Supplementary Note 13: Dorsal proteins

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Introduction

Rel/NFκB transcription factors are widely conserved in the animal kingdom. These proteins are defined by the presence of a Rel Homology Domain (RHD), which includes a DNA binding domain and a protein dimerization domain. In insects, Rel proteins play important roles in both development and immunity. *Drosophila melanogaster*, where these genes have been best studied, has three Rel genes: *dorsal* (*dl*), *Dorsal-related immunity factor* (*Dif*) and *Relish* (Ganesan et al., 2011). Dorsal and DIF both respond to Toll signaling (Belvin and Anderson, 1996; Meng et al., 1999), while Relish responds to IMD signaling (Hedengren et al., 1999; Khush et al., 2001). Relish also differs in that it contains ankyrin repeats (Dushay et al., 1996).

Methods

Rel genes from other insects were used to query the predicted *D. citri* protein sets (Diacit_International_psyllid_consortium_proteins_v1 and Diacit_RefSeq_proteins_Release_100) at i5k@NAL. The locus encoding the matching protein was identified and manually annotated in Web Apollo using RNA-Seq data to deduce the structure of transcripts. Multiple alignments of the predicted *D. citri* isoforms and their homologs were performed in Muscle (http://www.ebi.ac.uk/Tools/msa/muscle/). A phylogenetic tree was constructed using the neighbor-joining method in MEGA7. All sequences for multiple alignment and phylogenetic analysis were obtained from NCBI, FlyBase, ImmunoDB and the Bordenstein Lab (NSF DEB-1046149).

Results and Discussion

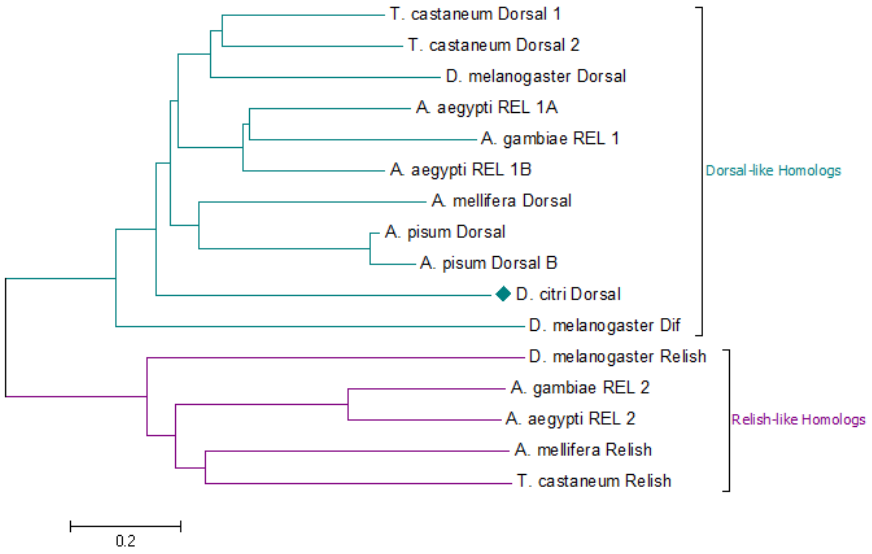

Figure 1: Phylogenetic tree depicting clustering of insect Rel proteins

In *D. citri*, BLASTp searches with known Rel proteins identified only one locus encoding a predicted Rel family member. The predicted RHD is much more similar to Dorsal than to either DIF or Relish. Moreover, the locus does not seem to encode the ankyrin repeats that are found in Relish orthologs. Consistent with these observations, phylogenetic analysis shows that the *D. citri* protein clusters with Dorsal proteins from other insects. Note that *dorsal* and *Dif* seem to be the result of a duplication in the *Drosophila* lineage (Meng et al., 1999; Christophides et al., 2004). *Dif* has diverged more rapidly (hence its position as an outgroup), so homologs in other insects have generally been named *dorsal* (Chen et al., 2000; Ursic-Bedoya et al., 2009; Gerardo et al., 2010). Following this convention, we will refer to this gene as *D. citri dorsal* (*dorsal*).

		D. citri Dorsal isoform A	*D. citri* Dorsal isoform B
D. melanogaster **Dorsal isoform A**	**Bit score**	254	244
	QC (Identity)	58% (45%)	45%(44%)
	E value	1.00E-76	3.00E-71
D. melanogaster **Dorsal isoform B**	**Bit score**	249	258
	QC (Identity)	58% (44%)	73% (45%)
	E value	1.00E-72	4.00E-74
D. melanogaster **Dif**	**Bit score**	184	180
	QC (Identity)	59% (38%)	42% (40%)
	E value	2.00E-50	5.00E-48
D. melanogaster **Relish**	**Bit score**	127	126
	QC (Identity)	59% (33%)	42% (34%)
	E value	1.00E-30	1.00E-29
A. gambiae **REL 1**	**Bit score**	171	171
	QC (Identity)	37% (46%)	29% (46%)
	E value	9.00E-51	1.00E-49
A. gambiae **REL 2**	**Bit score**	55.5	54.7
	QC (Identity)	14% (38%)	11% (38%)
	E value	1.00E-08	1.00E-08
A. aegypti **REL 1A**	**Bit score**	287	281
	QC (Identity)	57% (50%)	43% (50%)
	E value	3.00E-90	1.00E-86
A. aegypti **REL 1B**	**Bit score**	282	262
	QC (Identity)	55% (51%)	45% (48%)
	E value	4.00E-86	5.00E-80
A. aegypti **REL 2**	**Bit score**	102	105
	QC (Identity)	59% (31%)	49% (31%)
	E value	5.00E-23	2.00E-23
A. mellifera **Dorsal Isoform A**	**Bit score**	290	274
	QC (Identity)	97% (36%)	44% (49%)
	E value	7.00E-91	3.00E-83
A. mellifera **Dorsal Isoform B**	**Bit score**	271	287
	QC (Identity)	56% (49%)	74% (50%)
	E value	7.00E-82	3.00E-86
A. mellifera **Relish**	**Bit score**	48.1	45.1
	QC (Identity)	12% (42%)	7% (45%)
	E value	1.00E-05	1.00E-04
T. castaneum **Dorsal 1**	**Bit score**	270	256
	QC (Identity)	98% (37%)	45% (46%)
	E value	1.00E-83	7.00E-77
T. castaneum **Dorsal 2**	**Bit score**	194	191
	QC (Identity)	56% (39%)	44% (39%)
	E value	6.00E-57	1.00E-54
T. castaneum **Relish**	**Bit score**	99.8	99.8
	QC (Identity)	54% (30%)	42% (30%)
	E value	8.00E-22	2.00E-21
A. pisum **Dorsal**	**Bit score**	279	274
	QC (Identity)	56% (51%)	46% (49%)
	E value	3.00E-88	1.00E-84
A. pisum **Dorsal B**	**Bit score**	282	275
	QC (Identity)	97% (39%)	50% (47%)
	E value	2.00E-88	8.00E-84

Table 1: Bit score, query coverage, identity and e-value results from blast analysis of predicted dorsal_RA and dorsal_RB proteins

In several insect species, *dorsal* genes produce alternative transcripts that generate different protein isoforms (Gross et al., 1999; Shin et al., 2005; Zou et al., 2007). Therefore, it was not surprising that RNA-Seq data suggests that *dorsal* produces at least two alternative transcripts (*dorsal_RA* and *dorsal_RB*) that are very similar in structure to those seen in other insects.

Species	**# Dorsal-like genes**	**# Relish-like genes**
Drosophila melanogaster	2	1
Anopheles gambiae	1	1
Aedes aegypti	2	1
Apis mellifera	1	1
Tribolium castaeum	2	1
Acyrthosiphon pisum	2	0
Diaphorina citri	1	0

Table 2: Rel/NFkB transcription factors in *D. citri* and related organisms

No ortholog of *Relish* was found in the *D. citri* genome. While it is possible that we were unable to find *Relish* due to gaps in the *D. citri* genome, the fact that *Relish* is missing in the pea aphid genome (Gerardo et al., 2010) suggests that it could be absent in *D. citri* as well. It is interesting to note that both the pea aphid and the Asian citrus psyllid, two organisms known for their ability to host a variety of bacterial symbionts, appear to be lacking multiple members of the IMD pathway (including *Relish*) (Gerardo et al., 2010). The IMD pathway is known to be involved in the detection and elimination of Gram negative bacteria (Hedengren et al., 1999). Several Gram negative bacteria have been identified as *D. citri* symbionts, including the bacteria *Wolbachia*, *Candidatus* Carsonella, *Candidatus* Profftella armature, an as yet unidentified enteric bacteria closely related to *Klebsiella variicola* and *Salmonella enterica*, and *Candidatus* Liberibacter asiaticus, which causes citrus greening disease (Jagoueix et al., 1994; Nakabachi et al., 2006; Hilgenboecker et al., 2008; Saha et al., 2012; Sloan and Moran, 2012; Nakabachi et al., 2013). Given this information it is tempting to speculate that a reduction in IMD pathway genes may in fact aid in *D. citri's* ability to acquire and harbor these Gram negative symbionts.

Reference

1. Belvin, M. P. and Anderson, K. V. (1996) 'A conserved signaling pathway: the Drosophila toll-dorsal pathway', *Annu Rev Cell Dev Biol* 12: 393-416.

2. Chen, G., Handel, K. and Roth, S. (2000) 'The maternal NF-kappaB/dorsal gradient of Tribolium castaneum: dynamics of early dorsoventral patterning in a short-germ beetle', *Development* 127(23): 5145-56.

3. Christophides, G. K., Vlachou, D. and Kafatos, F. C. (2004) 'Comparative and functional genomics of the innate immune system in the malaria vector Anopheles gambiae', *Immunol Rev* 198: 127-48.

4. Dushay, M. S., Asling, B. and Hultmark, D. (1996) 'Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of Drosophila', *Proc Natl Acad Sci U S A* 93(19): 10343-7.

5. Ganesan, S., Aggarwal, K., Paquette, N. and Silverman, N. (2011) 'NF-kappaB/Rel proteins and the humoral immune responses of Drosophila melanogaster', *Curr Top Microbiol Immunol* 349: 25-60.

6. Gerardo, N. M., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S. M., de Vos, M., Duncan, E. J., Evans, J. D., Gabaldon, T., Ghanim, M. et al. (2010) 'Immunity and other defenses in pea aphids, Acyrthosiphon pisum', *Genome Biol* 11(2): R21.

7. Gross, I., Georgel, P., Oertel-Buchheit, P., Schnarr, M. and Reichhart, J. M. (1999) 'Dorsal-B, a splice variant of the Drosophila factor Dorsal, is a novel Rel/NF-kappaB transcriptional activator', *Gene* 228(1-2): 233-42.

8. Hedengren, M., Asling, B., Dushay, M. S., Ando, I., Ekengren, S., Wihlborg, M. and Hultmark, D. (1999) 'Relish, a central factor in the control of humoral but not cellular immunity in Drosophila', *Mol Cell* 4(5): 827-37.

9. Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. and Werren, J. H. (2008) 'How many species are infected with Wolbachia?--A statistical analysis of current data', *FEMS Microbiol Lett* 281(2): 215-20.

10. Jagoueix, S., Bove, J. M. and Garnier, M. (1994) 'The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria', *Int J Syst Bacteriol* 44(3): 379-86.

11. Khush, R. S., Leulier, F. and Lemaitre, B. (2001) 'Drosophila immunity: two paths to NF-kappaB', *Trends Immunol* 22(5): 260-4.

12. Meng, X., Khanuja, B. S. and Ip, Y. T. (1999) 'Toll receptor-mediated Drosophila immune response requires Dif, an NF-kappaB factor', *Genes Dev* 13(7): 792-7.

13. Nakabachi, A., Ueoka, R., Oshima, K., Teta, R., Mangoni, A., Gurgui, M., Oldham, N. J., van Echten-Deckert, G., Okamura, K., Yamamoto, K. et al. (2013) 'Defensive bacteriome symbiont with a drastically reduced genome', *Curr Biol* 23(15): 1478-84.

14. Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H. E., Moran, N. A. and Hattori, M. (2006) 'The 160-kilobase genome of the bacterial endosymbiont Carsonella', *Science* 314(5797): 267.

15. Saha, S., Hunter, W. B., Reese, J., Morgan, J. K., Marutani-Hert, M., Huang, H. and Lindeberg, M. (2012) 'Survey of endosymbionts in the Diaphorina citri metagenome and assembly of a Wolbachia wDi draft genome', *PLoS One* 7(11): e50067.

16. Shin, S. W., Kokoza, V., Bian, G., Cheon, H. M., Kim, Y. J. and Raikhel, A. S. (2005) 'REL1, a homologue of Drosophila dorsal, regulates toll antifungal immune pathway in the female mosquito Aedes aegypti', *J Biol Chem* 280(16): 16499-507.

17. Sloan, D. B. and Moran, N. A. (2012) 'Genome reduction and co-evolution between the primary and secondary bacterial symbionts of psyllids', *Mol Biol Evol* 29(12): 3781-92.

18. Ursic-Bedoya, R., Buchhop, J. and Lowenberger, C. (2009) 'Cloning and characterization of Dorsal homologues in the hemipteran Rhodnius prolixus', *Insect Mol Biol* 18(5): 681-9.

19. Zou, Z., Evans, J. D., Lu, Z., Zhao, P., Williams, M., Sumathipala, N., Hetru, C., Hultmark, D. and Jiang, H. (2007) 'Comparative genomic analysis of the Tribolium immune system', *Genome Biol* 8(8): R177.