

Article

Cloning, expression analysis, 20-hydroxyecdysone induction, and RNA interference study of autophagy-related gene 8 from *Heortia vitessoides* Moore

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Supplementary Materials

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TATTGGAATACAACACTGCTTCCTGCATTGTCATATCAGCTGATTGCGTCATTGGGTTTA 60
TAGACAATAACGCAAAAACAAAAACAAGTCGTAGTATCCAGTTTGTCTATATTTGATT 120
ATCTCGAAATTTTCAGTGCATTTTCATTCTCAAGATGAAATCCAATATAAAGAGGAACAC 181
                                     M K F Q Y K E E H
TCGTTTGAAAAAGGAAGACTGAGGGAGAAAAATACGCAGGAAGTATCCGGACCGAGTT 241
S F E K R K T E G E K I R R K Y P D R V
CCAGTAATTGTTGAGAAAGCCCGAAGGCTAGACTGGAGACCTCGATAAGAAGAAGTAT 301
P V I V E K A P K A R L G D L D K K K Y
TTAGTGGCCTCTGATTTAACTGTTGGCAGTCTACTTCTTGATCAGAAAACGCATTAC 361
L V P S D L T V G Q F Y F L I R K R I H
TTGCGGCCCGAAGATGCGCTGTTCTTTGTCAACAATGTAATCCCTCCTACATCGGCC 421
L R P E D A L F F F V N N V I P P T S A
ACCATGGGCTCTTTGTACCAGGAACACCATGACGAAGATTTTTCTCTACATAGCATT 481
T M G S L Y Q E H H D E D F F L Y I A F
TCCGACGAGAATGTTTATGGATATTTTGAATCCACGATTTTCATCACCAGAATGTGAA 540
S D E N V Y G Y F *
ATTATATGATCATAATGGTGAACAGATTAAAGGCTTAGCTTAATGATTGATGATTAT 600
GTTATAATGGTGATAATTAATTTAATTGCAATTCTACAACAACCTGTCAAATCACA 660
TTGTTAGAGTTTAAACATCGGTTTAAATGAAAATATTGCATTGTTTAAATCTCTTTGTTG 720
GTTTAGGAGTGTGATTATTTCTGTATCAAAATAAATAAAAAAGTACAAAAAATGCGTC 780
TTTCTTTCATTCTAAAGGTATTAAGACCTTATTTTATGTGCTAAAGTATGGTTCATATA 840
GTTCACTTATATTATGAGACAGTAATGTCGTGAATTGTTCTATAAACCATCTACTG 900
GTCTAATTTACACAATTAATAATTCGGAAAACAACATACATATTAGGGAAAATACTATTA 960
ATATAATATTTACTCTGTATGAACAGAACATCATAATTAACAGTAAATCATTCTTA 1020
TACTTTAGATTTTATAGGTATTAATCTATTTCATGCG 1057

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Figure S1. Sequence analysis of HvATG8. Nucleotide sequence of the cDNA encoding Atg8 isolated from *Heortia vitessoides* and the deduced amino acid sequence. The initiation codon and the termination codon are marked with a red underlines.

HvATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
SfATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
OfATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
SlATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
HaATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
TnATG8	MKFQYKEEHSFEKRRKTEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
BmATG8	MKFQYKEEHSFEKRAEGEKIRRKYPDRVPVIVEKAPKARLGDLDKKKYL	VPSDLTVGQF	60
AaATG8	MKFQYKEEHPFEKRAEGDKIRKYPDRVPVIVEKAPKARIGDLDKKEYL	VPSDLTVGQF	60
TmATG8	MKFQYKEEHPFEKRRSEGEKIRRKYPDRVPVIVEKAPRARIGDLDKKKYL	VPSDLTVGQF	60
PaATG8	MKFQYKEEHPFEKRAEGEKIRRKYPDRVPVIVEKAPKARIGDLDKKKYL	VPSDLTVGQF	60
	***** ***:**:*:*****:*****:***:*****:*****		
HvATG8	YFLIRKRIHLRPEDALFFFV--NVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGYF--	118
SfATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGY--	118
OfATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGSF--	117
SlATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGY---	117
HaATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGY---	117
TnATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGY---	117
BmATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHDDEFFLYIAFS	DENVYGN---	117
AaATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYSEHHEEDYFLYIAYS	DENVYGNK--	118
TmATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHEEDFFLYIAYS	DENVYGGDEL	120
PaATG8	YFLIRKRIHLRPEDALFFFVNNVIPPTSATMGSLYQEHHEEDFFLYIAYS	DENVYQG---	117
	*****	***** ***:**:*:*****:*****	

Figure S2. Comparison of amino acid sequences of HvATG8 with Atg8 in other insects. SfATG8, *Spodoptera frugiperda* (SPODOBASE: Sf2M09420-5-1); OfATG8, *Ostrinia furnacalis* (AYU75107.1)SlATG8, *Spodoptera litura* (JX183217); HaATG8, *Helicoverpa armigera* (JQ739159); TnATG8, *Trichoplusia ni* (JX183216); BmATG8, *Bombyx mori* (NP_001040244.1); TmATG8, *Tenebrio molitor* (KM676434.1); AaATG8, *Aedes aegypti* (AY736002.1) and PaATG8, *Periplaneta americana* (AB856588). Tyr49 and Leu50, which are required for the activation of the lipidated form of Atg8 by Atg7 and Atg3, are boxed in red. Phe77 and Phe 79, which are parts of the recognition site for Atg4, are boxed in pink (* indicate identical residues).

A Test of Between-Subjects Effects

Development Variable: Expression

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1157.455 ^a	5	231.491	7.243	0.000
intercept	768.239	1	768.239	24.038	0.000
species	404.772	1	404.772	12.665	0.002
Time	752.683	4	188.171	5.888	0.002
Error	767.029	24	31.960		
Total	2692.723	30			
Corrected Total	1924.484	29			

a. R Squared = 0.601 (Adjusted R Squared = 0.518)

B Test of Between-Subjects Effects

Development Variable: Expression

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.749 ^a	4	0.187	9.266	0.000
intercept	17.710	1	17.710	876.353	0.000
species	0.511	1	0.511	25.279	0.000
Time	0.238	3	0.079	3.928	0.024
Error	0.384	19	0.020		
Total	18.843	24			
Corrected Total	1.133	23			

a. R Squared = 0.661 (Adjusted R Squared = 0.590)

Figure S3. Some statistical results of two-way ANOVA. A represents the result of RNA interference; B represents the result of 20-hydroxyecdysone treatment.

Table S1. PCR primers used in this study.

Primers	Primer sequence (5'–3')
For real-time PCR	
HvATG1-F	CGCACACCTCAACCCTC
HvATG1-R	GTCCGCTTTGGCATCAT
HvATG2-F	CTCGACAGACGACACCCAC
HvATG2-R	GCTGACAAGTCCACACGCT
HvATG3-F	TTACCGCTTAGTTCCGT
HvATG3-R	CCTGTGTTGCCTTTCTC
HvATG4-F	CGAATCCCGCCTACTAT
HvATG4-R	TCCTTCAATGCCCTCTC
HvATG5-F	ATGTCAACTATGCAGAAGAAGG
HvATG5-R	CAGTGGAGTATCCACAGGAAG
HvATG6-F	CATTATGTGCCTCCTTTTCG
HvATG6-R	CCTCCTTGTCATCTTCCAGC
HvATG7-F	CCTCCTTGTCATCTTCCAGC
HvATG7-R	TGGAATGTGGTATGGGA
HvATG8-F	TGAAAAAAGGAAGACTGAGGG
HvATG8-R	CGGAGAATGCTATGTAGAGG
HvATG9-F	TGCCGTGTGTTGGAGAT
HvATG9-R	GGGCGAAGTGAAGCTAG
HvATG12-F	TCGGGTGGATTATGGAGTT
HvATG12-R	ATTTGGTCTGGAGAGGGTG
HvATG13-F	CGAGGATGGAGACGAAA
HvATG13-R	ATAGGCGACCGAAAAAC
HvATG16-F	CTGGTCTCCTGGCGTCTGT
HvATG16-R	CGCTTGCTTCCTCCTTTTT
HvATG18-F	AGCCACAAAACCTTAC
HvATG18-R	GCACACCAGATCCAGAG
For dsRNA	
dsATG8-F	TAATACGACTCACTATAGGGAAAAGGAAGACTGAGGGAGA
dsATG8-R	TAATACGACTCACTATAGGGCGGAGAATGCTATGTAGAGG
dsGFP-F	TAATACGACTCACTATAGGGCAGTTCCTTGTTGAATTAGATG
dsGFP-R	TAATACGACTCACTATAGGGTTTGGTTTGTCTCCCATGATG

F: forward primer; R: reverse primer.

Table S2. GenBank accession number in this study.

Gene	Genbank number
HvATG1	MN788355
HvATG2	MN788356
HvATG3	MN788357
HvATG4	MN788358
HvATG5	MN788359
HvATG6	MN788360

HvATG7	MN788361
HvATG8	MN788362
HvATG9	MN788363
HvATG12	MN788364
HvATG13	MN788365
HvATG16	MN788366
HvATG18	MN788367
α -tubulin	MG132200
