

Supplementary material

Article

Lysine Acetyltransferase p300/CBP Plays an Important Role in Reproduction, Embryogenesis and Longevity of the Pea Aphid *Acyrtosiphon pisum*

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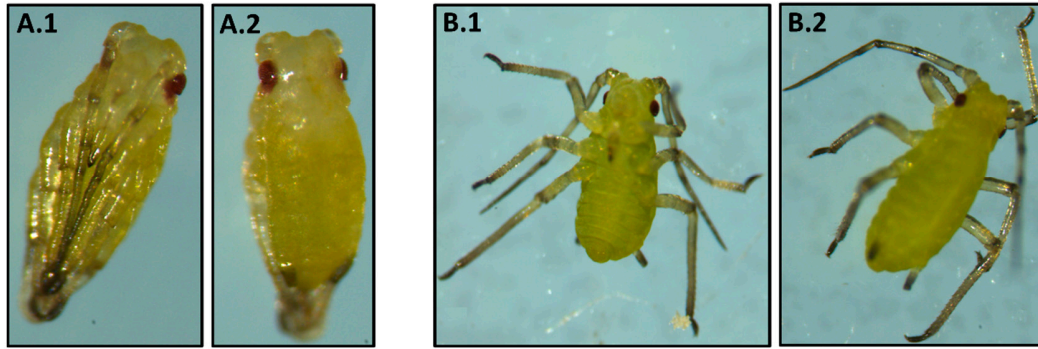


Figure S1. Nymphs of *p300/CBP* dsRNA treated mothers. A prematurely born nymph (A) in ventral (A.1) and dorsal (A.2) view. The legs and antenna remain unfolded and the nymphs die after eclosion. In comparison, a viable nymph (B) in ventral (B.1) and dorsal (B.2) view.

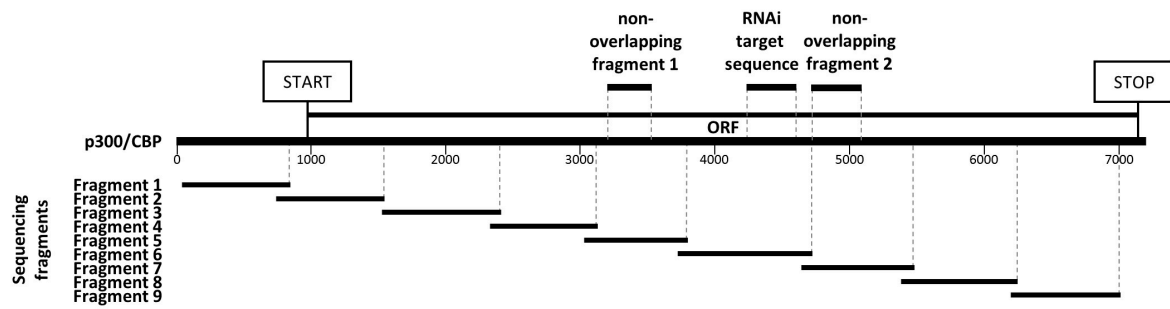


Figure S2. The *A. pisum* *p300/CBP* mRNA sequence from clone LL01 contains the 5'-UTR, start codon and most of the open reading frame (ORF), but the stop codon and 3'-UTR were not represented in our assembly (Tab. S1). The RNAi target site is also shown. Additional RNAi target sites used in this study are indicated as non-overlapping fragments 1 and 2.

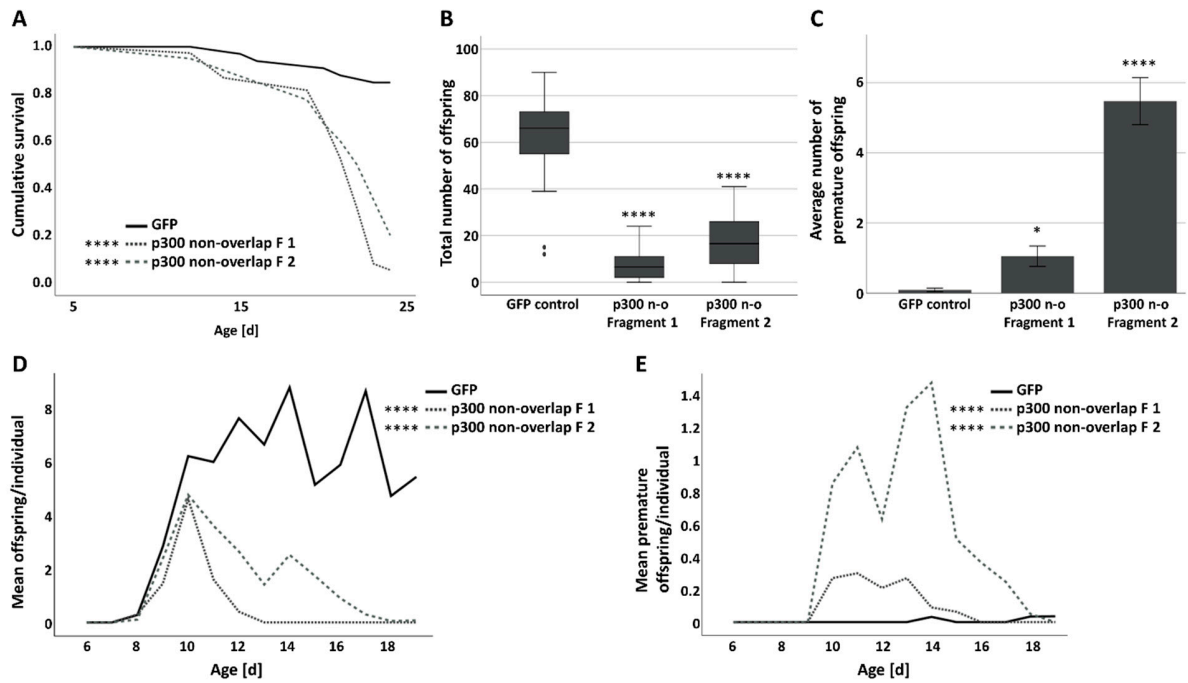


Figure S3: Life history parameters following the RNAi-mediated silencing of p300/CBP with the additionally tested non-overlapping fragments (called p300 non-overlap F1 and p300 non-overlap F2) as specified in Figure S2. We injected 40 individuals each with 25nl of a 3000 ng/ μ l solution. (A) survival, (B) total number of offspring, (C) average number of premature offspring, (D) number of viable offspring per day and (E) number of premature offspring per day is shown. Significances were tested using (A) the log-rank test, (B,C) the Kruskal-Wallis-test followed by Bonferroni corrections for pairwise analysis ($*p < 0.05$, $****p < 0.0001$) and (D,E) two-way-ANOVA ($****p < 0.000001$).

Table S1. Primer sequences used in this study.

	Primer name	Accession No. and residues (nt) sequences	Sequence	Frag. size (bp)	purpose
Fragment1	seq_AP_p300_fwd_1	MN734788	5'CTG ACC TTG GAC CGT GAG TT3'	807	sequencing
	seq_AP_p300_rev_1	nt1 – nt807	5'CAA ATT GAG AAA CAA AAT AGT TAA ACA3'		
Fragment2	seq_AP_p300_fwd_2	MN734788	5'AAT CAG CCT CCG ACT TAG CA3'	856	sequencing
	seq_AP_p300_rev_2	nt700 – nt1555	5'CTG GTT GCG AAT TTG TTG TG3'		
Fragment3	seq_AP_p300_fwd_3	MN734788	5'AGC AAC AAC AAA ACG GAC CT3'	887	sequencing
	seq_AP_p300_rev_3	nt1485 – nt2371	5'TAC CTG GGG TTG TGA ATG TG3'		
Fragment4	seq_AP_p300_fwd_4	MN734788	5'CTC AAA TGC CAC ATC CTC CT3'	801	sequencing
	seq_AP_p300_rev_4	nt2286 – nt3086	5'TTG GAA AAT GTA CCC TGT CTG A3'		
Fragment5	seq_AP_p300_fwd_5	MN734788	5'TCA ATT CAA CCC AAT CAG CA3'	769	sequencing
	seq_AP_p300_rev_5	nt2990 – nt3758	5'CCA TTT TCT TTG AAC TTG AAC CA3'		
Fragment6	seq_AP_p300_fwd_6	MN734788	5'AAC ACC GTC GGC ATC TGT GA3'	1002	sequencing
	seq_AP_p300_rev_6	nt3685 – nt4687	5'AGC AAC TTC ACC AGC GCC TG3'		
Fragment7	seq_AP_p300_fwd_7	MN734788	5'ATT GGC TAT AAC AAA ATT AGG TAC G3'	839	sequencing
	seq_AP_p300_rev_7	nt4600 – nt5438	5'TGC TCT TTC TCT GGC TGG AC3'		
Fragment8	seq_AP_p300_fwd_8	MN734788	5'GAA GTT GAA ATT GGC CCT GA3'	865	sequencing
	seq_AP_p300_rev_8	nt5351 – nt6215	5'GCT GTG CTT GTT GCA GTC TT3'		
Fragment9	seq_AP_p300_fwd_9	MN734788	5'TGT CCA GTG TTG TTC TGT CCA3'	811	sequencing
	seq_AP_p300_rev_9	nt6137 – nt6947	5'GGA ACA TTT GCC TTT GTC GT3'		
Assembly	Fragment1-Fragment9	MN734788			Assembling
Reference sequences	Protein	XP_008187184			
	Nucleotide	XM_008188962			
	RNAi_AP_p300_fwd		5' TZ G TCT TGG TTA TTG CTG TGG TCG T3'	367	RNAi
	RNAi_AP_p300_rev		5' TZ T CAC CGC GCT TCT TCA AAC AGT3'		
	RNAi_AP_p300_fwd_1		5' TZ AGC CAC CTG TCC CAA GTC CA3'		RNAi non-overlapping Fragment 1
	RNAi_AP_p300_rev_1		5' TZ GGT ACA CTT GTA GTT GAT GGC AC3'		
	RNAi_AP_p300_fwd_2		5' TZ GCG CTG GTG AAG TTG CTA TAC G3'		RNAi non-overlapping Fragment 2
	RNAi_AP_p300_rev_2		5' TZ GCA TGC CCA AAT GTG AGC CA3'		
	Rpl32_F		5'AGT ATC GCC CAA CAA TTA TCA3'		qPCR reference gene [1]
	Rpl32_R		5'CTT GAA TCG TCT TCG GAC T3'		
	qPCR_AP_p300_fwd4		5'GTT GCG AAC TCT AGG TCA GAA TA3'		qPCR target gene
	qPCR_AP_p300_rev4		5'GCT GTT GCA TTT GTT GTT GTT C3'		

Table S2. Statistical analysis of RNAi data for survival compared to the GFP control.

Treatment	Mean survival [d]	Standard error [d]	Chi-square	Significance
Control (GFP)	13.88	0.44		
p300/CBP 3000 ng/μl	10.23	0.34	63.95	p<0.0000
p300/CBP 1000 ng/μl	10.26	0.39	49.03	p<0.0000
p300/CBP 250 ng/μl	10.93	0.40	38.21	p<0.0000
p300/CBP 50 ng/μl	11.08	0.47	30.65	p<0.0000

Table S3. Statistical analysis of RNAi data for start of reproduction compared to the GFP control.

treatment	Mean start [d]	Standard error [d]	Chi-square	significances
Control (GFP)	4.93	0.07		
p300/CBP 3000 ng/μl	5.13	0.25	9	p=0.003
p300/CBP 1000 ng/μl	5.79	0.24	15.16	p<0.0000
p300/CBP 250 ng/μl	5.18	0.19	2.02	ns
p300/CBP 50 ng/μl	5.18	0.15	1.6	ns

Table S4. Statistical analysis of RNAi data for average number of offspring compared to the GFP control.

Treatment	Mean number	Standard error	Kruskal-Wallis test value	Significances
Control (GFP)	62.38	1.84		
p300/CBP 3000 ng/μl	11.11	0.81	317.24	p<0.000
p300/CBP 1000 ng/μl	11.84	1.17	306.36	p<0.000
p300/CBP 250 ng/μl	21.38	1.42	209.96	p<0.000
p300/CBP 50 ng/μl	23.02	1.31	184.17	p<0.000

Table S5. Reproductive parameters evaluated during the RNAi experiments including viviparous offspring determined by two-way ANOVA.

Treatment		Statistical parameters	Significances
p300/CBP 3000 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=463.702 F=98.235	p<0.0000
	Age of reproduction	df=28 mean square=349.784 F=74.102	p<0.0000
	Aphid line x Age of reproduction	df=27 mean square=262.781 F=55.67	p<0.0000
p300/CBP 1000 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=2462.32 F=596.994	p<0.0000
	Age of reproduction	df=30 mean square=204.834 F=49.662	p<0.0000
	Aphid line x Age of reproduction	df=16 mean square=203.719 F=49.392	p<0.0000
p300/CBP 250 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=1829.640 F=373.094	p<0.0000
	Age of reproduction	df=30 mean square=289.921 F=59.120	p<0.0000
	Aphid line x Age of reproduction	df= 17 mean square=167.724 F=34.202	p<0.0000
p300/CBP 50 ng/μl	Aphid line (control vs p300/CBP)	df=1 mean square=1173.761 F=242.996	p<0.0000
	Age of reproduction	df=30 mean square=297.476 F=61.584	p<0.0000
	Aphid line x Age of reproduction	df=20 mean square=132.424 F=27.415	p<0.0000

Table S6. Statistical analysis of RNAi data for average number of premature offspring compared to the GFP control.

Treatment	Mean number	Standard error	Kruskal-Wallis test value	Significances
Control (GFP)	0.43	0.05		
p300/CBP 3000 ng/ μ l	2.06	0.14	-185.09	p<0.000
p300/CBP 1000 ng/ μ l	2.98	0.17	-242.40	p<0.000
p300/CBP 250 ng/ μ l	1.89	0.24	-185.41	p<0.000
p300/CBP 50 ng/ μ l	1.57	0.24	-145.93	p<0.000

Table S7. Reproductive parameters evaluated during the RNAi experiments including premature offspring determined by two-way ANOVA.

Treatment		Statistical parameters	Significances
p300/CBP 3000 ng/ μ l	Aphid line (control vs p300/CBP)	df=1 mean square=0.836 F=5.994	P=0.014
	Age of reproduction	df=28 mean square=2.472 F=17.722	p<0.0000
	Aphid line x Age of reproduction	df=21 mean square=3.745 F=26.848	p<0.0000
p300/CBP 1000 ng/ μ l	Aphid line (control vs p300/CBP)	df=1 mean square=8.954 F=41.950	p<0.0000
	Age of reproduction	df=30 mean square=1.702 F=7.973	p<0.0000
	Aphid line x Age of reproduction	df=16 mean square=3.119 F=14.614	p<0.0000
p300/CBP 250 ng/ μ l	Aphid line (control vs p300/CBP)	df=1 mean square=3.658 F=27.031	p<0.0000
	Age of reproduction	df=30 mean square=0.823 F=6.083	p<0.0000
	Aphid line x Age of reproduction	df=17 mean square=1.237 F=9.144	p<0.0000
p300/CBP 50 ng/ μ l	Aphid line (control vs p300/CBP)	df=1 mean square=0.842 F=6.774	p<0.009
	Age of reproduction	df=30 mean square=0.643 F=5.171	p<0.0000
	Aphid line x Age of reproduction	df=20 mean square=0.924 F=7.435	p<0.0000

Table S8. Statistical analysis of RNAi data for body weight [mg] compared to the GFP control (dsRNA concentration = 3000 ng/ μ l).

Time after injection [d]	Body weight p300/CBP [mg]		Body weight GFP control [mg]		Significances
	Mean	Standard error	Mean	Standard error	
0	1	0.02	0.92	0.02	ns
3	2.10	0.07	2.07	0.09	ns
8	2.86	0.09	2.89	0.07	ns

ns = not significant

Table S9. Statistical analysis of RNAi data for body size (length*width)[mm²] compared to the GFP control (dsRNA concentration = 3000 ng/ μ l).

Time after injection [d]	Body size p300/CBP [mm ²]		Body size GFP control [mm ²]		Significances
	mean	Std. error	mean	Std. error	
3	2.43	0.08	2.45	0.1	ns
8	3.07	0.07	3.07	0.06	ns

ns = not significant

Table S10. Statistical analysis of RNAi data for body color (grayscale) compared to the GFP control (dsRNA concentration = 3000 ng/ μ l).

Time after injection [d]	Body color p300/CBP - grayscale		Body color GFP control - grayscale		Significances
	Mean	Standard error	Mean	Standard error	
3	107.22	1.33	101.15	1.56	ns
8	75.59	2.4	95.41	0.95	p<0.01

ns = not significant

AACATGTTAACTATGTAAGTCAAATCTAATGGCAACTGTCCGATTTGTAACAATATATTGCACCTTGCTTTCATCATGCTAAGTATTGTACTGAAGCTAAGTGTCCAGTGTGTTCTGTCCAAATATGAAACACAAGAT
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CGCCTAAGAGAGATTCTGAGGAGCAACCCACAGCTGTTGGCCCAATTCATTAGGAACCAACGACAAAGGCAATGTTCC

References

1. Sapountzis, P.; Duport, G.; Balmand, S.; Gaget, K.; Jaubert-Possamai, S.; Febvay, G.; Charles, H.; Rahbé, Y.; Colella, S.; Calevro, F. New insight into the RNA interference response against cathepsin-L gene in the pea aphid, *Acyrtosiphon pisum*: Molting or gut phenotypes specifically induced by injection or feeding treatments. *Insect Biochemistry and Molecular Biology* **2014**, *51*, 20–32.