ARTICLE

Knockdown of *TOR* causing ovarian diapause in a genetically stable brachypterous strain of *Nila a a a l gen*

¹Hbil R Uiiaia Saiab P Maa_K Laba,C Pa SiaT, Ha AiaUii,Wa,Cia ²C LiSiaT, Ha AiaUii,Wa,Cia

Correspondence

HiaHa,C PaSia T,HaAiaUii, Wa430070,Cia. E_al:a ia@_al.a. Ga:NaiaNaaSiFa

Ga:NaiaNaaSiFaiCia;Ga_b:31171846.

Abstract

В	а		(BPH),	Nilapar	vata lugens (S) (H _ i	a: D -			
a i a), í		-	a_	ai	Í.	. BPH i			
a_i a	Í		í a	а	aia	Í	_i a			
аi	а		Í	ia a	.T _	а_	ai_			
	- Í	ia a	_ (ai	a,a		1 <u>-</u> 1			

^{2 of 11} WILL

ii ii , iiai i BPH.A ii ii a i MF, ia ii iia i BF (J , 1963; Kii_ , 1956). I abiaiaab, BPH i BF i __ia iaiai (S b, Miia, Ya_ai, Maai, & Ma_a, 2002); abia ii, __BPH i MF i abii aiia_iai (Kii_ , 1956).

I a iii , MF _ a a ai ii ii a BF _ a (Ki i _ , 1965).T i i a bi ia a _ i a _ a , aia _ i ai _ i a i (C , C , Ya , & Yi , 1979). BPH i _ i _ i b Insulin Receptor 1 a InsulinReceptor 2

TABLE1 Li i.

GenBank accession			Length of PCR
number	Name of primer	Primer sequences	product
JQ793898.1	NITOR-F	GATCGGCATGAGGGAGGGAGACA	6,768 b
	NITOR-R	CGACGACGGTACACTGCGTTTGG	
	1NITOR-F	TAATACGACTCACTATAGGACCAG TGAAATGCTCGTAAACA	550 b
	1NITOR-R	TAATACGACTCACTATAGGCCAGGTGCAGG TAATCGTCCAG	
	2NITOR-F	TAATACGACTCACTATAGGTTGACGG TCACTCACTACTGCA	492 b
	2NITOR-R	TAATACGACTCACTATAGGGCTCTTTGTT TCGTCCCATACC	
U76561	GFP-F	TAATACGACTCACTATAGGGTAAA CGGCCACAAGTTCAG	400 b
	GFP-R	TAATACGACTCACTATAGGTCGGC CATGATATAGACGTT	
EU179846.1	qPCR-NIActin1-F	CCAACCGTGAGAAGATGACC	256 b
	qPCR-NIActin1-R	GATGTCACGCACGATTTCAC	
JQ793898.1	qPCR-NITOR-F	AACGCCATGGAGGTGACAGG	143 b
	qPCR-NITOR-R	ATGAGGCGCCAGTTGAGCAG	
KY827832	qPCR-NIE74B-F	AACAACATAATAGGCACAGTC	175 b
	qPCR-NIE74B-R	GGAATGGCGAAGAAGTATC	
FJ263049.1	qPCR-NIEcR-F	GCCAGAAAGTACGACGTGAA	234 b
	qPCR-NIEcR-R	TTGGATCTTCTCCACCTTCC	
19JF345255	qPCR-NIFoxA-F	GCGGAGGTTATGTTGTGTTGTA	193 b
	qPCR-NIFoxA-R	CTGAGCCTTGTAGCATGTTGAA	
AB353856.1	qPCR-NIVg-F	TCTTCATCATCCTCCTCCTCTTC	173 b
	qPCR-NIVg-R	TCCTGGTTGTTGTCATTGTCATT	

2.3 | Synthesis of dsRNA

T b-a RNA (RNA) i b)a b 2,764 a 3,313 i ,a NI1TOR. T RNA (1NITOR, 550 RNA(2*NITOR*, 492 b) a b 1,736 a 2,227 i .T7 _ a _ a a a 5'- - i i_ .T i_ a_ i DNA NITOR.T i PCR aii T7 _ a _ a b 5'a _ a RNA í.QIAgíTMPCR í ai í (C N .: 28104,Qia-, D, G_a) i i a T7 Rib MAX E I PCR . RNA RNAIS _ (C N .: P1700, P _ a, Ma i) i _aa'i i.GFP (G Baai.U76561)RNA(GFP)aaai.Tq í. í RNA a 1NITOR-F/R a 2NITOR-F/R (Tab 1).

2.4 | RNAi using microinjection

 T
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i
 i

 GFP) ai
 i a _ a i
 a a _ a.A a 250 _

 aia
 iai
 ia a _ (i..., ia 750 _ i a

 RNA).T
 a _ a ib b Li a. (2015).T
 ia BPH a ai

 aii.A
 a_i_, i a_ a _ qRT-PCR aa ia 3, 6, 7, 9, 11 a ai

 ii.

2.5 | Developmental duration and ovary observation

Ea BPH _ a i RNA a b a 6 i_ i a a_i i i .N _ _a i i - _a .T BPH ai i i i - ii (WPI, Sa a a) a 1, 3, 5, a 8 a a a _ _, i 1 × a -b ai (PBS) b ai i 4% _a i 1 × PBS 20_i a _ _ a .Di ai a i_ 10_i i 0.2% Ti -X 100 (C N ::T9824, Sa a a a, Si_a) i 1 × PBS.A a i , ai a i a 0 _ _ i (SZX16, O _ ,T ,Ja a) a P90 P Di i a Ca_ a (P90, Ni ,T ,Ja a).Ri i i BPH i _ a _ b .

2.6 | qRT-PCR

TRI a (C N .: 15596018, I i , Ca ba) a a a RNA. Actin1 (G Ba a i _b EU179846.1) a aaia .SYBRP_iE Tag (C N .:RR420A, Taaa, Daia, Cía) a gRT-PCR aa i a ABIP i_ 7300 i _ (A i Bi _)a i _a a 'i i.Ea ai i_ a i a_ ia i_a 100 300 b PCR (Tab 1), a PCR a_ i a i i _ .0 i_ aia i aa Ĺ i qRT-PCR a a i.Ea RNA a_ 85 110% í a a 98% a í а í aí, a í aí í a a a_i b a aa a.T bi ia ia a a_ .T ai i a a a _ Liaa S_i (2001).Sq i_ qRT-PCRa i Tab 1. a í

2.7 | Sequence comparison and phylogenetic relationship

Pi ba a_iaiq __, i, _ibib-ii_ i MaEiaGiAai(MEGA)a. Tba aii1,000 ia.

2.8 | Data analysis

Aaa aa i SPSS i 18.0-aANOVAa aa i b_a, a a-_a ANOVAa aa ia RNA.P aa aib aiiaaai.

3 | RESULTS

3.1 | NITOR expression patterns during different developmental stages in BS and MS BPH

Т		a NITOR a				í b				аа		а	i BPH	í BPH;í			а		_ ai		
	Í		a a	. D	Í.	-	а	а	,	NITO	R	Í		a i	Ĺ	а		Í	В	S	а
MS	ia	Í	a,b			ĹĹ	а	Ĺ		Ĺ	NITO	2	Í	а				-	а	а	
Т	NITOR		Í.	Í	BS a		Í	Í	а	Ĺ	a	a	Í	MS a	а	1, 2,	а	4	а	а	



Development stages

FIGURE 1TaiiaNITORai_aASABS; BPH actin 1aa a.Tia q a iaiaBS. Taia babiiaia.Ebaiiaaa.Baabiaa iia babiiaia.Ebaiiaaa.Baabiaa iiia bBPHMSaBS, a_ii- aANOVA (P < 0.05)</td>



FIGURE 2 Sia a BSBPHi 1NITOR a 2NITORii. GFPiia aa .Ea i 45_.Sia GFP, 1NITOR, a 2NITOR aa i a-_a ANOVA

_ , a NITOR i a iBS a MS3 a a _ (Fi 1).T NITOR i i i a a a 5 a _ b BS a MS (Fi 1).

3.2 | Effects of knockdown of *NITOR* on the survival of BS BPH

Ba i NITOR i BSa i i a i MSa a 1,2,a 4 a a _ , NITOR a i i _ BS i i a NITOR i i iaa i BPH.



FIGURES Tai i NITORa i RNAi i-ia _ iBS.E baiia aa .T i aqai ai a _ i i GFP.Baab i i iia a iia i a_ a_ a_ aa _i i - aANOVA (P<0.05).Taa i aba biia ia

BPH i _aa, i _8 15 aa i i, a BPH i a a.Ti iia a *NITOR* a a BPH a b BPH _ .

3.3 Effects of dsRNA injection on NITOR gene expression in BS

IBS, i i 1NITOR a 2NITOR i i a _ i i a _ i NITOR _ RNA a a _ i i .F _ 3 11 a a i i NITOR, a i NITOR a i i a b 36.9 94.1% (Fi 3) (P <



1NITOR a 2NITOR i BS BPH FIGURE 4 T Í ai а _ Í iiaa.A baiia aa -Í ia.Ba iiaa iiai ab i i a_ a_ ,a _i - a ANOVA (P < 0.05) Í

3.6 Knockdown of NITOR inhibited ovarian development

a _a BS _ i í. W a i 1NITOR ai а а ,_a BSi GFPai_a a a _b _. (ab Н Ĺ 100 а , i ai _a a 1,3,5,a 8 a a / _a).T a_i а -. .T aaia _aiibiai a a . Í a b 1NITORí í,_aí a _i aia ba3,5,a 8a.Ai . í ía BPH aia b L (2011), aí NITOR-a BS _ a ai аí baí í.Ba, Í... а GFP- a _ a аí i aia ba 3, 5, a 8 a (Fi 6). T _a ,a _____a í NITOR _ai BS _ a i ia a aа ai С ba a.(1979).

3.7 | Effects of in vivo knockdown of *NITOR* on the expression of *NIFo* A and *NIVg* related to ovarian development

 P
 i
 a
 a
 FoxA a
 Vg (Vitellogenin) a i i aia _ (D a., 2011).

 T
 i
 NIFoxA a
 NIVg _ i i _ a b qRT-PCR a 1, 3, a 5 a a _ .
 .

 A
 1, 3, a 5 a a _ .
 , NIFoxA i i _ a a i 1NITOR a b 95.9, 89.7,
 .
 .

 a
 25.5%, i (Fi 7A) i _ ai .
 .
 .
 A 1, 3, a 5 a a _ .
 , NIVg i

 i _ a a i 1NITOR a i i a b 71.6, 60.8, a 51.2%, i (Fi 7B), _ a
 .
 .
 A 1, 3, a 5 a a _ .
 .

 i _ a a i 1NITOR a i i a b 71.6, 60.8, a 51.2%, i (Fi 7B), _ a
 .
 .
 .
 .

4 | DISCUSSION

TOR a iiaiaia a,i,a а ____iii ai__(Oa_ Í, Í a., 2000; Z a a., 2000). I i , TOR a (L a., 2016; -Ma a., 2009; Pa a., 2006). NITOR a b i BPH а ÍÍ a a (La., 2016; Zaia., 2015; Za., 2017). Di _iai, a BPH_i a _a ii _ a , aí, í iaa a (C a., 1979). W a í аí b NITOR a BPH i iaa _ai .l a NITORi ,



9

AL. WILEY MUSCICAL PHYSIOLOGY Iday 3day 5day 8day ds GFP ds INTOR

 FIGURE 6
 E
 1NITOR
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 a
 <



 FIGURE 7
 Tai
 i
 NIFoxA a
 NIVg a
 i
 RNA. (A) Tai
 i

 NIFoxA a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 i
 NIVg

 a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 i
 NIVg

 a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 i
 NIVg

 a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 i
 NIVg

 a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 i
 NIVg

 a
 i
 1NITORi
 _
 a
 i
 a.(B) Tai
 a
 a

 qai
 a
 _
 i
 a.(B) Tai
 a
 a
 a
 a

 b
 a_
 a_
 _
 i
 GFP. Baab
 a a
 i
 a

 b
 a_
 a_
 _
 i
 a
 ANOVA (P < 0.05). Tai</td>
 a

 a
 b
 i
 i
 i
 i
 a

_ a a a , i i i i NITORb MSa BSa _ a a a a 1,2,a 4 a i .l i NITORi BS _ a a _ i BSBPH _a i a_a i a i iaa .T i i ai b NITORa BPH i iaa .

 BPH i
 i
 _i
 b
 Insulin Receptor 1 a
 Insulin Receptor 2 a
 _ a a
 (X a., 2015), i

 ai
 a
 _i
 iaa
 _iaa
 _iaa
 _iba
 _.Ti
 i

 ai
 a
 _iaa
 _iaa
 _iaa
 _iaa
 _iaa
 _iba
 _.Ti
 i

 a
 _aaa
 i
 iaaa, ia
 _iaa
 _aBS_b
 b
 _iaa
 _aBS_b
 b

 i
 iai<.A</td>
 5 a
 i
 iaaa, iaa
 _aBPH ai
 _ia
 MF (MS, ~85% MF) a
 a

 BPH ai
 i
 BF (BS, 100% BF)
 i
 ab.T
 ai
 i
 a__

 i
 NITOR
 _
 ai
 _iab.T
 ai
 i
 a__

 i
 NITOR
 _
 ai
 _iaa
 aa
 ba
 .T
 aa
 i

 i
 NITOR
 _
 ai
 _iaa
 ab
 73.7%.T
 ,
 BS<_aa</td>

 _
 _
 _
 _
 aa
 _
 iaaaaaai
 _________iai.

 </tbr>
 </tbr>

T NITOR i i a 300, i i baia i a a i X,X,L,Za, Z,a Za (2015).Li a (2015) a 300 NlapA; , NlapA a a BPH. T , bi a _ ai i a b i b b NITOR i .l a, NITOR a

, BPH _ BPH a . W а Í а а a NITOR a a ai _ Í Í Ĺ NITOR i a Ĺ. i a a i b í. .

Pi _ a aNITOR a_aia a iJHai_ a a(NIJHAMT),a aiai JHIII RNAi(NITOR)_a aia

b RNAi,í í a í TOR a a i JH bi í a AA -_ ia V ίí а а N. lugens (L a ., 2016). O a NITOR a i i a a a .NITOR NIE74Ba NIEcR.T íía i a_ TOR a а Í aí а. а b í JH bi i,a i a a a .

, NITOR NIVg. T i NIFoxA a а í a Zai a. (2015). Wai a TORiai í í BPH а í í _ , ai i_ a i i iaa i_ia _a b ai Ĺ NIFoxA a NIVg.

ACKNOWLEDGMENTS

Ti a baa NaiaNaaSi Fai Cia(N.31171846).

LITERATURE CITED

- C , R., C , X., Ya , L., & Yi , X. (1979). B a aia _ ai ai i i . Acta Entomologica Sinica, 22, 280 288. (I C i).
- D, X., Zai, Y., Za, J., S, Z., C, J., C, J., & Za, W. (2011). F a a i i a i g i aia _ a i b a , Nilaparvata lugens (S). BMC Molecular Biology, 12, 53.
- J , S. (1963). A a i i a a _ii a i _i b a , Nia a a a . Japanese Journal of Applied Entomology and Zoology, 7.1, 45–48.
- Kii_, R. (1956). E i i aa i _iai i _aa a . Nature, 178(4534), 641 642.
- Ki I_, R. (1965). S I __ I_A I AI I AI B A , Nilaparvata lugens (S a). Bulletin of the Shikoku National Agricultural Experiment Station, 13, 101 106.
- Li, F., Li, K., Li, J., H, D., Za, J., H, Y., ... Ha, H. (2015). Apterous A_aii, bi_aiaaii Nilaparvata lugens. Scientific Reports, 5, 10526.
- Lia, J. K., & S_i, T. D. (2001). A a i ai i a a i a i_ qaiai PCR a $2^{-\Delta\Delta C}$, *Methods*, 25, 402 408.
- L, F. (2011). T _____ i a a a i i ia a ia ___ i b a . Chinese Journal of Applied Entomology, 48, 1394 1400.
- L, K., C, X., Li, W., & Z, Q. (2016). TOR a a -_ ia i __ i a i -_ a i Nilaparvata lugens (S). International Journal of Molecular Sciences, 17(4), 438.
- Maai, S. A. P., Raa a, D., H_aaa, P. V., & Saaa, S. G. J. N. (2012). Eaai ba Nilaparvata lugens (Sa) ia i Oryza nivara i ia i SiLaa. Proceedings of International Forestry & Environment Symposium, 15, 172–175.
- Ma , J. L., C b , J., & B , X. (2009). Target of rapamycin (TOR) _ ia a i ii a i a i i _ i . Journal of Biological Chemistry, 284, 5506 5513.
- M a, S., & T , S. (1992). Mai a a i ai ibii i i _ a b i i ii i b a - , Nilaparvata lugens (H _ a: D a i a). Applied Entomology and Zoology, 27, 445 445.
- O a_, S., M a , J., Ra i_ i, T., T _a , G., & Ha , E. (2000). G i a bi _i a a a i a i TOR, Drosophila _ target of rapamycin. Genes & Development, 14, 2689 2694.
- Pa, J.H., Aa, G.M., Ha, I.A., & Rai, A.S. (2006). GATA aaaiiaaaiiaaaa

LIU ET AL.

- S b , S., Mi i a, H., Ya_a i, J., Ma a i, M., & Ma _ a, M. (2002). F a i a a a i i _ a i b a , Nilaparvata lugens S a i i . Japanese Journal of Applied Entomology and Zoology, 46(3), 135 143.
- T ____, C.S. (2002). E a 2000. Insect Biochemistry and Molecular Biology, 32(2), 113 120.
- X, H., X, J., L, B., Za, X., Z, J., H, S., ... Za, C. (2015). Tii __iaaii_i a...*Nature*, 519, 464–467.
- Y, R., X, X., Lia, Y., Tia, H., Pa, Z., Ji, S., ... Z a, W. (2014). T i i a ia a RNAi-ba . International Journal of Biological Sciences, 10(10), 1171–1180.
- Zai, Y., S, Z., Za, J., Ka, K., C, J., & Za, W. (2015). A iai TOR iai a ab a_i a i . Scientific Reports, 5, 10694.
- Z a , H., S a , J. P., N , J. C., R i a , C., & N , T. P. (2000). R a i a b Drosophila a a _ i TOR. Genes & Development, 14, 2712 2724.
- Z , J., X , J., L , B., H a , H., X , H., & Z a , C. (2017). E RNAI-_ ia NITOR ii _ a Nia a a a . Journal of Insect Physiology, 98, 149 159.

 How to cite this article:
 I
 F, Li K, Cai W, Z a J, Z Y, H a H. K
 TOR a i
 a ia
 ia a

 i a
 i a
 ab b a
 ai
 Nilaparvata lugens. Arch Insect Biochem Physiol. 2017;95: 21400.

 :// i.
 /10.1002/a
 .21400