCCG <mark>TTAGGC</mark> ACCTAATCCTTATGT <mark>CATAAT</mark> AGATCCGAACAC	
OspsaA	TCCAAGATAAAAAAAG-TCCG <mark>TTAGGC</mark> ACCCAATCCTTATGT <mark>CATAAT</mark> AGATC <mark>C</mark> GAACAC	
HvpsaA	TTCAAGATAAACAATG-TCCG <mark>TTAGGC</mark> ACCTAACCTTTATGT <mark>CATAAT</mark> AGATCC <mark>G</mark> AACAC	
AtpsaA	AATTATAAAAAAGGGTCCG <mark>TTGAGC</mark> ACCCTATGGATATGT <mark>CATAAT</mark> AGATCC <mark>G</mark> AACAC	
	* **** ** * ****	
	psaAB-231	
	-10 -35 T SS2 (-129)	
ZmpsaA	TTGCCT <mark>CGGATTGACTTCAATA-</mark> TATAAT <mark>TGCTCCAGTGAATAACTAAAAAAAA-AAATA</mark>	
OspsaA	TTGCCT <mark>CAGATTGACTTCAATA-</mark> TATAAT <mark>TGCTCCAGTG</mark> ATAACTAAAAAAAA-ATA	
HvpsaA	TTGCCTCGGATTGACTTTAATA-TATAATTGCTCCAGTGAATAACTAAAAAAAA-A	
AtpsaA	TTGCCC <mark>CAGATCGACTTCCAGAT</mark> CATAAT <mark>TGCTCTAGT</mark> GAATAACTAAAGAAAATAGATG	
	***** * *** ***** * * *****************	
	psaAB-190	
7 mp $c > \lambda$		
2mpsaA OspsaA		
UspsaA		
IIVPSaA Atpaal		
Alpsak	* * ** ** ** **** * ***** * * **** ** *	
	nsa4R-150	
ZmpsaA	GACAGTTGGCGGGTC	
OspsaA	GTTGGCGGGTC	
HvpsaA	TATCTTTCAAAATCTATCTTTCAAAGATTCACTAAAAAAAAAGACAGTTGGCGGGTC	
AtpsaA	GGTTCACTAATTACTTGAGTGACTGTTGGCGGGTT	
L	* *** * * * ******	
ZmpsaA	TCTTTGTATGTCTTGTCCGGAAAGAGGAGGACTTAATGATTATTCGTTCG	
OspsaA	TCTTTGTATGTCTTGTCCGGAAAGAGGAGGACTTAATGATGATTCGTTCG	
HvpsaA	TCTTTGTATGTCTTGTCCGGAAAGAGGAGGAGGTTAATGATTATTCGTTCG	
AtpsaA	TCTTTGTATGTGTTGTCCGGAAAGAGGAGGACTCAATGATTATTCGTTCG	
T	***************************************	

Fig. S6 Comparison of the *psaAB* promoter sequences from maize (Zm), rice (Os), barley (Hv) and *Arabidopsis* (At).

The known transcription start sites TSS1 and TSS2 (black shaded), translation start sites (ATG) and prokaryotic-like promoter elements (gray boxes) are indicated. Bent arrows denote mapped transcription start sites with the nucleotide positions relative to the ATG site of the rice gene (Chen et al., 1993). The positions of probes (double-headed arrows) used in binding assays are diagrammed below the sequence.

Chen MC, Cheng MC, Chen SC. 1993. Characterization of the promoter of rice plastid *psaA-psaB-rps14* operon and the DNA-specific binding proteins. *Plant Cell Physiol* 34: 577-584.