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Review Article

The Bic-C Family of Developmental Translational Regulators

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Regulation of mRNA translation is especially important during cellular and developmental processes. Many evolutionarily conserved proteins act in the context of multiprotein complexes and modulate protein translation both at the spatial and the temporal levels. Among these, Bicaudal C constitutes a family of RNA binding proteins whose founding member was first identified in *Drosophila* and contains orthologs in vertebrates. We discuss recent advances towards understanding the functions of these proteins in the context of the cellular and developmental biology of many model organisms and their connection to human disease.

1. Introduction

Translational regulation of mRNA distributed asymmetrically in the early *Drosophila* embryo underlies pattern formation and germ cell specification. Furthermore, expression of certain proteins occurs only at definite stages of development. Exquisite, often partially redundant mechanisms of control ensure the coordination of the spatial and temporal expression of proteins with morphogenetic potential. These mechanisms have been reviewed recently [6]. Here we will discuss the case of one of such translational regulators, Bicaudal C (Bic-C), which is evolutionarily conserved, and for which there is recent accumulating functional evidence from both invertebrate and vertebrate model organisms suggesting that Bic-C is a fundamental regulator of cellular processes and an outstanding example of the fascinating complexity of the developmental mechanisms.

2. Materials and Methods

The sequences shown in this paper are listed in Table 1, and they were recovered by running BLAST [7] with the *Drosophila* sequence and the NCBI sequence database, using the Homologene feature at the NCBI. The sequences for the different *Drosophila* species were retrieved from FlyBase [8]. Sequences were aligned with Clustal W [1, 2].

3. Results and Discussion

3.1. Bic-C . The *Bic-C* gene was originally identified during a *Drosophila* screen for maternal genes affecting embryonic polarity [9]. In fact, adult females bearing *Bic-C* mutations in one of their second chromosomes produce embryos exhibiting anterior-posterior defects of severity ranging from anterior defects, to the development of bicaudal embryos composed of as few as four segments arranged as two, mirror-image posterior ends, to embryos that fail to cellularize [3]. This pleiotropy indicates that Bic-C participates in (or influences) many different pathways.

Early work demonstrated that Bic-C is required during oogenesis to establish anterior-posterior polarity in the oocyte [3, 5, 9, 10]. It encodes a 905-amino-acid (aa) RNA binding protein containing two canonical and three noncanonical KH RNA binding domains (KH2, 4 and KH 1, 3, 5, resp., aa 56–524) [3, 11, 12], a C-terminal Sterile Alpha Motif domain (SAM domain, aa 805–868, Prosite) [13], and a region rich in serine and glycine (aa 598–693). In the Bic-C protein, both the region containing the KH domains and the full-length, recombinant protein possess affinity for RNA [14, 15] with the full-length protein exhibiting more selective binding of synthetic probes *in vitro*. RNA binding is likely important to Bic-C function in fruit flies, as a spontaneous mutation (G296R) that affects the third KH domain, decreases RNA affinity *in vitro,* and exhibits

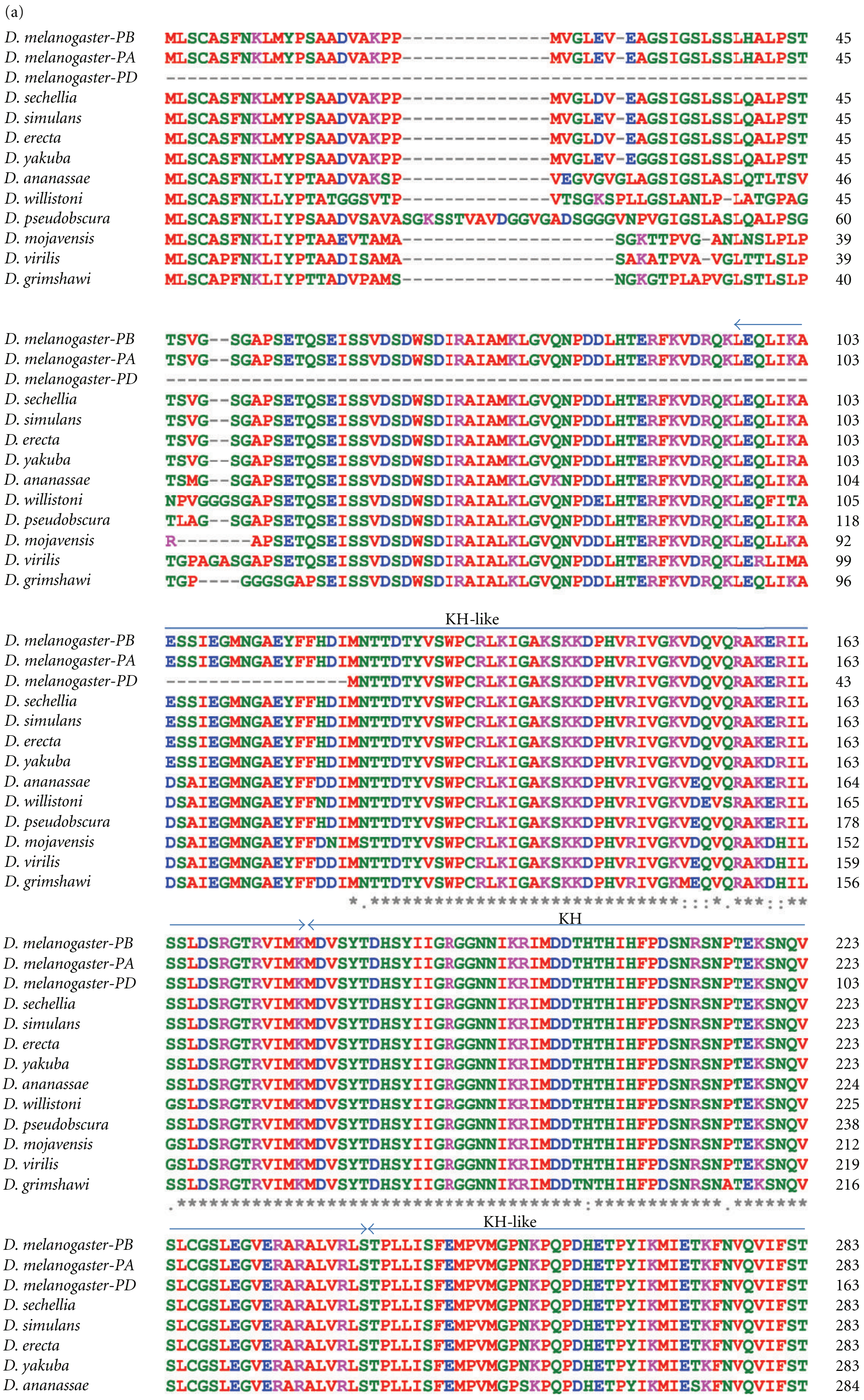
Table 1: Sequences used in this study.

Sequences	Species
Bic-C	
Gene Bank ID	
gi|24584539	*D. melanogaster* B isoform
gi|158300058	*A. gambiae*
gi|13994223	*M. musculus*
gi|109509376	*R. norvegicus*
gi|122937472	*H. sapiens*
gi|114631037	*P. troglodytes*
gi|73953060	*C. familiaris*
gi|194679417	*B. taurus*
gi|292623098	*D. rerio*
gi|212646112	*C. elegans*
gi|118092391	*G. gallus*
FlyBase ID	
FBpp0080362	*D. melanogaster* B isoform
FBpp0080363	*D. melanogaster* D isoform
FBpp0080361	*D. melanogaster* A isoform
FBpp0118127	*D. ananassae*
FBpp0143734	*D. erecta*
FBpp0144300	*D. grimshawi*
FBpp0166588	*D. mojavensis*
FBpp0179414	*D. persimilis*
FBpp0287937	*D. pseudobscura*
FBpp0200128	*D. sechellia*
FBpp0222439	*D. simulans*
FBpp0232468	*D. virilis*
FBpp0253912	*D. willistoni*
FBpp0266309	*D. yakuba*
Not3/5	
Gene Bank ID	
gi|39945962	*Magnaportae oryzae*
gi|85075997	*Neurospora crassa*
gi|19115701	*S. pombe*
gi|19921660	*D. melanogaster*
gi|158299738	*A. gambiae*
gi|22122717	*M. musculus*
gi|34854462	*R. norvegicus*
gi|7657387	*H. sapiens*
gi|114678945	*P. troglodytes*
gi|73946891	*C. familiaris*
gi|119911200	*B. taurus*
gi|53933228	*D. rerio*
gi|133901756	*C. elegans*
gi|238481292	*A. thaliana*
gi|115454389	*O. sativa japonica*
FlyBase ID	
FBpp0085398	*D. melanogaster*
FBpp0125948	*D. ananassae*
FBpp0129398	*D. erecta*
FBpp0147530	*D. grimshawi*
FBpp0160933	*D. mojavensis*
FBpp01852	*D. persimilis*
FBpp0288020	*D. pseudobscura*
FBpp0197981	*D. sechellia*
FBpp0208756	*D. simulans*
Bpp0227498	*D. virilis*
FBpp0243918	*D. willistoni*
FBpp0264455	*D. yakuba*

a strong phenotype *in vivo* [3]. However, this mutation may be affecting more than RNA binding of the whole protein, for example, by perturbing secondary structure in its neighbourhood, as it may be the case for a similar mutation occurring in another KH domain [12]. If this were the case, the severity of the phenotype may be due to the combination of lack of RNA interaction and other defective pathways under Bic-C control in the wild type. The region containing the KH domains in two Bic-C orthologs shows conserved RNA binding capability in the mouse Bicc1 [16] and, surprisingly, not in the *C. elegans* GLD-3 [12].

SAM domains are ancient modules present in most species that are commonly engaged in mediating protein-protein interaction [13, 17] and can multimerize [18, 19]. Multimerization of RNA binding proteins and RNA is most likely the basis for building RNP particles and a target of regulation. Interestingly, the SAM domain of the human BICC1 can form polymers *in vitro* [20] and some KH domains can mediate interactions between proteins [21, 22]. This is also the case for the C. elegans GLD-3 that interacts with the GLD-2 polymerase via its first KH domain [23] therefore it is likely that Bic-C is part of multiprotein complexes such as cellular RNPs. Certain SAM domains have also been implicated in RNA binding, as the case of *Drosophila* Smaug and *S. cerevisiae* Vts1 [24]. Interestingly, among all the *Drosophila* SAM domains, Bic-C contains the one most similar to Smaug's, which includes the critical residues for RNA interaction [25], suggesting the possibility that it may contribute to the Bic-C RNA binding capacity in the cell [17]. Studies of the vertebrate Bic-C homologs, whose targets are largely unknown, have suggested that presence of the SAM domain may mediate association with the P-bodies [26, 27]. Another interesting possibility is that the putative RNA binding and protein-protein interaction capabilities of the SAM domain may be regulated, possibly via posttranslational modifications. In this scenario protein modification in this domain may change the specificity and/or affinity of Bic-C for RNA to switch between protein and RNA binding activities in certain cellular or developmental contexts. Interestingly, a tyrosine residue in position 822 that can be phosphorylated in other SAM domains to regulate their activity is also conserved [28] (Figure 1).

3.2. Evolutionary Conservation of the Bic-C Protein. Bic-C is found in all the sequenced *Drosophila* species and its homologs are virtually identical to each other, except for regions of



(a)

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D. melanogaster-PB      MLSCASFNKLMYPSAADVAKPP--------------MVGLEV-EAGSIGSLSSLHALPST    45
D. melanogaster-PA      MLSCASFNKLMYPSAADVAKPP--------------MVGLEV-EAGSIGSLSSLHALPST    45
D. melanogaster-PD      ------------------------------------------------------------
D. sechellia            MLSCASFNKLMYPSAADVAKPP--------------MVGLDV-EAGSIGSLSSLQALPST    45
D. simulans             MLSCASFNKLMYPSAADVAKPP--------------MVGLEV-EAGSIGSLSSLQALPST    45
D. erecta               MLSCASFNKLMYPSAADVAKPP--------------MVGLDV-EAGSIGSLSSLQALPST    45
D. yakuba               MLSCASFNKLMYPSAADVAKPP--------------MVGLEV-EGGSIGSLSSLQALPST    45
D. ananassae            MLSCASFNKLIYPTAADVAKSP--------------VEGVGVGLAGSIGSLASLQTLTSV    46
D. willistoni           MLSCASFNKLLYPTATGGSVTP--------------VTSGKSPLLGSLANLP-LATGPAG    45
D. pseudobscura         MLSCASFNKLIYPSAADVSAVASGKSSTVAVDGGVGADSGGGVNPVGIGSLASLQALPSG    60
D. mojavensis           MLSCASFNKLIYPTAAEVTAMA--------------------SGKTTPVG-ANLNSLPLP    39
D. virilis              MLSCAPFNKLIYPTAADISAMA--------------------SAKATPVA-VGLTTLSLP    39
D. grimshawi            MLSCAPFNKLIYPTTADVPAMS--------------------NGKGTPLAPVGLSTLSLP    40

D. melanogaster-PB      TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIKA    103
D. melanogaster-PA      TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIKA    103
D. melanogaster-PD      ------------------------------------------------------------
D. sechellia            TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIKA    103
D. simulans             TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIKA    103
D. erecta               TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIKA    103
D. yakuba               TSVG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQLIRA    103
D. ananassae            TSMG--SGAPSETQSEISSVDSDWSDIRAIAMKLGVKNPDDLHTERFKVDRQKLEQLIKA    104
D. willistoni           NPVGGGSGAPSETQSEISSVDSDWSDIRAIALKLGVQNPDELHTERFKVDRQKLEQFITA    105
D. pseudobscura         TLAG--SGAPSETQSEISSVDSDWSDIRAIALKLGVQNPDDLHTERFKVDRQKLEQLIKA    118
D. mojavensis           R-------APSETQSEISSVDSDWSDIRAIALKLGVQNVDDLHTERFKVDRQKLEQLLKA    92
D. virilis              TGPAGASGAPSETQSEISSVDSDWSDIRAIALKLGVQNPDDLHTERFKVDRQKLERLIMA    99
D. grimshawi            TGP----GGGSGAPSEISSVDSDWSDIRAIALKLGVQNPDDLHTERFKVDRQKLEQLIKA    96

                                                    KH-like
D. melanogaster-PB      ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    163
D. melanogaster-PA      ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    163
D. melanogaster-PD      -----------------MNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    43
D. sechellia            ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    163
D. simulans             ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    163
D. erecta               ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKERIL    163
D. yakuba               ESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKDRIL    163
D. ananassae            DSAIEGMNGAEYFFDDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVEQVQRAKERIL    164
D. willistoni           DSAIEGMNGAEYFFNDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDEVSRAKERIL    165
D. pseudobscura         DSAIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVEQVQRAKERIL    178
D. mojavensis           DSAIEGMNGAEYFFDNIMSTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVDQVQRAKDHIL    152
D. virilis              DSAIEGMNGAEYFFDDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKVEQVQRAKDHIL    159
D. grimshawi            DSAIEGMNGAEYFFDDIMNTTDTYVSWPCRLKIGAKSKKDPHVRIVGKMEQVQRAKDHIL    156
                                         *.*****************************:::*.***::**
                                                                     KH
D. melanogaster-PB      SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. melanogaster-PA      SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. melanogaster-PD      SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    103
D. sechellia            SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. simulans             SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. erecta               SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. yakuba               SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    223
D. ananassae            SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    224
D. willistoni           GSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    225
D. pseudobscura         SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    238
D. mojavensis           GSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    212
D. virilis              GSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTHTHIHFPDSNRSNPTEKSNQV    219
D. grimshawi            SSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRIMDDTNTHIHFPDSNRSNATEKSNQV    216
                        .**************************************:************.*******
                                                 KH-like
D. melanogaster-PB      SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. melanogaster-PA      SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. melanogaster-PD      SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    163
D. sechellia            SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. simulans             SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. erecta               SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. yakuba               SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFST    283
D. ananassae            SLCGSLEGVERARALVRLSTPLLISFEMPVMGPSKPQPDHETPYIKMIESKFNVQVIFST    284
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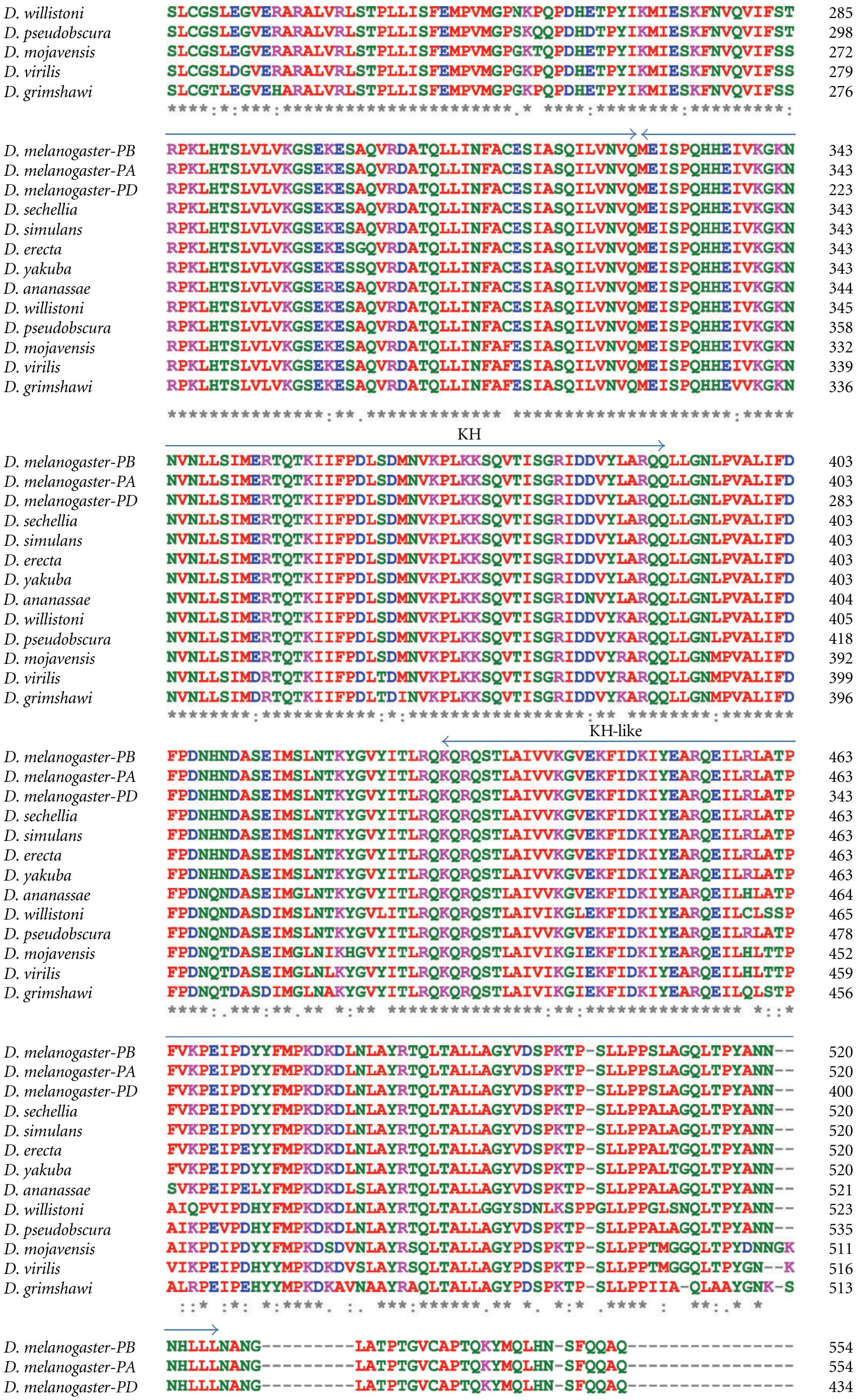
Figure 1: Continued.



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D. willistoni          SLCGSLEGVERARALVRLSTPLLISFEMPVMGPNKPQPDHETPYIKMIESKFNVQVIFST   285
D. pseudobscura        SLCGSLEGVERARALVRLSTPLLISFEMPVMGPSKQQPDHDTPYIKMIESKFNVQVIFST   298
D. mojavensis          SLCGSLEGVERARALVRLSTPLLISFEMPVMGPGKTQPDHETPYIKMIESKFNVQVIFSS   272
D. virilis             SLCGSLDGVERARALVRLSTPLLISFEMPVMGPGKPQPDHETPYIKMIESKFNVQVIFSS   279
D. grimshawi           SLCGTLEGVEHARALVRLSTPLLISFEMPVMGPGKPQPDHETPYIKMIESKFNVQVIFSS   276
                       ****:*:***:**********************.* ****:*********:*********:

D. melanogaster-PB     RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. melanogaster-PA     RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. melanogaster-PD     RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   223
D. sechellia           RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. simulans            RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. erecta              RPKLHTSLVLVKGSEKESGQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. yakuba              RPKLHTSLVLVKGSEKESSQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   343
D. ananassae           RPKLHTSLVLVKGSERESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   344
D. willistoni          RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   345
D. pseudobscura        RPKLHTSLVLVKGSEKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKN   358
D. mojavensis          RPKLHTSLVLVKGSEKESAQVRDATQLLINFAFESIASQILVNVQMEISPQHHEIVKGKN   332
D. virilis             RPKLHTSLVLVKGSEKESAQVRDATQLLINFAFESIASQILVNVQMEISPQHHEIVKGKN   339
D. grimshawi           RPKLHTSLVLVKGSEKESAQVRDATQLLINFAFESIASQILVNVQMEISPQHHEVVKGKN   336

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                                              KH
D. melanogaster-PB     NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. melanogaster-PA     NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. melanogaster-PD     NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   283
D. sechellia           NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. simulans            NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. erecta              NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. yakuba              NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLPVALIFD   403
D. ananassae           NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDNVYLARQQLLGNLPVALIFD   404
D. willistoni          NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYKARQQLLGNLPVALIFD   405
D. pseudobscura        NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYKARQQLLGNLPVALIFD   418
D. mojavensis          NVNLLSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYRARQQLLGNMPVALIFD   392
D. virilis             NVNLLSIMDRTQTKIIFPDLTDMNVKPLKKSQVTISGRIDDVYRARQQLLGNMPVALIFD   399
D. grimshawi           NVNLLSIMDRTQTKIIFPDLTDINVKPLKKSQVTISGRIDDVYKARQQLLGNMPVALIFD   396
                       ********:***********:*:*****************:** ********:*******
                                                                     KH-like
D. melanogaster-PB     FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. melanogaster-PA     FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. melanogaster-PD     FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   343
D. sechellia           FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. simulans            FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. erecta              FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. yakuba              FPDNHNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   463
D. ananassae           FPDNQNDASEIMGLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILHLATP   464
D. willistoni          FPDNQNDASDIMSLNTKYGVLITLRQKQRQSTLAIVIKGLEKFIDKIYEARQEILCLSSP   465
D. pseudobscura        FPDNQNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKFIDKIYEARQEILRLATP   478
D. mojavensis          FPDNQTDASEIMGLNIKHGVYITLRQKQRQSTLAIVIKGIEKFIDKIYEARQEILHLTTP   452
D. virilis             FPDNQTDASEIMGLNLKYGVYITLRQKQRQSTLAIVIKGIEKFIDKIYEARQEILHLTTP   459
D. grimshawi           FPDNQTDASDIMGLNAKYGVYITLRQKQRQSTLAIVIKGIEKFIDKIYEARQEILQLSTP   456
                       ****:.***:**.** *:** ***************:**:*************** *::*

D. melanogaster-PB     FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPSLAGQLTPYANN--   520
D. melanogaster-PA     FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPSLAGQLTPYANN--   520
D. melanogaster-PD     FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPSLAGQLTPYANN--   400
D. sechellia           FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPALAGQLTPYANN--   520
D. simulans            FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPALAGQLTPYANN--   520
D. erecta              FVKPEIPEYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPALTGQLTPYANN--   520
D. yakuba              FVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPALTGQLTPYANN--   520
D. ananassae           SVKPEIPELYFMPKDKDLSLAYRTQLTALLAGYVDSPKTP-SLLPPALAGQLTPYANN--   521
D. willistoni          AIQPVIPDHYFMPKDKDLNLAYRTQLTALLGGYSDNLKSPPGLLPPGLSNQLTPYANN--   523
D. pseudobscura        AIKPEVPDHYFMPKDKDLNLAYRTQLTALLAGYVDSPKTP-SLLPPALAGQLTPYANN--   535
D. mojavensis          AIKPDIPDYYFMPKDSDVNLAYRSQLTALLAGYPDSPKTP-SLLPPTMGGQLTPYDNNGK   511
D. virilis             VIKPEIPDHYYMPKDKDVSLAYRSQLTALLAGYPDSPKTP-SLLPPTMGGQLTPYGN--K   516
D. grimshawi           ALRPEIPEHYYMPKDKAVNAAYRAQLTALLAGYPDSPKTP-SLLPPIIA-QLAAYGNK-S   513
                        ::* :*: *:****. :. ***:******.** *. *:* .**** :  **:.* *

D. melanogaster-PB     NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQAQ---------------   554
D. melanogaster-PA     NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQAQ---------------   554
D. melanogaster-PD     NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQAQ---------------   434
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Figure 1: Continued.

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D. sechellia          NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQTQ----------------    554
D. simulans           NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQTQ----------------    554
D. erecta             NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQTQ----------------    554
D. yakuba             NHLLLNANG---------LATPTGVCAPTQKYMQLHN-SFQQTQ----------------    554
D. ananassae          NHLLLNANGGV---AVGGLATPTGVCAPTQKYMQLHNSAFQQGQ----------------    562
D. willistoni         NHLLLNANASVNGSGGGGLSTPTGICAPTQKYMQMHN-NFQQAQ----------------    566
D. pseudobscura       NHLLLNANAAVG-----GLATPTGICAPTQKYMQLHNSAFQHQQ----------------    574
D. mojavensis         GHMLLGA---------AGLATPTGICAPTQKYMQLHNNNYQPRPLSAIN-----------    551
D. virilis            AHMLLAANVG------VGLTTPTGICAPTQKYMQLHNSSYQPRQVSTMNNLSNCSNNNSS    570
D. grimshawi          HNVLLGNSVG------VGLATPTGICAPTQKYMQLHNSNYQPR-----------------    550
                       ::**             *:****:*********:**  :*

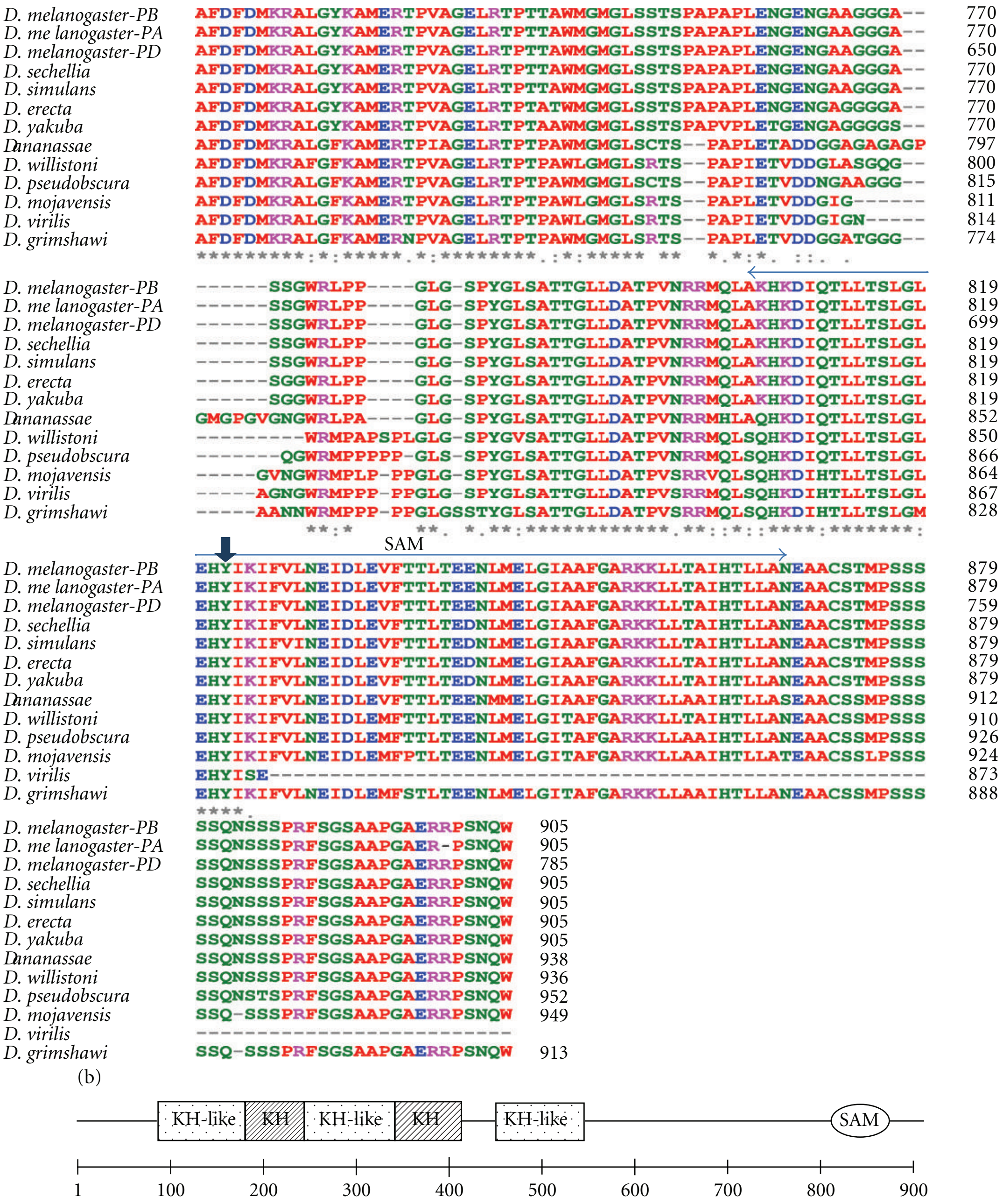
D. melanogaster-PB    ----------------NRSMVAG-----------GQSNNGNYLQVPG------AVAPP--    579
D. melanogaster-PA    ----------------NRSMVAG-----------GQSNNGNYLQVPG------AVAPP--    579
D. melanogaster-PD    ----------------NRSMVAG-----------GQSNNGNYLQVPG------AVAPP--    459
D. sechellia          ----------------NRSMVAG-----------GQNNNGNYLQVPG------AVAP---    578
D. simulans           ----------------NRSMVAG-----------GQNNNGNYLQVPG------AVAP---    578
D. erecta             ----------------GRSMVAG-----------GQSSNGNYLQVPG------AVAP---    578
D. yakuba             ----------------GRSMVAG-----------GQSSNCNYLQVPG------AVAP---    578
D. ananassae          ----------------VGTVQAGR--------PLGVNHNNNYLQVPGGL---GGVAGNG-    594
D. willistoni         ----------------AQQQQQQQQHVQQVAPRQSVVANNNYLQVPGS---KPPLNVG--    605
D. pseudobscura       ----------------LQGQGQVQGP-GQGRPGPVPNHNNNYLQVPGTANAGAGVGAGAG    617
D. mojavensis         NNNNNSSSNNNNNTTTTSNNISNNNNNNNNNINN---NNNNYLQVPGAGLLKPPANLPPT    608
D. virilis            NNNNNNNSNNNNCSSNNNNNISNNSNNINNNNNNNISNNNNYLQVPGSGLLKPPPAPMPS    630
D. grimshawi          ---------------QPLAPVAGTGNG------------------TGTGSVATAPA----    573
                                                                   .*

D. melanogaster-PB    ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    627
D. melanogaster-PA    ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    627
D. melanogaster-PD    ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    507
D. sechellia          ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    626
D. simulans           ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    626
D. erecta             ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    626
D. yakuba             ------------LKPPTVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSS    626
D. ananassae          ---------QLKPLPMNVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLQGAVSSTSS    645
D. willistoni         ------------SNTVNVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLQATVSSTSS    653
D. pseudobscura       MLKPPPPPSSGVGGGMNVSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQLQGAVSSTSS    677
D. mojavensis         ISVTG---------SINLSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYGQVQTTVSSTSS    659
D. virilis            TNVGPP-----PTVGVNLSPRNSCSQNTSGYQSFSSSTTSLEQSYPPYAQVQAAVSSTSS    685
D. grimshawi          --------------AVQLSPRNSCSQNTSGYQSFSSSTTSLEQSYPPFAQVQTVVSSTSS    619
                                       :*****************************:.*:  .******

D. melanogaster-PB    STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLSN-----    675
D. melanogaster-PA    STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLSN-----    675
D. melanogaster-PD    STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLSN-----    555
D. sechellia          STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLGN-----    674
D. simulans           STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLGN-----    674
D. erecta             STAG------SQNRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLGN-----    674
D. yakuba             STAG------SQSRAHYSPDSTYGSEGGGV-GGGGGGGARLGRRLSDGVLLGLGN-----    674
D. ananassae          S-AG------CANRAHYSPDSTYGSEAGSVPGGGGGGGARLGRRLSDGVLLGLGN-----    693
D. willistoni         S-SS------CANRAHYSPDSTYSSEGGGG-GLGMGASARLGRRLSDGVLLGLSNAAGGV    705
D. pseudobscura       T--G------CGSRAHYSPDSTYSSEAGSI-----GGAARLGRRLSDGVLLGLGN-----    719
D. mojavensis         S-SG-------ANRAHYSPDSTYNSEVGGSIVG----AARLGRRLSDGVLLGLSN-----    702
D. virilis            SSAG-------ANRAHYSPDSTYSSEAGSIAGA----AARLGRRLSDGVLLGLGN-----    729
D. grimshawi          SSGGGAGGLGCASRSHYSPDSTYSSEAGSIAG-----AARLGRRLSDGVLLGLGS-----    669
                      :  .        .*:********.** *.        .***************..

D. melanogaster-PB    -SNGGGGNSGG-AHLLPGSAESYRSLHYDLGG--------------------NKHS-GHR    712
D. melanogaster-PA    -SNGGGGNSGG-AHLLPGSAESYRSLHYDLGG--------------------NKHS-GHR    712
D. melanogaster-PD    -SNGGGGNSGG-AHLLPGSAESYRSLHYDLGG--------------------NKHS-GHR    592
D. sechellia          -SSGGGGNSGGGAHLLPGSAESYRSLHYDLGG--------------------NKHS-GHR    712
D. simulans           -SSGGGGNSGGGAHLLPGSAESYRSLHYDLGG--------------------NKHS-GHR    712
D. erecta             -SSGGGGNAGGGAHLLPGSAESYRSLHYDLGG--------------------NKHS-SHR    712
D. yakuba             -SSGGGANSGGGAHLLPGSAESYRSLHYDLGG--------------------NKHS-SHR    712
D. ananassae          -GSSGG------APLLPGSAESYRSLHYDLTGS-------GSISGSGTGAAGNKHTNIHR    739
D. willistoni         GGSMGGAGGGGGAHLLPGSAESYRSLHYDLAG---------------------NGQLTHR    744
D. pseudobscura       --SGGGG-----AHLLPGSAESYRSLHYDLAGG-------GG------GAKHHHQHATHR    759
D. mojavensis         ---ANNGINSGGAHLLPGSAESYRNLHYDLAAVAGKQQQHQQQQQQHQQQQQQQQQQQQR    759
D. virilis            ---ATG----GGAHLLPGSAESYRNLHYDLAA---------------------QQQQQQR    761
D. grimshawi          ---ATT----GGAHLLPGSAESYRNLHYEHQQ----QQQQQQHQQQHHHQQQQQQQQQQR    718
                                  * **********.***:                        :    :*
```

Figure 1: Continued.



```
D. melanogaster-PB  AFDFDMKRALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAPAPLENGENGAAGGGA--  770
D. me lanogaster-PA AFDFDMKRALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAPAPLENGENGAAGGGA--  770
D. melanogaster-PD  AFDFDMKRALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAPAPLENGENGAAGGGA--  650
D. sechellia        AFDFDMKRALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAPAPLENGENGAAGGGA--  770
D. simulans         AFDFDMKRALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAPAPLENGENGAAGGGA--  770
D. erecta           AFDFDMKRALGYKAMERTPVAGELRTPTATWMGMGLSSTSPAPAPLENGENGAGGGGA--  770
D. yakuba           AFDFDMKRALGYKAMERTPVAGELRTPTAAWMGMGLSSTSPAPVPLETGENGAGGGGS--  770
D.ananassae         AFDFDMKRALGFKAMERTPIAGELRTPTPAWMGMGLSCTS--PAPLETADDGGAGAGAGP  797
D. willistoni       AFDFDMKRAFGFKAMERTPVAGELRTPTPAWLGMGLSRTS--PAPIETVDDGLASGQG--  800
D. pseudobscura     AFDFDMKRALGFKAMERTPVAGELRTPTPAWMGMGLSCTS--PAPIETVDDNGAAGGG--  815
D. mojavensis       AFDFDMKRALGFKAMERTPVAGELRTPTPAWLGMGLSRTS--PAPLETVDDGIG------  811
D. virilis          AFDFDMKRALGFKAMERTPVAGELRTPTPAWLGMGLSRTS--PAPIETVDDGIGN-----  814
D. grimshawi        AFDFDMKRALGFKAMERNPVAGELRTPTPAWMGMGLSRTS--PAPLETVDDGGATGGG--  774
                    *********:*:*****.*:********.:*:***** **  *.*:*. ::. .

D. melanogaster-PB  ------SSGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D. me lanogaster-PA ------SSGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D. melanogaster-PD  ------SSGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  699
D. sechellia        ------SSGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D. simulans         ------SSGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D. erecta           ------SGGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D. yakuba           ------SGGWRLPP----GLG-SPYGLSATTGLLDATPVNRRMQLAKHKDIQTLLTSLGL  819
D.ananassae         GMGPGVGNGWRLPA----GLG-SPYGLSATTGLLDATPVNRRMHLAQHKDIQTLLTSLGL  852
D. willistoni       ---------WRMPAPSPLGLG-SPYGVSATTGLLDATPVNRRMQLSQHKDIQTLLTSLGL  850
D. pseudobscura     -------QGWRMPPPPP-GLS-SPYGLSATTGLLDATPVNRRMQLSQHKDIQTLLTSLGL  866
D. mojavensis       -----GVNGWRMPLP-PPGLG-SPYGLSATTGLLDATPVSRRVQLSQHKDIHTLLTSLGL  864
D. virilis          -----AGNGWRMPPP-PPGLG-SPYGLSATTGLLDATPVSRRMQLSQHKDIHTLLTSLGL  867
D. grimshawi        -----AANNWRMPPP-PPGLGSSTYGLSATTGLLDATPVSRRMQLSQHKDIHTLLTSLGM  828
                             **:*     **. *.**:************.**::*::****:*******:

D. melanogaster-PB  EHYIKIFVLNEIDLEVFTTLTEENLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D. me lanogaster-PA EHYIKIFVLNEIDLEVFTTLTEENLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D. melanogaster-PD  EHYIKIFVLNEIDLEVFTTLTEENLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  759
D. sechellia        EHYIKIFVLNEIDLEVFTTLTEDNLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D. simulans         EHYIKIFVINEIDLEVFTTLTEDNLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D. erecta           EHYIKIFVLNEIDLEVFTTLTEDNLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D. yakuba           EHYIKIFVLNEIDLEVFTTLTEDNLMELGIAAFGARKKLLTAIHTLLANEAACSTMPSSS  879
D.ananassae         EHYIKIFVLNEIDLEVFTTLTEENMMELGIAAFGARKKLLAAIHTLLASEAACSSMPSSS  912
D. willistoni       EHYIKIFVLNEIDLEMFTTLTEENLMELGITAFGARKKLLTAIHTLLANEAACSSMPSSS  910
D. pseudobscura     EHYIKIFVLNEIDLEMFTTLTEENLMELGITAFGARKKLLAAIHTLLANEAACSSMPSSS  926
D. mojavensis       EHYIKIFVLNEIDLEMFPTLTEENLMELGITAFGARKKLLAAIHTLLATEAACSSLPSSS  924
D. virilis          EHYISE------------------------------------------------------  873
D. grimshawi        EHYIKIFVLNEIDLEMFSTLTEENLMELGITAFGARKKLLAAIHTLLANEAACSSMPSSS  888
                    ****.

D. melanogaster-PB  SSQNSSSPRFSGSAAPGAERRPSNQW  905
D. me lanogaster-PA SSQNSSSPRFSGSAAPGAER-PSNQW  905
D. melanogaster-PD  SSQNSSSPRFSGSAAPGAERRPSNQW  785
D. sechellia        SSQNSSSPRFSGSAAPGAERRPSNQW  905
D. simulans         SSQNSSSPRFSGSAAPGAERRPSNQW  905
D. erecta           SSQNSSSPRFSGSAAPGAERRPSNQW  905
D. yakuba           SSQNSSSPRFSGSAAPGAERRPSNQW  905
D.ananassae         SSQNSSSPRFSGSAAPGAERRPSNQW  938
D. willistoni       SSQNSSSPRFSGSAAPGAERRPSNQW  936
D. pseudobscura     SSQNSTSPRFSGSAAPGAERRPSNQW  952
D. mojavensis       SSQ-SSSPRFSGSAAPGAERRPSNQW  949
D. virilis          --------------------------
D. grimshawi        SSQ-SSSPRFSGSAAPGAERRPSNQW  913
```

Figure 1: (a) Alignment of Bic-C sequences from 11 *Drosophila* species. Clustal W [1, 2] was used to align sequences extracted from FlyBase. Amino acid (aa) color coding is from Clustal W: red, small aliphatic, hydrophobic, and aromatics; blue, acidic; magenta, basic; green, hydroxyl, sulphydryl, amine, and glycine; grey, unusual aa. Symbols for aa conservation are from Clustal W: (asterisk ∗): positions with a single, fully conserved residue. (Colon :): conservation between groups of strongly similar properties scoring >0.5 in the Gonnet PAM 250 matrix. (Period .): conservation between groups of weakly similar properties scoring ≤0.5 in the Gonnet PAM 250 matrix. All three *D. melanogaster* Bic-C isoforms are shown (*PA*, *PB*, *PD*). The two canonical (KH) and three noncanonical (KH-like) KH RNA-binding modules are indicated (arrows, top). Domain assignment is as in [3] except for the fourth KH-related motif and the SAM domains, that are labelled according to the Pfam database [4]. A conserved, potentially phosphorylated, tyrosine is also indicated (arrowhead, top). Divergence occurs in regions of low complexity in the encoding DNA. Relative to the numbering of the *Drosophila* sequence: insertion at 555, variable length of the serine stretches around aa 623, and between aa 647–658 in the serine-glycine rich region. Further, after aa 715 there seems to be insertions of glutamine stretches of various lengths in *D. mojavensis*, *D. virilise*, and *D. grimshawi*. Finally, *D. ananassae* shows a short insertion at aa 770. The *D. virilise*, sequence results truncated. A TBLASTn search with the C-terminal region of Bic-C from *D. melanogaster* reveals many ESTs with similarity to the *D. melanogaster* sequence, suggesting a possible misannotation (not shown). Another region of possible sequencing misannotation in the *D. virilis* and the *D. mojavensis* Bic-C is italicized and not in bold type. Note that the *Bic-C* gene in *D. melanogaster* has nine mapped introns [5], and there is the possibility that the sequence was misannotated with this respect. (b) Block structure of the *D. melanogaster* Bic-C highlighting the protein motifs described in the text.

low complexity, where there are stretches of adjacent identical amino acids whose number varies in different species, the possible result of evolutionary mechanisms acting on triplet repeats or of stuttering sequencing polymerases (Figure 1).

An alignment of Bic-C orthologs from different animals reveals extensive sequence conservation from aa 83 to 268, (referring to the *Drosophila* sequence). Between aa 269 and 303, the vertebrate proteins lack the acidic residues present in the two Dipterans (*D. melanogaster and Anopheles gambiae*) while the basic residues between aa 281 and 286 are conserved (Figure 2).

Similarly, between aa 417 and 423 the acidic residues are exchanged with a basic (K) or a neutral (G) residues, while the adjacent phenylalanine 424 is changed conservatively into a tyrosine, suggesting that the overall protein folding may be preserved and that the electrostatic environment may be different between the insect and the vertebrate proteins. Since this region contains possible KH-domain-like modules, this may influence their ability to interact with RNA by contributing positive charges that might help retain or stabilize the interaction with RNA. At aa 458, vertebrate sequences diverge from those of *Drosophila*, *Anopheles*, and *Caenorhabditis elegans*. These sequences show blocks of conservation (aa 712–737 and 815–863) interspersed with regions of divergence and one insertion of 38 residues at aa 778. The SAM domain is one such block of conservation, with its phosphorylatable tyrosine [28] that is invariant in all the sequences analysed and the identity (or conservative substitution) of most of the amino acids that contribute to create an environment conducive to RNA binding in the case of Smaug [24].

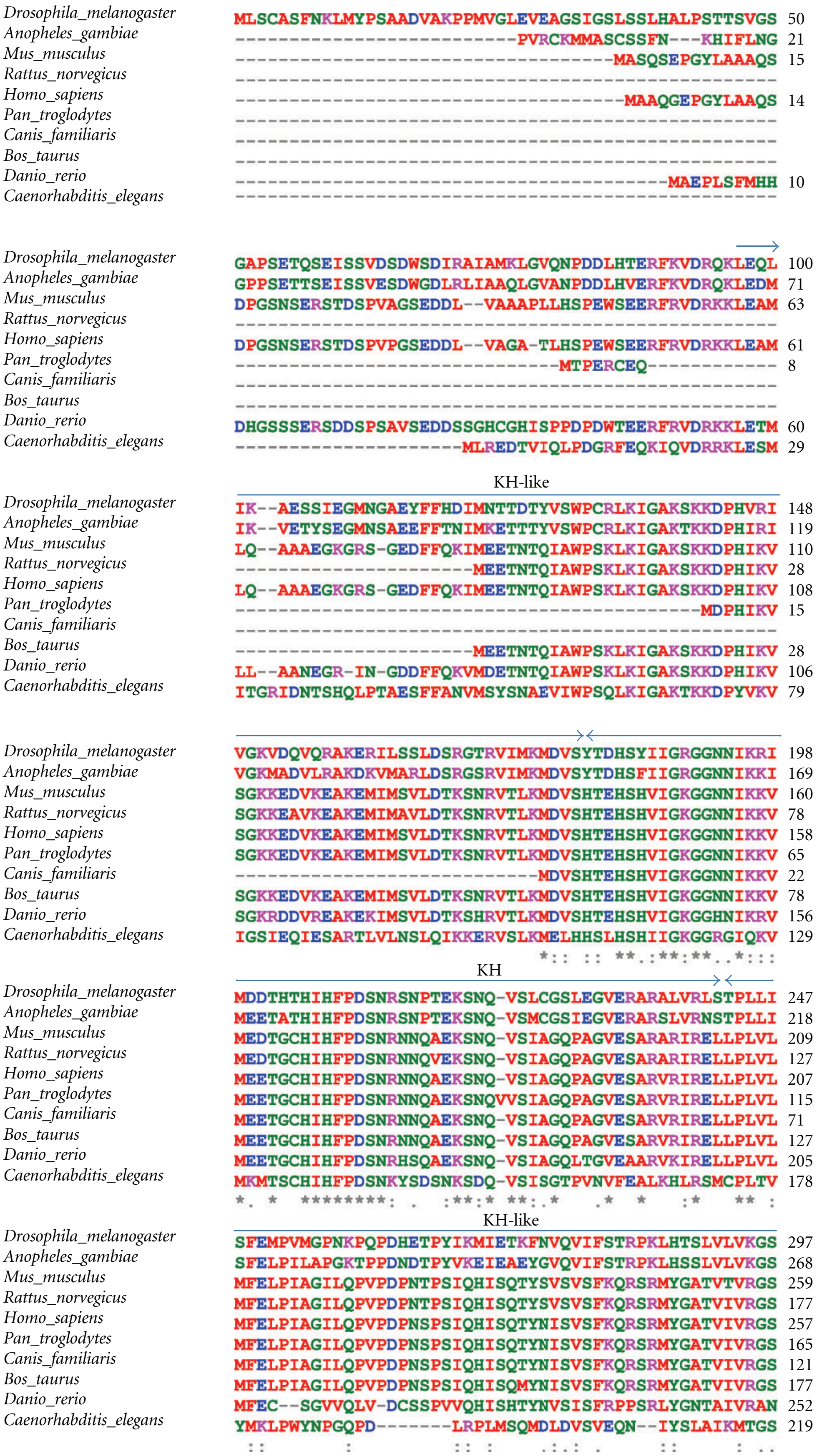
3.3. Bic-C and Translational Regulation. Evidence that Bic-C was involved in control of mRNA translation came first from studies in *Drosophila* where it was observed that Oskar, a well-studied morphogen, was upregulated in ovaries from Bic-C mutated females [14]. The identification of other mRNA targets coimmunoprecipitated with Bic-C yielded the *Bic-C* mRNA itself and several mRNAs encoding factors involved in the Wnt pathway, vesicular trafficking, and organization of the actin cytoskeleton [15]. Bic-C interacts directly with the Not3/5 subunit of the CCR4 deadenylase complex, and it is believed that, when bound to its target RNA, it is able to recruit the deadenylase. This shifts the cellular balance between polyadenylation and deadenylation towards the latter, impairing translation [15]. Since Not3/5 is also evolutionarily conserved, it is discussed below in the perspective of its contribution to the Bic-C complexes.

The other invertebrate family member for which there is substantial functional information is the *C. elegans* GLD-3. GLD-3 is involved in germline development and embryogenesis by regulating the time of expression of developmental factors [23, 29, 30]. GLD-3, via its first KH domain, interacts with GLD-2, a noncanonical polyA polymerase devoid of an RNA interaction domain of its own [23, 30]. Although it was expected that GLD-3 may tether GLD-2 to the RNA, a recent structural study could not find any RNA binding activity for the GLD-3 KH region [12]; therefore further studies are needed to elucidate how GLD-3 participates to *C. elegans* development.

In the *Drosophila* ovary Bic-C is present in cytoplasmic granules enriched for Trailer Hitch (Tral) and Me31B [31, 32], two proteins marking sponge bodies, ovarian organelles related to the repression of mRNA translation [33–35]. Mouse and *Xenopus* Bicc1 in cultured cells are also found within subcellular structures associated with mRNA silencing, the processing granules (P granules, [26, 27, 36]), strongly suggesting that the members of the Bic-C protein family may share a conserved function in translational control. For example, P bodies may destabilize mRNAs via the action of decapping enzymes such as Dcp1 in many tissues undergoing rapid mRNA turnover, while certain yeast mRNAs can be reversibly associated with P-bodies [37]. Further, in metazoans, deadenylation is often the rate-limiting, first step of mRNA decay [38]. While in the kidney, high turnover of certain mRNA may be instrumental to rapidly adapt organ function to the environmental changes, in tissues with a strong “anabolic” activity such as the ovary it would not be surprising to find that some maternal mRNAs are silenced and stored in cellular compartments refractory to translation during oogenesis, to be deployed later in the early embryo. Consistent with the possibility that Bic-C may not function by destabilizing its mRNA targets, no global changes in *Bic-C* mRNA stability were observed in the *Drosophila* ovary, neither by quantitative RT-PCR of ovarian total mRNA nor by *in situ* hybridization (Bic-C negatively regulates its own mRNA) [15]. While there seems to be a mild effect on stability of the polycystic kidney disease 2 (*Pkd2*) mRNA in the kidneys of the *Bicc1*$^{-/-}$ KO mice, in this case, no direct association of this mRNA with the Bicc1 protein was formally demonstrated [27]. It is also possible that only a fraction of the cellular Bic-C pool is involved in destabilization and degradation of mRNA targets, possibly constituting a distinct compartment. This scenario would have escaped detection via traditional biochemical methods because they cannot preserve the integrity of the tissues analyzed. Until more regulatory targets for the Bic-C family members will be identified, validated, and characterized functionally, this current puzzle will remain unanswered.

3.4. Not3/5: An Evolutionarily Conserved Bic-C Partner Affecting mRNA Translation. Not3 is one of the subunits of the CCR4-NOT deadenylase, which is the predominant deadenylase, at least in the yeast *S. cerevisiae* [39–41]. Other subunits include CCR4, CAF1, NOT1-5 [40–44]. In *Drosophila* homologous genes are present for each of these subunits, with the exception of NOT3 and NOT5, for which there is only one gene displaying homology to both proteins [45]. Interestingly, Not3/5 does not contain any known protein domain, as identified via Prosite [46].

Drosophila Not3/5 proteins are virtually identical in 12 species, the differences being concentrated in areas of low-sequence complexity (Figure 3). A BLAST search [7] reveals that besides insects and vertebrates, there are Not3/5 orthologs, in *fungi* (*S. cerevisiae*, *Schizosaccharomyces pombe*, as well as the mushrooms *Laccaria bicolor*, *Coprinopsis*



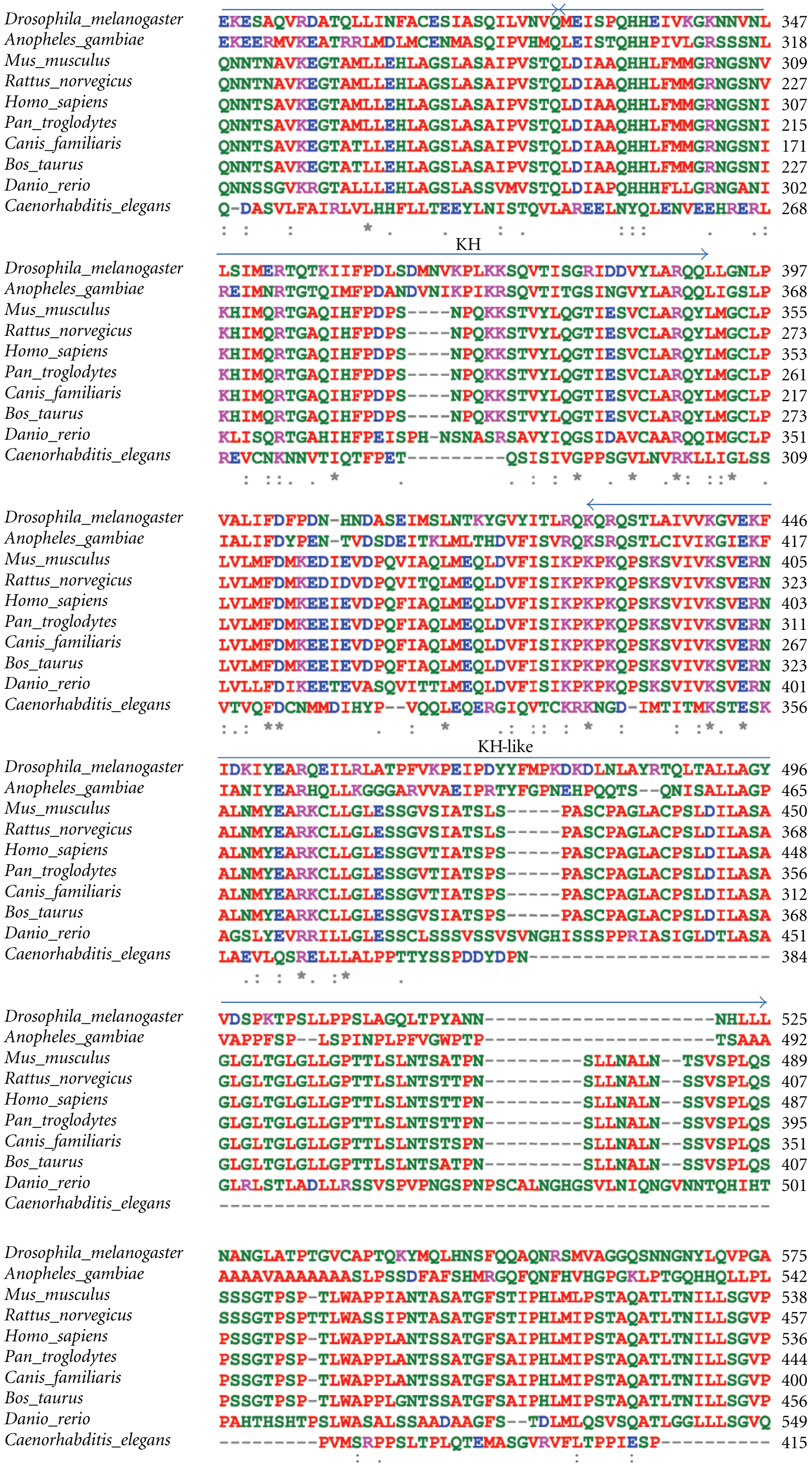
```
Drosophila_melanogaster  MLSCASFNKLMYPSAADVAKPPMVGLEVEAGSIGSLSSLHALPSTTSVGS 50
Anopheles_gambiae        --------------------------PVRCKMMASCSSFN---KHIFLNG 21
Mus_musculus             -----------------------------------MASQSEPGYLAAAQS 15
Rattus_norvegicus        --------------------------------------------------
Homo_sapiens             ------------------------------------MAAQGEPGYLAAQS 14
Pan_troglodytes          --------------------------------------------------
Canis_familiaris         --------------------------------------------------
Bos_taurus               --------------------------------------------------
Danio_rerio              ----------------------------------------MAEPLSFMHH 10
Caenorhabditis_elegans   --------------------------------------------------

Drosophila_melanogaster  GAPSETQSEISSVDSDWSDIRAIAMKLGVQNPDDLHTERFKVDRQKLEQL 100
Anopheles_gambiae        GPPSETTSEISSVESDWGDLRLIAAQLGVANPDDLHVERFKVDRQKLEDM 71
Mus_musculus             DPGSNSERSTDSPVAGSEDDL--VAAAPLLHSPEWSEERFRVDRKKLEAM 63
Rattus_norvegicus        --------------------------------------------------
Homo_sapiens             DPGSNSERSTDSPVPGSEDDL--VAGA-TLHSPEWSEERFRVDRKKLEAM 61
Pan_troglodytes          ------------------------------MTPERCEQ------------ 8
Canis_familiaris         --------------------------------------------------
Bos_taurus               --------------------------------------------------
Danio_rerio              DHGSSSERSDDSPSAVSEDDSSGHCGHISPPDPDWTEERFRVDRKKLETM 60
Caenorhabditis_elegans   ---------------------MLREDTVIQLPDGRFEQKIQVDRRKLESM 29

                                              KH-like
Drosophila_melanogaster  IK--AESSIEGMNGAEYFFHDIMNTTDTYVSWPCRLKIGAKSKKDPHVRI 148
Anopheles_gambiae        IK--VETYSEGMNSAEEFFTNIMKETTTYVSWPCRLKIGAKTKKDPHIRI 119
Mus_musculus             LQ--AAAEGKGRS-GEDFFQKIMEETNTQIAWPSKLKIGAKSKKDPHIKV 110
Rattus_norvegicus        ----------------------MEETNTQIAWPSKLKIGAKSKKDPHIKV 28
Homo_sapiens             LQ--AAAEGKGRS-GEDFFQKIMEETNTQIAWPSKLKIGAKSKKDPHIKV 108
Pan_troglodytes          -------------------------------------------MDPHIKV 15
Canis_familiaris         --------------------------------------------------
Bos_taurus               ----------------------MEETNTQIAWPSKLKIGAKSKKDPHIKV 28
Danio_rerio              LL--AANEGR-IN-GDDFFQKVMDETNTQIAWPSKLKIGAKSKKDPHIKV 106
Caenorhabditis_elegans   ITGRIDNTSHQLPTAESFFANVMSYSNAEVIWPSQLKIGAKTKKDPYVKV 79

Drosophila_melanogaster  VGKVDQVQRAKERILSSLDSRGTRVIMKMDVSYTDHSYIIGRGGNNIKRI 198
Anopheles_gambiae        VGKMADVLRAKDKVMARLDSRGSRVIMKMDVSYTDHSFIIGRGGNNIKKI 169
Mus_musculus             SGKKEDVKEAKEMIMSVLDTKSNRVTLKMDVSHTEHSHVIGKGGNNIKKV 160
Rattus_norvegicus        SGKKEAVKEAKEMIMAVLDTKSNRVTLKMDVSHTEHSHVIGKGGNNIKKV 78
Homo_sapiens             SGKKEDVKEAKEMIMSVLDTKSNRVTLKMDVSHTEHSHVIGKGGNNIKKV 158
Pan_troglodytes          SGKKEDVKEAKEMIMSVLDTKSNRVTLKMDVSHTEHSHVIGKGGNNIKKV 65
Canis_familiaris         ----------------------------MDVSHTEHSHVIGKGGNNIKKV 22
Bos_taurus               SGKKEDVKEAKEMIMSVLDTKSNRVTLKMDVSHTEHSHVIGKGGNNIKKV 78
Danio_rerio              SGKRDDVREAKEKIMSVLDTKSHRVTLKMDVSHTEHSHVIGKGGHNIKRV 156
Caenorhabditis_elegans   IGSIEQIESARTLVLNSLQIKKERVSLKMELHHSLHSHIIGKGGRGIQKV 129
                                                     *:: :: **.:**:**..*:::
                                        KH
Drosophila_melanogaster  MDDTHTHIHFPDSNRSNPTEKSNQ-VSLCGSLEGVERARALVRLSTPLLI 247
Anopheles_gambiae        MEETATHIHFPDSNRSNPTEKSNQ-VSMCGSIEGVERARSLVRNSTPLLI 218
Mus_musculus             MEDTGCHIHFPDSNRNNQAEKSNQ-VSIAGQPAGVESARARIRELLPLVL 209
Rattus_norvegicus        MEDTGCHIHFPDSNRNNQVEKSNQ-VSIAGQPAGVESARARIRELLPLVL 127
Homo_sapiens             MEETGCHIHFPDSNRNNQAEKSNQ-VSIAGQPAGVESARVRIRELLPLVL 207
Pan_troglodytes          MEETGCHIHFPDSNRNNQAEKSNQVVSIAGQPAGVESARVRIRELLPLVL 115
Canis_familiaris         MEETGCHIHFPDSNRNNQAEKSNQ-VSIAGQPAGVESARVRIRELLPLVL 71
Bos_taurus               MEETGCHIHFPDSNRNNQAEKSNQ-VSIAGQPAGVESARVRIRELLPLVL 127
Danio_rerio              MEETGCHIHFPDSNRHSQAEKSNQ-VSIAGQLTGVEAARVKIRELLPLVL 205
Caenorhabditis_elegans   MKMTSCHIHFPDSNKYSDSNKSDQ-VSISGTPVNVFEALKHLRSMCPLTV 178
                         *. *  ********: .  :**:* **:.*   .*  *   :*   ** :
                                           KH-like
Drosophila_melanogaster  SFEMPVMGPNKPQPDHETPYIKMIETKFNVQVIFSTRPKLHTSLVLVKGS 297
Anopheles_gambiae        SFELPILAPGKTPPDNDTPYVKEIEAEYGVQVIFSTRPKLHSSLVLVKGS 268
Mus_musculus             MFELPIAGILQPVPDPNTPSIQHISQTYSVSVSFKQRSRMYGATVTVRGS 259
Rattus_norvegicus        MFELPIAGILQPVPDPNTPSIQHISQTYSVSVSFKQRSRMYGATVIVRGS 177
Homo_sapiens             MFELPIAGILQPVPDPNSPSIQHISQTYNISVSFKQRSRMYGATVIVRGS 257
Pan_troglodytes          MFELPIAGILQPVPDPNSPSIQHISQTYNISVSFKQRSRMYGATVIVRGS 165
Canis_familiaris         MFELPIAGILQPVPDPNSPSIQHISQTYNISVSFKQRSRMYGATVIVRGS 121
Bos_taurus               MFELPIAGILQPVPDPNSPSIQHISQMYNISVSFKQRSRMYGATVIVRGS 177
Danio_rerio              MFEC--SGVVQLV-DCSSPVVQHISHTYNVSISFRPPSRLYGNTAIVRAN 252
Caenorhabditis_elegans   YMKLPWYNPGQPD-------LRPLMSQMDLDVSVEQN--IYSLAIKMTGS 219
                          ::       :         :: :    .:.: .     ::     : ..
```

Figure 2: Continued.



```
Drosophila_melanogaster     EKESAQVRDATQLLINFACESIASQILVNVQMEISPQHHEIVKGKNNVNL 347
Anopheles_gambiae           EKEERMVKEATRRLMDLMCENMASQIPVHMQLEISTQHHPIVLGRSSSNL 318
Mus_musculus                QNNTNAVKEGTAMLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNV 309
Rattus_norvegicus           QNNTNAVKEGTAMLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNV 227
Homo_sapiens                QNNTSAVKEGTAMLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNI 307
Pan_troglodytes             QNNTSAVKEGTAMLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNI 215
Canis_familiaris            QNNTSAVKEGTATLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNI 171
Bos_taurus                  QNNTSAVKEGTATLLEHLAGSLASAIPVSTQLDIAAQHHLFMMGRNGSNI 227
Danio_rerio                 QNNSSGVKRGTALLLEHLAGSLASSVMVSTQLDIAPQHHHFLLGRNGANI 302
Caenorhabditis_elegans      Q-DASVLFAIRLVLHHFLLTEEYLNISTQVLAREELNYQLENVEEHRERL 268
                            : :   :      * .    .    : .        :::     .   .:
                                                      KH
Drosophila_melanogaster     LSIMERTQTKIIFPDLSDMNVKPLKKSQVTISGRIDDVYLARQQLLGNLP 397
Anopheles_gambiae           REIMNRTGTQIMFPDANDVNIKPIKRSQVTITGSINGVYLARQQLIGSLP 368
Mus_musculus                KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 355
Rattus_norvegicus           KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 273
Homo_sapiens                KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 353
Pan_troglodytes             KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 261
Canis_familiaris            KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 217
Bos_taurus                  KHIMQRTGAQIHFPDPS----NPQKKSTVYLQGTIESVCLARQYLMGCLP 273
Danio_rerio                 KLISQRTGAHIHFPEISPH-NSNASRSAVYIQGSIDAVCAARQQIMGCLP 351
Caenorhabditis_elegans      REVCNKNNVTIQTFPET---------QSISIVGPPSGVLNVRKLLIGLSS 309
                              : ::. . *     .         . : : *  . *  .*: ::*  .

Drosophila_melanogaster     VALIFDFPDN-HNDASEIMSLNTKYGVYITLRQKQRQSTLAIVVKGVEKF 446
Anopheles_gambiae           IALIFDYPEN-TVDSDEITKLMLTHDVFISVRQKSRQSTLCIVIKGIEKF 417
Mus_musculus                LVLMFDMKEDIEVDPQVIAQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 405
Rattus_norvegicus           LVLMFDMKEDIDVDPQVITQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 323
Homo_sapiens                LVLMFDMKEEIEVDPQFIAQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 403
Pan_troglodytes             LVLMFDMKEEIEVDPQFIAQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 311
Canis_familiaris            LVLMFDMKEEIEVDPQFIAQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 267
Bos_taurus                  LVLMFDMKEEIEVDPQFIAQLMEQLDVFISIKPKPKQPSKSVIVKSVERN 323
Danio_rerio                 LVLLFDIKEETEVASQVITTLMEQLDVFISIKPKPKQPSKSVIVKSVERN 401
Caenorhabditis_elegans      VTVQFDCNMMDIHYP--VQQLEQERGIQVTCKRKNGD-IMTITMKSTESK 356
                            :.: **        .  :  *    .: :: : *  :    : :*. *
                                                         KH-like
Drosophila_melanogaster     IDKIYEARQEILRLATPFVKPEIPDYYFMPKDKDLNLAYRTQLTALLAGY 496
Anopheles_gambiae           IANIYEARHQLLKGGGARVVAEIPRTYFGPNEHPQQTS--QNISALLAGP 465
Mus_musculus                ALNMYEARKCLLGLESSGVSIATSLS-----PASCPAGLACPSLDILASA 450
Rattus_norvegicus           ALNMYEARKCLLGLESSGVSIATSLS-----PASCPAGLACPSLDILASA 368
Homo_sapiens                ALNMYEARKCLLGLESSGVTIATSPS-----PASCPAGLACPSLDILASA 448
Pan_troglodytes             ALNMYEARKCLLGLESSGVTIATSPS-----PASCPAGLACPSLDILASA 356
Canis_familiaris            ALNMYEARKCLLGLESSGVTIATSPS-----PASCPAGLACPSLDILASA 312
Bos_taurus                  ALNMYEARKCLLGLESSGVSIATSPS-----PASCPAGLACPSLDILASA 368
Danio_rerio                 AGSLYEVRRILLGLESSCLSSSVSSVSVNGHISSSPPRIASIGLDTLASA 451
Caenorhabditis_elegans      LAEVLQSRELLLALPPTTYSSPDDYDPN---------------------- 384
                              .: : *. :*    .

Drosophila_melanogaster     VDSPKTPSLLPPSLAGQLTPYANN---------------------NHLLL 525
Anopheles_gambiae           VAPPFSP--LSPINPLPFVGWPTP---------------------TSAAA 492
Mus_musculus                GLGLTGLGLLGPTTLSLNTSATPN---------SLLNALN--TSVSPLQS 489
Rattus_norvegicus           GLGLTGLGLLGPTTLSLNTSTTPN---------SLLNALN--SSVSPLQS 407
Homo_sapiens                GLGLTGLGLLGPTTLSLNTSTTPN---------SLLNALN--SSVSPLQS 487
Pan_troglodytes             GLGLTGLGLLGPTTLSLNTSTTPN---------SLLNALN--SSVSPLQS 395
Canis_familiaris            GLGLTGLGLLGPTTLSLNTSTSPN---------SLLNALN--SSVSPLQS 351
Bos_taurus                  GLGLTGLGLLGPTTLSLNTSATPN---------SLLNALN--SSVSPLQS 407
Danio_rerio                 GLRLSTLADLLRSSVSPVPNGSPNPSCALNGHGSVLNIQNGVNNTQHIHT 501
Caenorhabditis_elegans      --------------------------------------------------

Drosophila_melanogaster     NANGLATPTGVCAPTQKYMQLHNSFQQAQNRSMVAGGQSNNGNYLQVPGA 575
Anopheles_gambiae           AAAAVAAAAAAAASLPSSDFAFSHMRGQFQNFHVHGPGKLPTGQHHQLLPL 542
Mus_musculus                SSSGTPSP-TLWAPPIANTASATGFSTIPHLMLPSTAQATLTNILLSGVP 538
Rattus_norvegicus           SSSGTPSPTTLWASSIPNTASATGFSTIPHLMIPSTAQATLTNILLSGVP 457
Homo_sapiens                PSSGTPSP-TLWAPPLANTSSATGFSAIPHLMIPSTAQATLTNILLSGVP 536
Pan_troglodytes             PSSGTPSP-TLWAPPLANTSSATGFSAIPHLMIPSTAQATLTNILLSGVP 444
Canis_familiaris            PSSGTPSP-TLWAPPLANTSSATGFSAIPHLMIPSTAQATLTNILLSGVP 400
Bos_taurus                  PSSGTPSP-TLWAPPLGNTSSATGFSAIPHLMIPSTAQATLTNILLSGVP 456
Danio_rerio                 PAHTHSHTPSLWASALSSAADAAGFS--TDLMLQSVSQATLGGLLLSGVQ 549
Caenorhabditis_elegans      ---------PVMSRPPSLTPLQTEMASGVRVFLTPPIESP---------- 415
                                        : .                 :    :
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Figure 2: Continued.

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Drosophila_melanogaster     VAPPLKPPTVSPRN--------------------------------SCSQ 593
Anopheles_gambiae           SLPPGLERTVPGGSS-----------------AGKMNHLSSPHLLLTVSQ 575
Mus_musculus                TYGHT-APSPPPGLTPVDVHINSMQTEGKNISASINGHVQPANMKYGPLS 587
Rattus_norvegicus           TYGHT-APSPPPGLTPVDVHINSMQTEGKNISASINGHVQPPNMKYGPLS 506
Homo_sapiens                TYGHT-APSPPPGLTPVDVHINSMQTEGKKISAALNGHAQSPDIKYGAIS 585
Pan_troglodytes             TYGHT-APSPPPGLTPVDVHINSMQTEGKKISAALNGHAQSPNIKYGAIS 493
Canis_familiaris            TYGHT-APSPPPGLTPVDVHINTMQTEGKKISASLNGHAQSPNIKYGAIP 449
Bos_taurus                  TYGHT-APSPPPGLTPVDVHINTMQAEGKKISAALNGHTQSPSLKYGAIS 505
Danio_rerio                 SQAHTHTPSLPPGLAPIHKTVS---------AEHLNGHLASS--VYSRIS 588
Caenorhabditis_elegans      --------------------------------------------------


Drosophila_melanogaster     N-------TSGYQSFSSSTTSLEQSYPPYAQLPGTVSSTSSSTAGSQNRA 636
Anopheles_gambiae           NSSHNDIHSSGYQSLNCSSNSLDQQFQSNSSASGSVSQVSSNSLLNNSPD 625
Mus_musculus                TSSLGEKVLSSNHGDPSMQTAGPEQASPKSNSVEGCNDAFVEVGMPRSPS 637
Rattus_norvegicus           TSSLGEKVLSSNHGDPSMQTAGPEQASPKSNSVEGCNDAFVEVGMPRSPS 556
Homo_sapiens                TSSLGEKVLSANHGDPSIQTSGSEQTSPKSSPTEGCNDAFVEVGMPRSPS 635
Pan_troglodytes             TSSLGEKVLSANHGDPSIQTSGSEQTSPKSSPTEGCNDAFVEVGMPRSPS 543
Canis_familiaris            TSSLGEKVLSGNHGDPSRQTTGPEQASPKSNPTEGCNDAFVEVGMPRSPS 499
Bos_taurus                  TSSLGEKVLSANHGDPSRQTAGSEQTSPKSNPTEGCNDAFVEVGMPRSPS 555
Danio_rerio                 SVSL-----NSAHCDTAQEGIGHTQSEAKS--TDEGSDTFVEVGMPRSPS 631
Caenorhabditis_elegans      ------------------KSPDPEDSPLAASILKGAKDISKNSDIWKKKS 447
                                                    .    :      ..   .    ..

Drosophila_melanogaster     HYSP--DSTYGSEGGGVGGGGGGGARLGRRLSDGVLLGLSNSNGGGGNSG 684
Anopheles_gambiae           HQSPGAAGTSGLNRCRLSVCTPESPHYQSELEQRTPLAFEQKVG-----V 670
Mus_musculus                HSGNAGDLKQMLGASKVSCAKRQTVELLQGTKNSHLHGTDRLLSDPELSA 687
Rattus_norvegicus           HSGNAGDLKQMLGPSKVSCAKRQTVELLQGTKNSHLHSTDRLLSDTELSA 606
Homo_sapiens                HSGNAGDLKQMMCPSKVSCAKRQTVELLQGTKNSHLHSTDRLLSDPELSA 685
Pan_troglodytes             HSGNAGDLKQMMCPSKVSCAKRQTVELLQGTKNSHLHSTDRLLSDPELSA 593
Canis_familiaris            HSGNAGDLKQMMGPSKVSCAKRQTVELLQGTKNSHLHSTDRLLSDPELST 549
Bos_taurus                  HSGNAGDLKQMMGPSKVACAKRQTVELLQGTKNSHLHSTDRLLSDPELSA 605
Danio_rerio                 HSANGSELKQMLASCTVSPGKRQTVELLQRTKNTLLH-VECVLAD----S 676
Caenorhabditis_elegans      KADRG--------------------------------------------- 452
                            :

Drosophila_melanogaster     GAHLLPGSAESYRSLHYDLGGNK--------------HSGHRAFDFDMKR 720
Anopheles_gambiae           VRRCLPVHLKRLTVLGNHLQSS---------------LADTFLFNLDPRV 705
Mus_musculus                TESPLADKKAPGSERAAERAAAAQQKSERARLASQPTYVHMQAFDYEQKK 737
Rattus_norvegicus           TESPLADKKAPGSERAAERAAAAQQNSERARLASQPPYVHMQAFDYEQKK 656
Homo_sapiens                TESPLADKKAPGSERAAERAAAAQQNSERAHLAPRSSYVNMQAFDYEQKK 735
Pan_troglodytes             TESPLADKKAPGSERAAERAAAAQQNSERAHLAPRSSYVNMQAFDYEQKK 643
Canis_familiaris            TESPLADKKAPGSERAAERAAAAQQNSERARLAPRPSYVNMQAFDYEQKK 599
Bos_taurus                  AESPLADKKAPGSERAAER--AAQQNNERARLAPRPSYVNMQAFDYEQKK 653
Danio_rerio                 DDNPMTDKRAPGSERAAER-----------RLAP-----HMQAFDYEKKK 710
Caenorhabditis_elegans      -----------------------------------------------EML 455


Drosophila_melanogaster     ALGYKAMERTPVAGELRTPTTAWMGMGLSSTSPAP--------------- 755
Anopheles_gambiae           VAGYKAMHMSPQQGEIRTPTLSWQGLGLSQSSPAPLE------------- 742
Mus_musculus                LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 787
Rattus_norvegicus           LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 706
Homo_sapiens                LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 785
Pan_troglodytes             LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 693
Canis_familiaris            LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 649
Bos_taurus                  LLATKAMLKKPVVTEVRTPTNTWSGLGFSKSMPAETIKELRRANHVSYKP 703
Danio_rerio                 LLATKAMLKKPVVTEIRTPTNTWSGLGFSKSMPAESIKELRRAHHVPYKP 760
Caenorhabditis_elegans      IKATQAIFDDSVLSSPRYPTDLWSGYGFSSSLPADLLKGMMDLSTNEPST 505
                              . :*:   .   . * **  * * *:*.: **

Drosophila_melanogaster     ------------APLENGENGAAGGGASSGWRLPP--------------- 778
Anopheles_gambiae           ----ACDLSWANTSSSSSTGGGRDGGGGSGCANTS--------------- 773
Mus_musculus                TMTTAYEGSSLSLSRSSSREHLASGSESDNWRDRN------GIGPMGHSE 831
Rattus_norvegicus           TMTTAYEGSSLSLSRSSSREHLASGSESDNWRDRN------GIGPMGHSE 750
Homo_sapiens                TMTTTYEGSSMSLSRSNSREHLGGGSESDNWRDRN------GIGPGSHSE 829
Pan_troglodytes             TMTTTYEGSSMSLSRSNSREHLGSGSESDNWRDRN------GIGPGSHSE 737
Canis_familiaris            TMTTTFEGSSMSLSRSNSREHLGSGSESDNWRDRN------GIGPPSPSE 693
Bos_taurus                  TMTTTFEGSSMSLSRSNSREHLGSGSESDNWRDRN------GIGPASHGE 747
Danio_rerio                 SMGTTYEDSHLSMSHSGIQEGLINDTKSDNWGDLNGNVNINGNGPSGNSE 810
Caenorhabditis_elegans      NGPPMMNHSQRGLCSVREEDEELSDFSASSTNYGMS-------------- 541
                                                   ..  ...
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Figure 2: Continued.

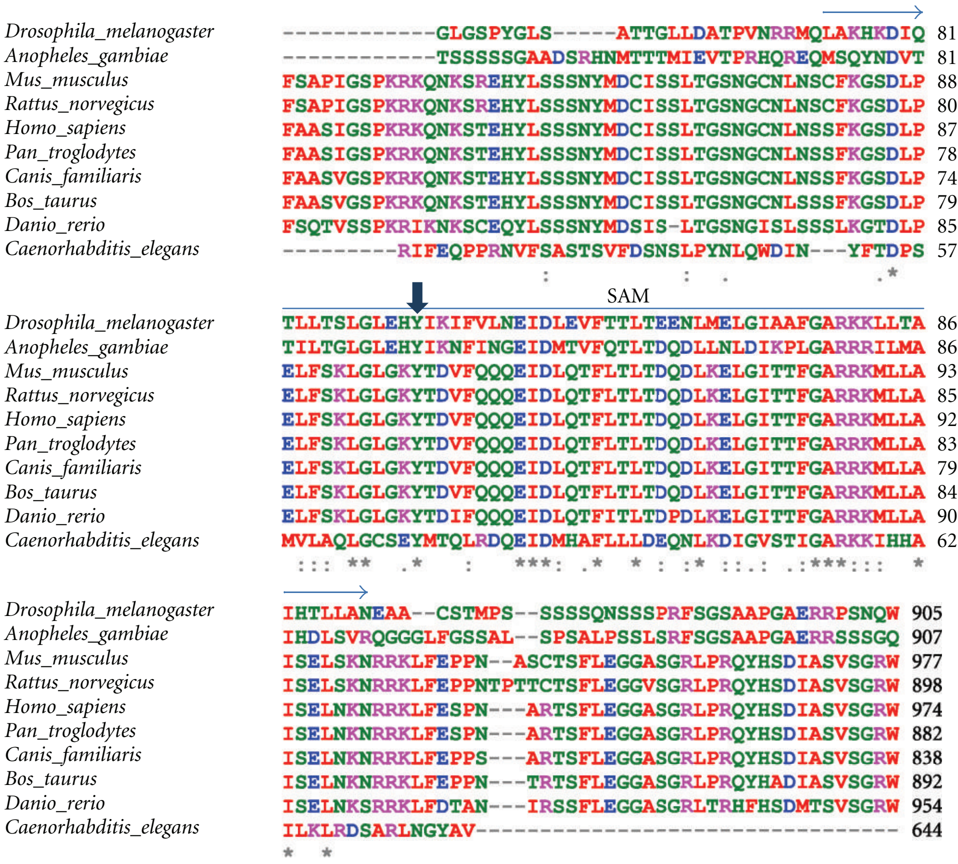


Figure 2: Bic-C orthologs. Clustal W [1, 2] was used to align sequences extracted from the NCBI sequence database. As in Figure 1, the two canonical (KH) and three noncanonical (KH-like) KH RNA-binding modules are indicated (arrows, top). Domain assignment is as in [3] except for the fourth KH-related motif and the SAM domains, that are labelled according to the Pfam database [4]. A conserved, potentially phosphorylated, tyrosine is also indicated (arrowhead, top). Amino acid (aa) color-coding is from Clustal W: red, small aliphatic, hydrophobic and aromatics; blue, acidic; magenta, basic; green, hydroxyl, sulphydryl, amine, and glycine; grey, unusual aa. Symbols for aa conservation are from Clustal W: (asterisk $*$): positions with a single, fully conserved residue. (Colon :): conservation between groups of strongly similar properties-scoring >0.5 in the Gonnet PAM 250 matrix. (Period .): conservation between groups of weakly similar properties-scoring ≤0.5 in the Gonnet PAM 250 matrix. Highlighted yellow: residues that contribute to RNA binding in the Smaug protein. Grey highlight denotes mild (versus strong) basic charges. Light blue highlights a charged aa in a conserved position, but an opposite electrical charge. The *Gallus gallus* genome also contains a predicted sequence with extensive homology to Bic-C (Table 1) and with a long extension at the N terminal end. Since there is no experimental evidence of the true starting methionine we did not include it in this alignment.

cinerea, and *Schizophyllum commune*), vascular plants (e.g., *Arabidopsis thaliana*, *Oryza sativa*, *Vitis vinifera*, and *Ustilago maydis*), and mosses (*Physcomitrella patens*). An alignment of complete sequences is shown in Figure 3. The conservation is highest at the N- and C-terminus of the protein (aa 1–238 and 680–844, with reference to the *Drosophila* sequence) where all the family members show extensive identity. Between aa 330 and 679 the sequences diverge with the orthologues from the two insects (*D. melanogaster* and *Anopheles gambiae*), the *fungi*, the higher eukaryotes, and the plants being more similar with each other than with members of a different group. Notably, the vertebrate sequences, with the exception of zebrafish that contains various small deletions, have blocks of almost complete identity in this region (Figure 4). The partial divergence in the central region of Not3/5 is likely due to the fact that the *Drosophila* gene is homologous to both the NOT3 and NOT5 genes and likely plays the functional roles of both yeast proteins, [45] a seemingly unique feature of *Drosophila [47]*. Not3/5 was recovered in a two-hybrid screen for proteins interacting with *Drosophila* Bic-C, and multiple pieces of evidence support the existence of this interaction *in vivo*: there is genetic interaction between *Bic-C* and *twin*, the *Drosophila* gene encoding for CCR4; other subunits of the CCR4-NOT complex can be coimmunoprecipitated with Bic-C from ovary extracts and the Bic-C target mRNAs that were tested were found with longer polyA tails in *Bic-C* mutants [15]. Although one study of vertebrate models could not detect differences in polyadenylation in a presumptive Bic-C target [27], due to the high homology of the Bic-C and NOT orthologs it is possible that Bic-C from other species can interact with NOT homologs and, possibly, other subunits of the deadenylase complex. These may contribute to the interaction only in the context of the assembled complex and

```
D. melanogaster  MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. sechellia     MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. simulans      MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. erecta        MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. yakuba        MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. persimilis    MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. pseudobscura  MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. grimshawi     MAATRKLQGEIDRCLKKVGEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. virilis       MAATRKLQGEIDRCLKKVGEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. mojavensis    MAATRKLQGEIDRCLKKVGEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. willistoni    MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
D. ananassae     MAATRKLQGEIDRCLKKVAEGVETFEDIWKKVHNATNTNQKQKHLQEKYEADLKKEIKKL 60
                 ******************.*****************************************

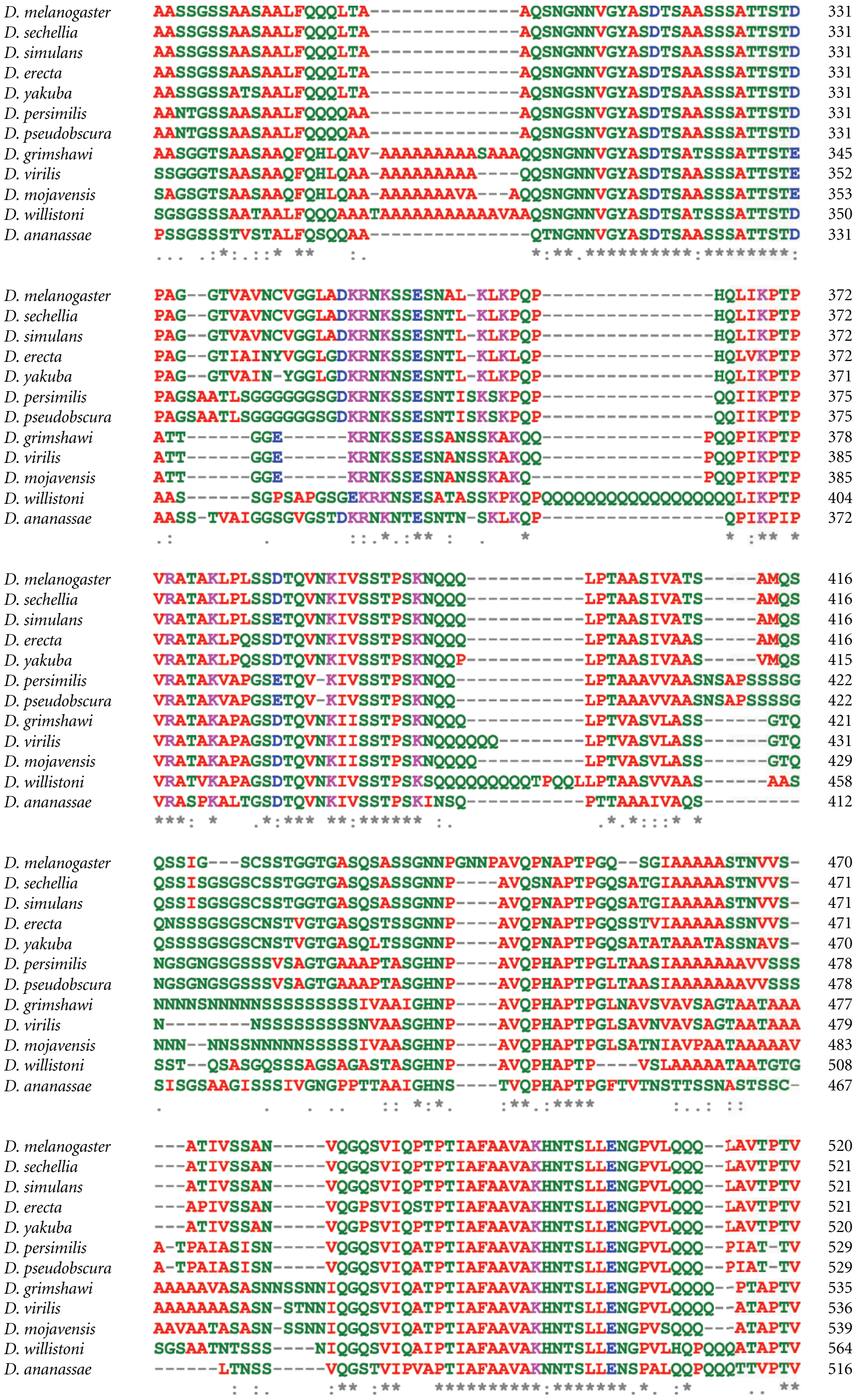
D. melanogaster  QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. sechellia     QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. simulans      QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. erecta        QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. yakuba        QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. persimilis    QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. pseudobscura  QRLRDQIKSWIASAEIKDKSSLLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. grimshawi     QRLRDQIKSWIASAEIKDKSALLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. virilis       QRLRDQIKSWIASAEIKDKSALLENRRLIETASCQMERFKVVERETKTKAYSKEGLGAAQ 120
D. mojavensis    QRLRDQIKSWIASAEIKDKSALLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. willistoni    QRLRDQIKSWIASAEIKDKSALLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
D. ananassae     QRLRDQIKSWIASAEIKDKSALLENRRLIET---QMERFKVVERETKTKAYSKEGLGAAQ 117
                 ********************:**********   **************************

D. melanogaster  KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
D. sechellia     KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
D. simulans      KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
D. erecta        KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
D. yakuba        KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
D. persimilis    KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRAKL 177
D. pseudobscura  KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRAKL 177
D. grimshawi     KMDPAQRIKDHARNWLTNSISALQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRSKL 177
D. virilis       KMDPAQRIKDHARNWLTGSISTLQIQIDQYESEIESLLAGKKKRVDRDKQERMDDLRSKL 180
D. mojavensis    KMDPAQRIKDHARNWLTGSISTLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRSKL 177
D. willistoni    KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRSKL 177
D. ananassae     KMDPAQRIKDDARNWLTSSISSLQIQIDQYESEIESLLAGKKKRLDRDKQERMDDLRGKL 177
                 **********.******.***:**********************:************.**

D. melanogaster  DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. sechellia     DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. simulans      DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. erecta        DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. yakuba        DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. persimilis    DRHKFHITKLETLLRLLDNDGVEADQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. pseudobscura  DRHKFHITKLETLLRLLDNDGVEADQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. grimshawi     DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. virilis       DRHKFHITKLETLLRLLDNDGVEADQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 240
D. mojavensis    DRHKFHITKLETLLRLLDNDGVEADQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. willistoni    DRHKFHITKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQEPDFEENEFIYDDIIGLD 237
D. ananassae     DRHKFHISKLETLLRLLDNDGVEAEQVNKIKDDVEYYIDSSQDPDFEENEFIYDDIIGLD 237
                 *******:****************:*****************:*****************

D. melanogaster  EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILNTSSQ------------G 285
D. sechellia     EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILNTSSQ------------G 285
D. simulans      EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILNTSSQ------------G 285
D. erecta        EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILNTSSQ------------G 285
D. yakuba        EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILNASSQ------------G 285
D. persimilis    EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPASVQ------------A 285
D. pseudobscura  EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPASVQ------------A 285
D. grimshawi     EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQILPSSSS-----------SG 286
D. virilis       EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPSSSTQPQ---SAMAGSS 297
D. mojavensis    EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPSSSSSAQQQQTSTAGSS 297
D. willistoni    EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPSGASSGG-------GSS 290
D. ananassae     EVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQVLPPSMP-----------VA 286
                 ****************************************:* ..              .
```

Figure 3: Continued.



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D. melanogaster  AASSGSSAASAALFQQQLTA--------------AQSNGNNVGYASDTSAASSSATTSTD 331
D. sechellia     AASSGSSAASAALFQQQLTA--------------AQSNGNNVGYASDTSAASSSATTSTD 331
D. simulans      AASSGSSAASAALFQQQLTA--------------AQSNGNNVGYASDTSAASSSATTSTD 331
D. erecta        AASSGSSAASAALFQQQLTA--------------AQSNGNNVGYASDTSAASSSATTSTD 331
D. yakuba        AASSGSSATSAALFQQQLTA--------------AQSNGSNVGYASDTSAASSSATTSTD 331
D. persimilis    AANTGSSAASAALFQQQQAA--------------AQSNGSNVGYASDTSAASSSATTSTD 331
D. pseudobscura  AANTGSSAASAALFQQQQAA--------------AQSNGSNVGYASDTSAASSSATTSTD 331
D. grimshawi     AASGGTSAASAAQFQHLQAV-AAAAAAAAASAAAQQSNGNNVGYASDTSATSSSATTSTE 345
D. virilis       SSGGGTSAASAAQFQHLQAA-AAAAAAAAA----QQSNGNNVGYASDTSAASSSATTSTE 352
D. mojavensis    SAGSGTSAASAAQFQHLQAA-AAAAAAAVA---AQQSNGNNVGYASDTSAASSSATTSTE 353
D. willistoni    SGSGSSSAATAALFQQQAAATAAAAAAAAAAAVAAQSNGNNVGYASDTSATSSSATTSTD 350
D. ananassae     PSSGSSSTVSTALFQSQQAA---------------QTNGNNVGYASDTSAASSSATTSTD 331
                 ... .:*:.::* **   :.               *:**.**********:********:

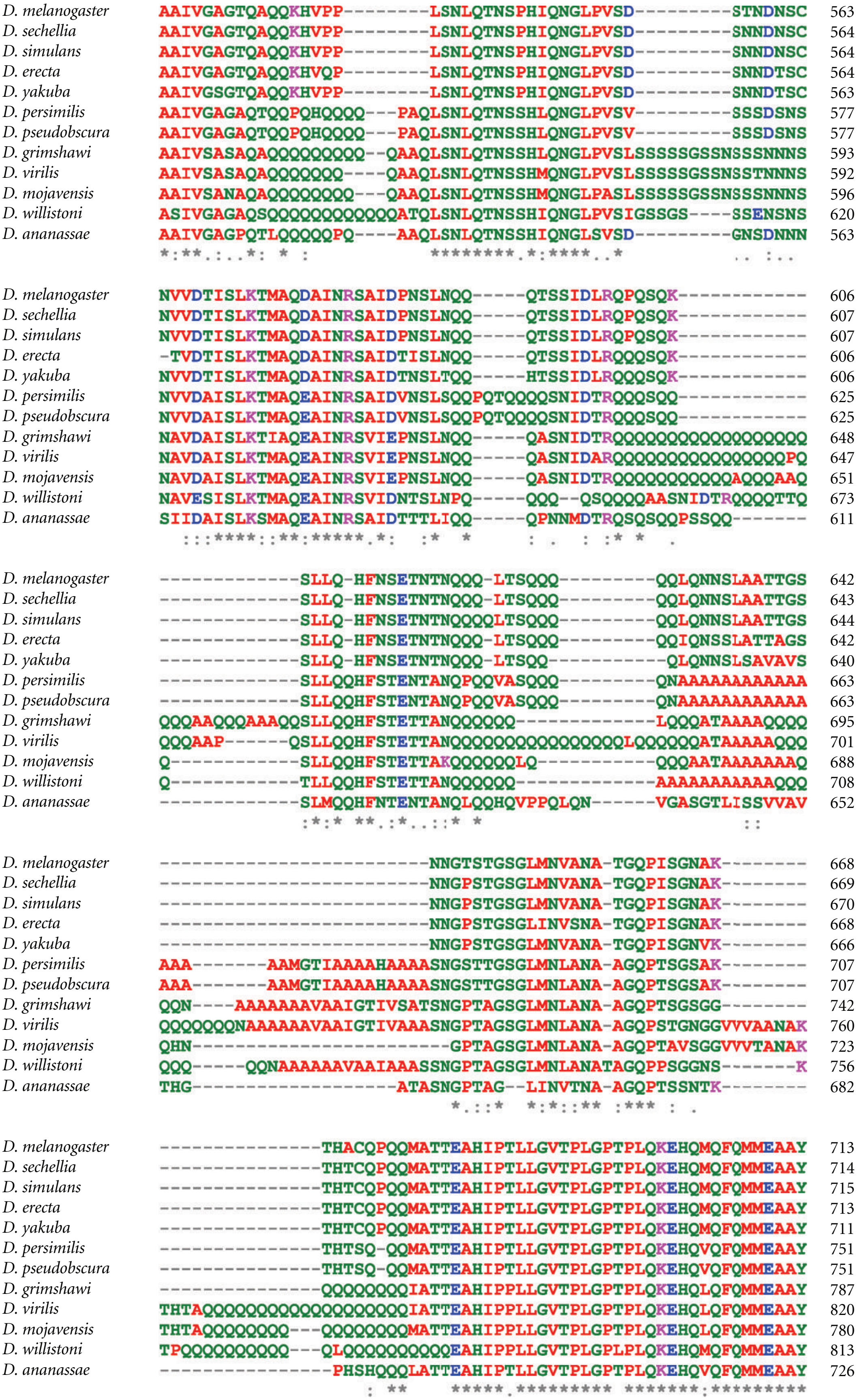
D. melanogaster  PAG--GTVAVNCVGGLADKRNKSSESNAL-KLKPQP----------------HQLIKPTP 372
D. sechellia     PAG--GTVAVNCVGGLADKRNKSSESNTL-KLKPQP----------------HQLIKPTP 372
D. simulans      PAG--GTVAVNCVGGLADKRNKSSESNTL-KLKPQP----------------HQLIKPTP 372
D. erecta        PAG--GTIAINYVGGLGDKRNKSSESNTL-KLKLQP----------------HQLVKPTP 372
D. yakuba        PAG--GTVAIN-YGGLGDKRNKNSESNTL-KLKPQP----------------HQLIKPTP 371
D. persimilis    PAGSAATLSGGGGGGSGDKRNKSSESNTISKSKPQP----------------QQIIKPTP 375
D. pseudobscura  PAGSAATLSGGGGGGSGDKRNKSSESNTISKSKPQP----------------QQIIKPTP 375
D. grimshawi     ATT------GGE------KRNKSSESSANSSKAKQQ---------------PQQPIKPTP 378
D. virilis       ATT------GGE------KRNKSSESNANSSKAKQQ---------------PQQPIKPTP 385
D. mojavensis    ATT------GGE------KRNKSSESNANSSKAKQ----------------PQQPIKPTP 385
D. willistoni    AAS------SGPSAPGSGEKRKNSESATASSKPKQPQQQQQQQQQQQQQQQQQQLIKPTP 404
D. ananassae     AASS-TVAIGGSGVGSTDKRNKNTESNTN-SKLKQP-----------------QPIKPIP 372
                 .:        .       ::.*.:** :  .   *                  * :** *

D. melanogaster  VRATAKLPLSSDTQVNKIVSSTPSKNQQQ-----------LPTAASIVATS-----AMQS 416
D. sechellia     VRATAKLPLSSDTQVNKIVSSTPSKNQQQ-----------LPTAASIVATS-----AMQS 416
D. simulans      VRATAKLPLSSETQVNKIVSSTPSKNQQQ-----------LPTAASIVATS-----AMQS 416
D. erecta        VRATAKLPQSSDTQVNKIVSSTPSKNQQQ-----------LPTAASIVAAS-----AMQS 416
D. yakuba        VRATAKLPQSSDTQVNKIVSSTPSKNQQP-----------LPTAASIVAAS-----VMQS 415
D. persimilis    VRATAKVAPGSETQV-KIVSSTPSKNQQ------------LPTAAAVVAASNSAPSSSSG 422
D. pseudobscura  VRATAKVAPGSETQV-KIVSSTPSKNQQ------------LPTAAAVVAASNSAPSSSSG 422
D. grimshawi     VRATAKAPAGSDTQVNKIISSTPSKNQQQ-----------LPTVASVLASS------GTQ 421
D. virilis       VRATAKAPAGSDTQVNKIISSTPSKNQQQQQQ--------LPTVASVLASS------GTQ 431
D. mojavensis    VRATAKAPAGSDTQVNKIISSTPSKNQQQQ----------LPTVASVLASS------GTQ 429
D. willistoni    VRATVKAPAGSDTQVNKIVSSTPSKSQQQQQQQQQTPQQLLPTAASVVAAS------AAS 458
D. ananassae     VRASPKALTGSDTQVNKIVSSTPSKINSQ-----------PTTAAAIVAQS--------- 412
                 ***: *   .*:*** **:****** :.             .*.*:::* *

D. melanogaster  QSSIG---SCSSTGGTGASQSASSGNNPGNNPAVQPNAPTPGQ--SGIAAAAASTNVVS- 470
D. sechellia     QSSISGSGSCSSTGGTGASQSASSGNNP----AVQSNAPTPGQSATGIAAAAASTNVVS- 471
D. simulans      QSSISGSGSCSSTGGTGASQSASSGNNP----AVQPNAPTPGQSATGIAAAAASTNVVS- 471
D. erecta        QNSSSGSGSCNSTVGTGASQSTSSGNNP----AVQPNAPTPGQSSTVIAAAAASSNVVS- 471
D. yakuba        QSSSSGSGSCNSTVGTGASQLTSSGNNP----AVQPNAPTPGQSATATAAATASSNAVS- 470
D. persimilis    NGSGNGSGSSSVSAGTGAAAPTASGHNP----AVQPHAPTPGLTAASIAAAAAAAVVSSS 478
D. pseudobscura  NGSGNGSGSSSVSAGTGAAAPTASGHNP----AVQPHAPTPGLTAASIAAAAAAAVVSSS 478
D. grimshawi     NNNNSNNNNNSSSSSSSSSIVAAIGHNP----AVQPHAPTPGLNAVSVAVSAGTAATAAA 477
D. virilis       N--------NSSSSSSSSSNVAASGHNP----AVQPHAPTPGLSAVNVAVSAGTAATAAA 479
D. mojavensis    NNN--NNSSNNNNNSSSSSIVAASGHNP----AVQPHAPTPGLSATNIAVPAATAAAAAV 483
D. willistoni    SST--QSASGQSSSAGSAGASTASGHNP----AVQPHAPTP----VSLAAAAATAATGTG 508
D. ananassae     SISGSAAGISSSIVGNGPPTTAAIGHNS----TVQPHAPTPGFTVTNSTTSSNASTSSC- 467
                 .         .   . ..   :: *:*.    :**.:****       :..: ::

D. melanogaster  ---ATIVSSAN-----VQGQSVIQPTPTIAFAAVAKHNTSLLENGPVLQQQ--LAVTPTV 520
D. sechellia     ---ATIVSSAN-----VQGQSVIQPTPTIAFAAVAKHNTSLLENGPVLQQQ--LAVTPTV 521
D. simulans      ---ATIVSSAN-----VQGQSVIQPTPTIAFAAVAKHNTSLLENGPVLQQQ--LAVTPTV 521
D. erecta        ---APIVSSAN-----VQGPSVIQSTPTIAFAAVAKHNTSLLENGPVLQQQ--LAVTPTV 521
D. yakuba        ---ATIVSSAN-----VQGPSVIQPTPTIAFAAVAKHNTSLLENGPVLQQQ--LAVTPTV 520
D. persimilis    A-TPAIASISN-----VQGQSVIQATPTIAFAAVAKHNTSLLENGPVLQQQ--PIAT-TV 529
D. pseudobscura  A-TPAIASISN-----VQGQSVIQATPTIAFAAVAKHNTSLLENGPVLQQQ--PIAT-TV 529
D. grimshawi     AAAAAVASASNNSSNNIQGQSVIQATPTIAFAAVAKHNTSLLENGPVLQQQQ--PTAPTV 535
D. virilis       AAAAAAASASN-STNNIQGQSVIQATPTIAFAAVAKHNTSLLENGPVLQQQQ--ATAPTV 536
D. mojavensis    AAVAATASASN-SSNNIQGQSVIQATPTIAFAAVAKHNTSLLENGPVSQQQ---ATAPTV 539
D. willistoni    SGSAATNTSSS----NIQGQSVIQAIPTIAFAAVAKHNTSLLENGPVLHQPQQQATAPTV 564
D. ananassae     ------LTNSS-----VQGSTVIPVAPTIAFAAVAKNNTSLLENSPALQQPQQQTTVPTV 516
                        : :.     :** :**   **********:*******.*. :*     .. **
```

Figure 3: Continued.



```
D. melanogaster   AAIVGAGTQAQQKHVPP--------LSNLQTNSPHIQNGLPVSD---------STNDNSC   563
D. sechellia      AAIVGAGTQAQQKHVPP--------LSNLQTNSPHIQNGLPVSD---------SNNDNSC   564
D. simulans       AAIVGAGTQAQQKHVPP--------LSNLQTNSPHIQNGLPVSD---------SNNDNSC   564
D. erecta         AAIVGAGTQAQQKHVQP--------LSNLQTNSPHIQNGLPVSD---------SNNDTSC   564
D. yakuba         AAIVGSGTQAQQKHVPP--------LSNLQTNSPHIQNGLPVSD---------SNNDTSC   563
D. persimilis     AAIVGAGAQTQQPQHQQQQ---PAQLSNLQTNSSHLQNGLPVSV---------SSSDSNS   577
D. pseudobscura   AAIVGAGAQTQQPQHQQQQ---PAQLSNLQTNSSHLQNGLPVSV---------SSSDSNS   577
D. grimshawi      AAIVSASAQAQQQQQQQQQ--QAAQLSNLQTNSSHLQNGLPVSLSSSSSGSSNSSSNNNS   593
D. virilis        AAIVSASAQAQQQQQQQ----QAAQLSNLQTNSSHMQNGLPVSLSSSSSGSSNSSTNNNS   592
D. mojavensis     AAIVSANAQAQQQQQQQQ---QAAQLSNLQTNSSHMQNGLPASLSSSSSGSSNSSSNNNS   596
D. willistoni     ASIVGAGAQSQQQQQQQQQQQQATQLSNLQTNSSHIQNGLPVSIGSSGS----SSENSNS   620
D. ananassae      AAIVGAGPQTLQQQQQPQ----AAQLSNLQTNSSHIQNGLSVSD---------GNSDNNN   563
                  *:**.:..*: * :           ********.*:****..*          .. :..

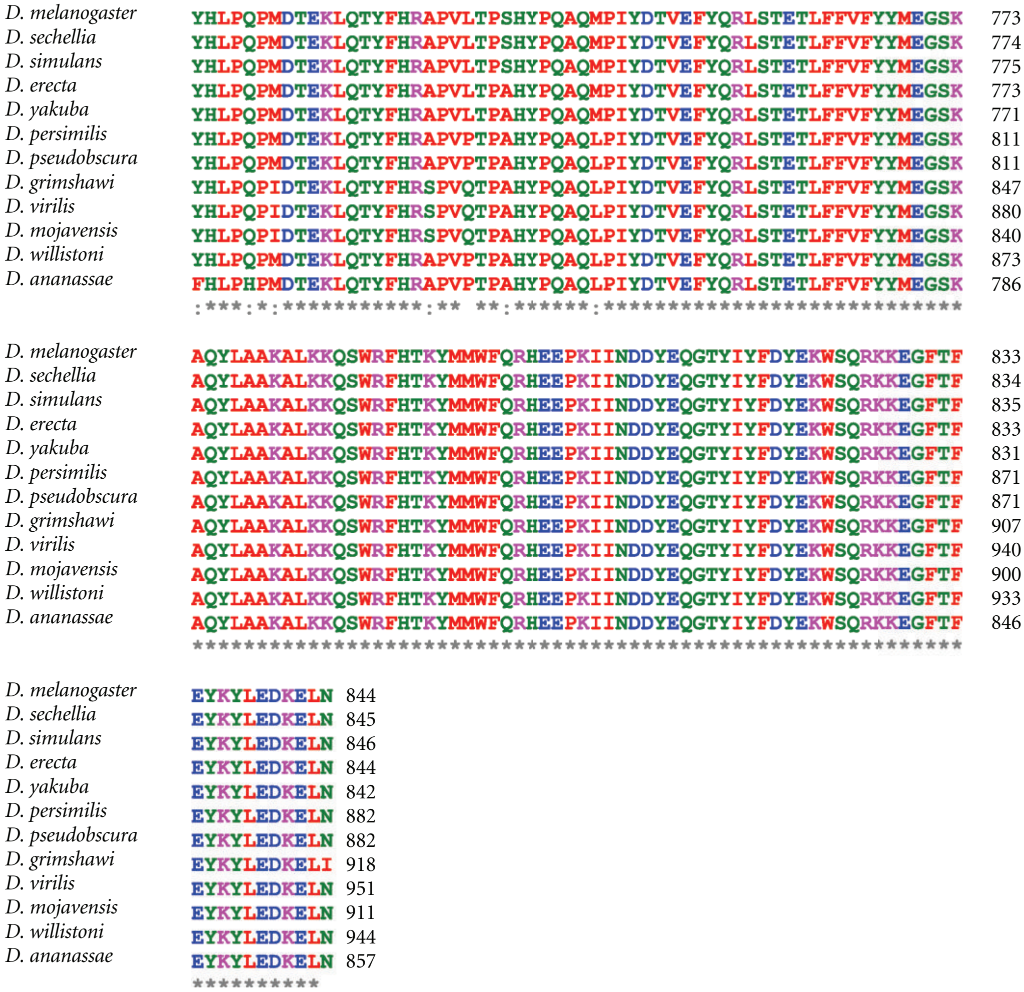
D. melanogaster   NVVDTISLKTMAQDAINRSAIDPNSLNQQ-----QTSSIDLRQPQSQK------------   606
D. sechellia      NVVDTISLKTMAQDAINRSAIDPNSLNQQ-----QTSSIDLRQPQSQK------------   607
D. simulans       NVVDTISLKTMAQDAINRSAIDPNSLNQQ-----QTSSIDLRQPQSQK------------   607
D. erecta         -TVDTISLKTMAQDAINRSAIDTISLNQQ-----QTSSIDLRQQQSQK------------   606
D. yakuba         NVVDTISLKTMAQDAINRSAIDTNSLTQQ-----HTSSIDLRQQQSQK------------   606
D. persimilis     NVVDAISLKTMAQEAINRSAIDVNSLSQQPQTQQQQSNIDTRQQQSQQ------------   625
D. pseudobscura   NVVDAISLKTMAQEAINRSAIDVNSLSQQPQTQQQQSNIDTRQQQSQQ------------   625
D. grimshawi      NAVDAISLKTIAQEAINRSVIEPNSLNQQ-----QASNIDTRQQQQQQQQQQQQQQQQQQ   648
D. virilis        NAVDAISLKTMAQEAINRSVIEPNSLNQQ-----QASNIDARQQQQQQQQQQQQQQQQPQ   647
D. mojavensis     NAVDAISLKTMAQEAINRSVIEPNSLNQQ-----QASNIDTRQQQQQQQQQQQAQQQAAQ   651
D. willistoni     NAVESISLKTMAQEAINRSVIDNTSLNPQ-----QQQ--QSQQQQAASNIDTRQQQQTTQ   673
D. ananassae      SIIDAISLKSMAQEAINRSAIDTTTLIQQ-----QPNNMDTRQSQSQQPSSQQ-------   611
                    :::****::**:*****.*:  :*  *     : .  : :* *  .

D. melanogaster   -------------SLLQ-HFNSETNTNQQQ-LTSQQQ---------QQLQNNSLAATTGS   642
D. sechellia      -------------SLLQ-HFNSETNTNQQQ-LTSQQQ---------QQLQNNSLAATTGS   643
D. simulans       -------------SLLQ-HFNSETNTNQQQQLTSQQQ---------QQLQNNSLAATTGS   644
D. erecta         -------------SLLQ-HFNSETNTNQQQ-LTSQQQ---------QQIQNSSLATTAGS   642
D. yakuba         -------------SLLQ-HFNSETNTNQQQ-LTSQQ-----------QLQNNSLSAVAVS   640
D. persimilis     -------------SLLQQHFSTENTANQPQQVASQQQ---------QNAAAAAAAAAAAA   663
D. pseudobscura   -------------SLLQQHFSTENTANQPQQVASQQQ---------QNAAAAAAAAAAAA   663
D. grimshawi      QQQAAQQQAAAQQSLLQQHFSTETTANQQQQQQ-------------LQQQATAAAAQQQQ   695
D. virilis        QQQAAP------QSLLQQHFSTETTANQQQQQQQQQQQQQQQQLQQQQQQATAAAAAQQQ   701
D. mojavensis     Q------------SLLQQHFSTETTAKQQQQQQLQ-----------QQQAATAAAAAAAQ   688
D. willistoni     Q------------TLLQQHFSTETTANQQQQQQ-------------AAAAAAAAAAAQQQ   708
D. ananassae      -------------SLMQQHFNTENTANQLQQHQVPPQLQN------VGASGTLISSVVAV   652
                               :*:* **.:*..::* *                        ::

D. melanogaster   -------------------------NNGTSTGSGLMNVANA-TGQPISGNAK--------   668
D. sechellia      -------------------------NNGPSTGSGLMNVANA-TGQPISGNAK--------   669
D. simulans       -------------------------NNGPSTGSGLMNVANA-TGQPISGNAK--------   670
D. erecta         -------------------------NNGPSTGSGLINVSNA-TGQPISGNAK--------   668
D. yakuba         -------------------------NNGPSTGSGLMNVANA-TGQPISGNVK--------   666
D. persimilis     AAA-------AAMGTIAAAAHAAAASNGSTTGSGLMNLANA-AGQPTSGSAK--------   707
D. pseudobscura   AAA-------AAMGTIAAAAHAAAASNGSTTGSGLMNLANA-AGQPTSGSAK--------   707
D. grimshawi      QQN----AAAAAAAVAAIGTIVSATSNGPTAGSGLMNLANA-AGQPTSGSGG--------   742
D. virilis        QQQQQQQNAAAAAAVAAIGTIVAAASNGPTAGSGLMNLANA-AGQPSTGNGGVVVAANAK   760
D. mojavensis     QHN------------------------GPTAGSGLMNLANA-AGQPTAVSGGVVVTANAK   723
D. willistoni     QQQ-----QQNAAAAAAVAAIAAASSNGPTAGSGLMNLANATAGQPPSGGNS-------K   756
D. ananassae      THG-------------------ATASNGPTAG--LINVTNA-AGQPTSSNTK--------   682
                                             *.::*  *:*::** :*** : .

D. melanogaster   ---------------THACQPQQMATTEAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAY   713
D. sechellia      ---------------THTCQPQQMATTEAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAY   714
D. simulans       ---------------THTCQPQQMATTEAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAY   715
D. erecta         ---------------THTCQPQQMATTEAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAY   713
D. yakuba         ---------------THTCQPQQMATTEAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAY   711
D. persimilis     ---------------THTSQ-QQMATTEAHIPTLLGVTPLGPTPLQKEHQVQFQMMEAAY   751
D. pseudobscura   ---------------THTSQ-QQMATTEAHIPTLLGVTPLGPTPLQKEHQVQFQMMEAAY   751
D. grimshawi      ---------------QQQQQQQQIATTEAHIPPLLGVTPLGPTPLQKEHQLQFQMMEAAY   787
D. virilis        THTAQQQQQQQQQQQQQQQQQQQIATTEAHIPPLLGVTPLGPTPLQKEHQLQFQMMEAAY   820
D. mojavensis     THTAQQQQQQQQ---QQQQQQQQMATTEAHIPPLLGVTPLGPTPLQKEHQLQFQMMEAAY   780
D. willistoni     TPQQQQQQQQQQ---QLQQQQQQQQQQEAHIPPLLGVTPLGPLPLQKEHQMQFQMMEAAY   813
D. ananassae      ----------------PHSHQQQLATTEAHIPTLLGVTPLGPTPLQKEHQVQFQMMEAAY   726
                                     : **    *****.********* *******:*********
```

Figure 3: Continued.



```
D. melanogaster   YHLPQPMDTEKLQTYFHRAPVLTPSHYPQAQMPIYDTVEFYQRLSTETLFFVFYYMEGSK  773
D. sechellia      YHLPQPMDTEKLQTYFHRAPVLTPSHYPQAQMPIYDTVEFYQRLSTETLFFVFYYMEGSK  774
D. simulans       YHLPQPMDTEKLQTYFHRAPVLTPSHYPQAQMPIYDTVEFYQRLSTETLFFVFYYMEGSK  775
D. erecta         YHLPQPMDTEKLQTYFHRAPVLTPAHYPQAQMPIYDTVEFYQRLSTETLFFVFYYMEGSK  773
D. yakuba         YHLPQPMDTEKLQTYFHRAPVLTPAHYPQAQMPIYDTVEFYQRLSTETLFFVFYYMEGSK  771
D. persimilis     YHLPQPMDTEKLQTYFHRAPVPTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  811
D. pseudobscura   YHLPQPMDTEKLQTYFHRAPVPTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  811
D. grimshawi      YHLPQPIDTEKLQTYFHRSPVQTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  847
D. virilis        YHLPQPIDTEKLQTYFHRSPVQTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  880
D. mojavensis     YHLPQPIDTEKLQTYFHRSPVQTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  840
D. willistoni     YHLPQPMDTEKLQTYFHRAPVPTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  873
D. ananassae      FHLPHPMDTEKLQTYFHRAPVPTPAHYPQAQLPIYDTVEFYQRLSTETLFFVFYYMEGSK  786
                  :***:*:***********:** **:******:****************************

D. melanogaster   AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  833
D. sechellia      AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  834
D. simulans       AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  835
D. erecta         AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  833
D. yakuba         AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  831
D. persimilis     AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  871
D. pseudobscura   AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  871
D. grimshawi      AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  907
D. virilis        AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  940
D. mojavensis     AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  900
D. willistoni     AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  933
D. ananassae      AQYLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYEQGTYIYFDYEKWSQRKKEGFTF  846
                  ************************************************************

D. melanogaster   EYKYLEDKELN  844
D. sechellia      EYKYLEDKELN  845
D. simulans       EYKYLEDKELN  846
D. erecta         EYKYLEDKELN  844
D. yakuba         EYKYLEDKELN  842
D. persimilis     EYKYLEDKELN  882
D. pseudobscura   EYKYLEDKELN  882
D. grimshawi      EYKYLEDKELI  918
D. virilis        EYKYLEDKELN  951
D. mojavensis     EYKYLEDKELN  911
D. willistoni     EYKYLEDKELN  944
D. ananassae      EYKYLEDKELN  857
                  **********
```

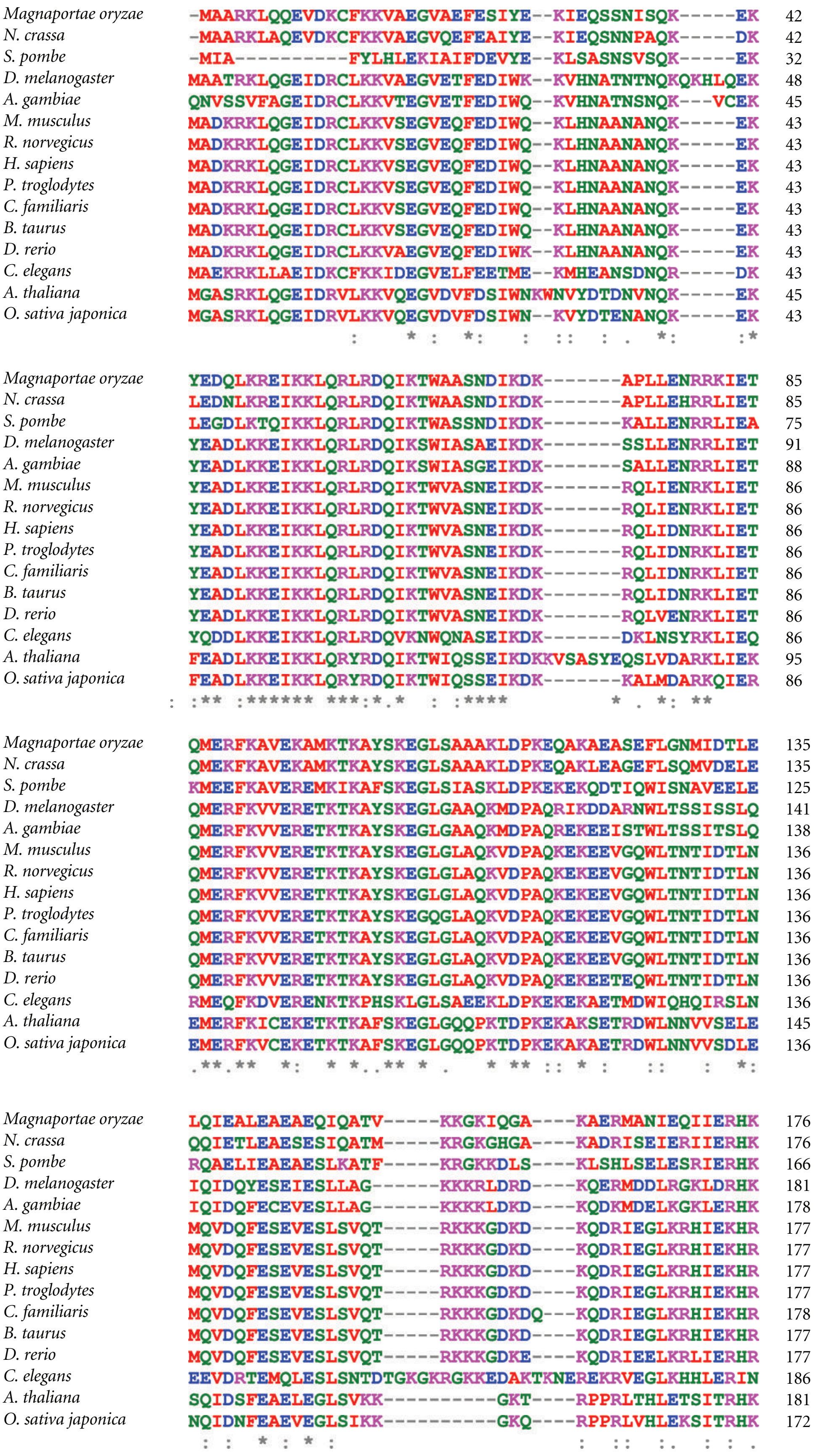
Figure 3: Not3/5 homologs from 12 *Drosophila* species. Clustal W [1, 2] was used to align sequences extracted from FlyBase. Amino acid (aa) color-coding is from Clustal W: red, small aliphatic, hydrophobic, and aromatics; blue, acidic; magenta, basic; green, hydroxyl, sulphydryl, amine, and glycine; grey, unusual aa. Symbols for aa conservation are from Clustal W: (asterisk ∗): positions with a single, fully conserved residue. (Colon :): conservation between groups of strongly similar properties-scoring >0.5 in the Gonnet PAM 250 matrix. (Period .): conservation between groups of weakly similar properties-scoring ≤0.5 in the Gonnet PAM 250 matrix.

may have therefore escaped detection in the *Drosophila* two-hybrid screen. Coimmunoprecipitation studies from tissue extracts and the precise mapping of the interaction domains on both proteins will be required to resolve this issue.

3.5. Multiple Bic-C Isoforms.

Drosophila Bic-C has three predicted mRNA isoforms, RA, RB, and RD, that encode two identical (RA and RB) and one shorter (RD) proteins lacking the first 120 aa (Figure 1). These mRNA isoforms are expressed at different times during development (FlyBase): *Bic-C-RA* is expressed in the early embryo (0–6 hrs old) and in the adult female (i.e., most likely in the ovary), and *Bic-C-RB* is found mostly in late embryogenesis (7–22 hrs old). This is also consistent with our earlier report of multiple protein isoforms [14]. During the larval phases *Bic-C* is undetectable, and during pupation *Bic-C* expression is resumed, with its *RD* isoform being the most abundant and remaining prominent in adult males (FlyBase). The presence of two distinct mRNAs encoding the same amino acid sequence at definite developmental stages also suggests the possibility that they may be subjected to distinct regulation(s) in different tissues or at different developmental times and that the Bic-C activity may be required in specific time windows. This is consistent with a report that *Bic-C* function is especially needed at embryonic day (*E*) 18.5 during mouse development [27].

Interestingly, the mouse *Bicc1* gene and human *BICC1* also produce two distinct mRNAs by alternative splicing, which differ for the presence of exon 21 [26, 48] although no further functional information is known to date, so it is difficult to speculate if the presence of multiple Bic-C isoforms has conserved functional roles.



```
Magnaportae oryzae  -MAARKLQQEVDKCFKKVAEGVAEFESIYE--KIEQSSNISQK-----EK  42
N. crassa           -MAARKLAQEVDKCFKKVAEGVQEFEAIYE--KIEQSNNPAQK-----DK  42
S. pombe            -MIA----------FYLHLEKIAIFDEVYE--KLSASNSVSQK-----EK  32
D. melanogaster     MAATRKLQGEIDRCLKKVAEGVETFEDIWK--KVHNATNTNQKQKHLQEK  48
A. gambiae          QNVSSVFAGEIDRCLKKVTEGVETFEDIWQ--KVHNATNSNQK---VCEK  45
M. musculus         MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
R. norvegicus       MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
H. sapiens          MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
P. troglodytes      MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
C. familiaris       MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
B. taurus           MADKRKLQGEIDRCLKKVSEGVEQFEDIWQ--KLHNAANANQK-----EK  43
D. rerio            MADKRKLQGEIDRCLKKVAEGVEQFEDIWK--KLHNAANANQK-----EK  43
C. elegans          MAEKRKLLAEIDKCFKKIDEGVELFEETME--KMHEANSDNQR-----DK  43
A. thaliana         MGASRKLQGEIDRVLKKVQEGVDVFDSIWNKWNVYDTDNVNQK-----EK  45
O. sativa japonica  MGASRKLQGEIDRVLKKVQEGVDVFDSIWN--KVYDTENANQK-----EK  43
                                  :    * :  *:   :  ::  : .  *:     :*

Magnaportae oryzae  YEDQLKREIKKLQRLRDQIKTWAASNDIKDK-------APLLENRRKIET  85
N. crassa           LEDNLKREIKKLQRLRDQIKTWAASNDIKDK-------APLLEHRRLIET  85
S. pombe            LEGDLKTQIKKLQRLRDQIKTWASSNDIKDK-------KALLENRRLIEA  75
D. melanogaster     YEADLKKEIKKLQRLRDQIKSWIASAEIKDK-------SSLLENRRLIET  91
A. gambiae          YEADLKKEIKKLQRLRDQIKSWIASGEIKDK-------SALLENRRLIET  88
M. musculus         YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIENRKLIET  86
R. norvegicus       YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIENRKLIET  86
H. sapiens          YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIDNRKLIET  86
P. troglodytes      YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIDNRKLIET  86
C. familiaris       YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIDNRKLIET  86
B. taurus           YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLIDNRKLIET  86
D. rerio            YEADLKKEIKKLQRLRDQIKTWVASNEIKDK-------RQLVENRKLIET  86
C. elegans          YQDDLKKEIKKLQRLRDQVKNWQNASEIKDK-------DKLNSYRKLIEQ  86
A. thaliana         FEADLKKEIKKLQRYRDQIKTWIQSSEIKDKKVSASYEQSLVDARKLIEK  95
O. sativa japonica  FEADLKKEIKKLQRYRDQIKTWIQSSEIKDK-------KALMDARKQIER  86
                  : :** :****** ***:*.*  : :****         * . *: **

Magnaportae oryzae  QMERFKAVEKAMKTKAYSKEGLSAAAKLDPKEQAKAEASEFLGNMIDTLE  135
N. crassa           QMEKFKAVEKAMKTKAYSKEGLSAAAKLDPKEQAKLEAGEFLSQMVDELE  135
S. pombe            KMEEFKAVEREMKIKAFSKEGLSIASKLDPKEKEKQDTIQWISNAVEELE  125
D. melanogaster     QMERFKVVERETKTKAYSKEGLGAAQKMDPAQRIKDDARNWLTSSISSLQ  141
A. gambiae          QMERFKVVERETKTKAYSKEGLGAAQKMDPAQREKEEISTWLTSSITSLQ  138
M. musculus         QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
R. norvegicus       QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
H. sapiens          QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
P. troglodytes      QMERFKVVERETKTKAYSKEGQGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
C. familiaris       QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
B. taurus           QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEEVGQWLTNTIDTLN  136
D. rerio            QMERFKVVERETKTKAYSKEGLGLAQKVDPAQKEKEETEQWLTNTIDTLN  136
C. elegans          RMEQFKDVERENKTKPHSKLGLSAEEKLDPKEKEKAETMDWIQHQIRSLN  136
A. thaliana         EMERFKICEKETKTKAFSKEGLGQQPKTDPKEKAKSETRDWLNNVVSELE  145
O. sativa japonica  EMERFKVCEKETKTKAFSKEGLGQQPKTDPKEKAKAETRDWLNNVVSDLE  136
                    .**.**  *:  * *..** * .   * ** :: * :   ::   :  *:

Magnaportae oryzae  LQIEALEAEAEQIQATV-----KKGKIQGA----KAERMANIEQIIERHK  176
N. crassa           QQIETLEAESESIQATM-----KRGKGHGA----KADRISEIERIIERHK  176
S. pombe            RQAELIEAEAESLKATF-----KRGKKDLS----KLSHLSELESRIERHK  166
D. melanogaster     IQIDQYESEIESLLAG------KKKRLDRD----KQERMDDLRGKLDRHK  181
A. gambiae          IQIDQFECEVESLLAG------KKKKLDKD----KQDKMDELKGKLERHK  178
M. musculus         MQVDQFESEVESLSVQT-----RKKKGDKD----KQDRIEGLKRHIEKHR  177
R. norvegicus       MQVDQFESEVESLSVQT-----RKKKGDKD----KQDRIEGLKRHIEKHR  177
H. sapiens          MQVDQFESEVESLSVQT-----RKKKGDKD----KQDRIEGLKRHIEKHR  177
P. troglodytes      MQVDQFESEVESLSVQT-----RKKKGDKD----KQDRIEGLKRHIEKHR  177
C. familiaris       MQVDQFESEVESLSVQT-----RKKKGDKDQ---KQDRIEGLKRHIEKHR  178
B. taurus           MQVDQFESEVESLSVQT-----RKKKGDKD----KQDRIEGLKRHIEKHR  177
D. rerio            MQVDQFESEVESLSVQT-----RKKKGDKE----KQDRIEELKRLIERHR  177
C. elegans          EEVDRTEMQLESLSNTDTGKGKRGKKEDAKTKNEREKRVEGLKHHLERIN  186
A. thaliana         SQIDSFEAELEGLSVKK----------GKT----RPPRLTHLETSITRHK  181
O. sativa japonica  NQIDNFEAEVEGLSIKK----------GKQ----RPPRLVHLEKSITRHK  172
                     : :  * : * :                     :  ::  :.  : : .
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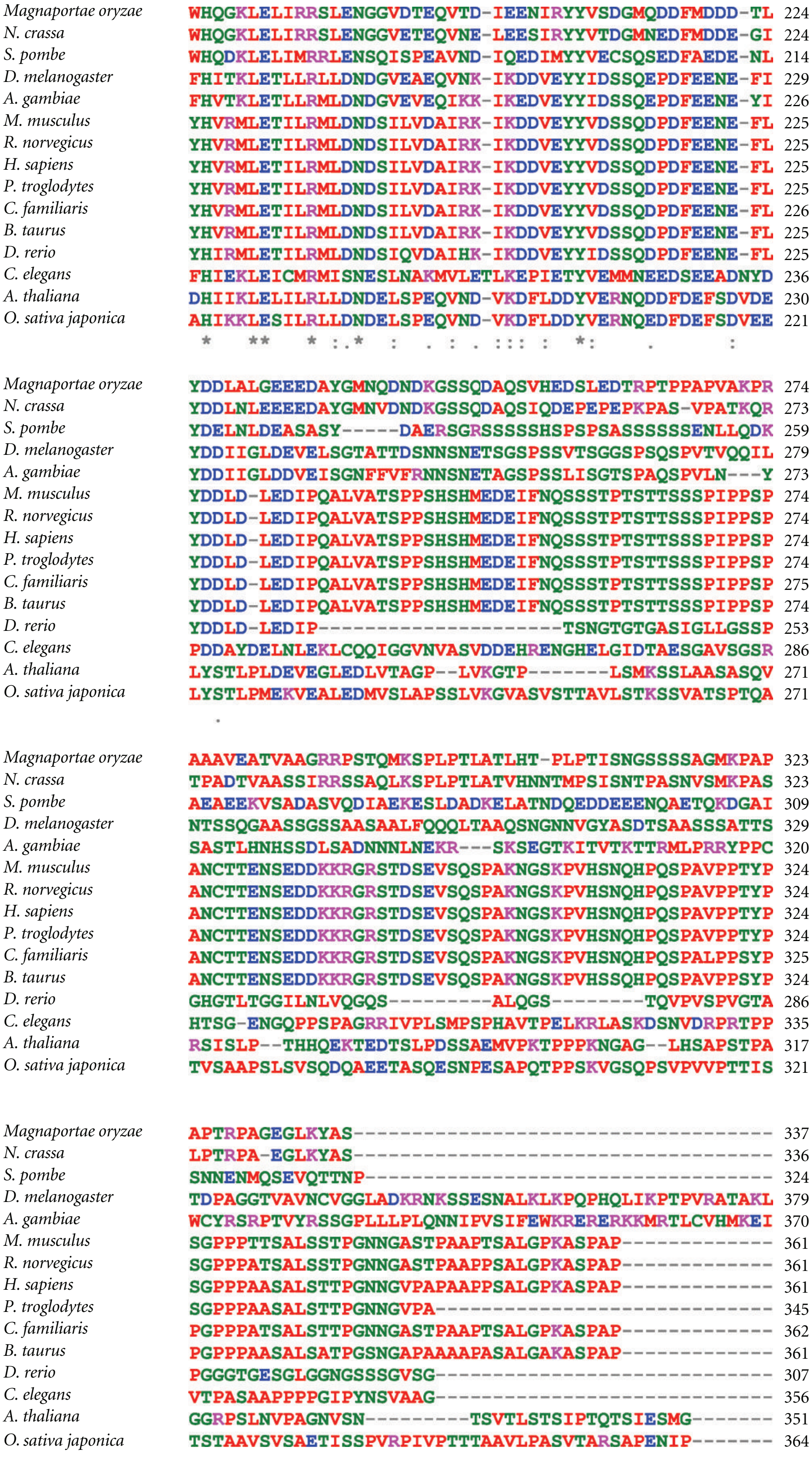
Figure 4: Continued.



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Magnaportae oryzae   WHQGKLELIRRSLENGGVDTEQVTD-IEENIRYYVSDGMQDDFMDDD-TL 224
N. crassa            WHQGKLELIRRSLENGGVETEQVNE-LEESIRYYVTDGMNEDFMDDE-GI 224
S. pombe             WHQDKLELIMRRLENSQISPEAVND-IQEDIMYYVECSQSEDFAEDE-NL 214
D. melanogaster      FHITKLETLLRLLDNDGVEAEQVNK-IKDDVEYYIDSSQEPDFEENE-FI 229
A. gambiae           FHVTKLETLLRMLDNDGVEVEQIKK-IKEDVEYYIDSSQEPDFEENE-YI 226
M. musculus          YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 225
R. norvegicus        YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 225
H. sapiens           YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 225
P. troglodytes       YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 225
C. familiaris        YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 226
B. taurus            YHVRMLETILRMLDNDSILVDAIRK-IKDDVEYYVDSSQDPDFEENE-FL 225
D. rerio             YHIRMLETILRMLDNDSIQVDAIHK-IKDDVEYYIDSSQDPDFEENE-FL 225
C. elegans           FHIEKLEICMRMISNESLNAKMVLETLKEPIETYVEMMNEEDSEEADNYD 236
A. thaliana          DHIIKLELILRLLDNDELSPEQVND-VKDFLDDYVERNQDDFDEFSDVDE 230
O. sativa japonica   AHIKKLESILRLLDNDELSPEQVND-VKDFLDDYVERNQEDFDEFSDVEE 221
                      *   **   * :.*  :  . : . ::: :  *:    .      :

Magnaportae oryzae   YDDLALGEEEDAYGMNQDNDKGSSQDAQSVHEDSLEDTRPTPPAPVAKPR 274
N. crassa            YDDLNLEEEEDAYGMNVDNDKGSSQDAQSIQDEPEPEPKPAS-VPATKQR 273
S. pombe             YDELNLDEASASY-----DAERSGRSSSSSHSPSPSASSSSSSENLLQDK 259
D. melanogaster      YDDIIGLDEVELSGTATTDSNNSNETSGSPSSVTSGGSPSQSPVTVQQIL 279
A. gambiae           YDDIIGLDDVEISGNFFVFRNNSNETAGSPSSLISGTSPAQSPVLN---Y 273
M. musculus          YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 274
R. norvegicus        YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 274
H. sapiens           YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 274
P. troglodytes       YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 274
C. familiaris        YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 275
B. taurus            YDDLD-LEDIPQALVATSPPSHSHMEDEIFNQSSSTPTSTTSSSPIPPSP 274
D. rerio             YDDLD-LEDIP---------------------TSNGTGTGASIGLLGSSP 253
C. elegans           PDDAYDELNLEKLCQQIGGVNVASVDDEHRENGHELGIDTAESGAVSGSR 286
A. thaliana          LYSTLPLDEVEGLEDLVTAGP--LVKGTP-------LSMKSSLAASASQV 271
O. sativa japonica   LYSTLPMEKVEALEDMVSLAPSSLVKGVASVSTTAVLSTKSSVATSPTQA 271
                       .

Magnaportae oryzae   AAAVEATVAAGRRPSTQMKSPLPTLATLHT-PLPTISNGSSSSAGMKPAP 323
N. crassa            TPADTVAASSIRRSSAQLKSPLPTLATVHNNTMPSISNTPASNVSMKPAS 323
S. pombe             AEAEEKVSADASVQDIAEKESLDADKELATNDQEDDEEENQAETQKDGAI 309
D. melanogaster      NTSSQGAASSGSSAASAALFQQQLTAAQSNGNNVGYASDTSAASSSATTS 329
A. gambiae           SASTLHNHSSDLSADNNNLNEKR---SKSEGTKITVTKTTRMLPRRYPPC 320
M. musculus          ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSNQHPQSPAVPPTYP 324
R. norvegicus        ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSNQHPQSPAVPPTYP 324
H. sapiens           ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSNQHPQSPAVPPTYP 324
P. troglodytes       ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSNQHPQSPAVPPTYP 324
C. familiaris        ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSNQHPQSPALPPSYP 325
B. taurus            ANCTTENSEDDKKRGRSTDSEVSQSPAKNGSKPVHSSQHPQSPAVPPSYP 324
D. rerio             GHGTLTGGILNLVQGQS---------ALQGS--------TQVPVSPVGTA 286
C. elegans           HTSG-ENGQPPSPAGRRIVPLSMPSPHAVTPELKRLASKDSNVDRPRTPP 335
A. thaliana          RSISLP--THHQEKTEDTSLPDSSAEMVPKTPPPKNGAG--LHSAPSTPA 317
O. sativa japonica   TVSAAPSLSVSQDQAEETASQESNPESAPQTPPSKVGSQPSVPVVPTTIS 321

Magnaportae oryzae   APTRPAGEGLKYAS------------------------------------ 337
N. crassa            LPTRPA-EGLKYAS------------------------------------ 336
S. pombe             SNNENMQSEVQTTNP----------------------------------- 324
D. melanogaster      TDPAGGTVAVNCVGGLADKRNKSSESNALKLKPQPHQLIKPTPVRATAKL 379
A. gambiae           WCYRSRPTVYRSSGPLLLPLQNNIPVSIFEWKRERERKKMRTLCVHMKEI 370
M. musculus          SGPPPTTSALSSTPGNNGASTPAAPTSALGPKASPAP------------- 361
R. norvegicus        SGPPPATSALSSTPGNNGASTPAAPPSALGPKASPAP------------- 361
H. sapiens           SGPPPAASALSTTPGNNGVPAPAAPPSALGPKASPAP------------- 361
P. troglodytes       SGPPPAASALSTTPGNNGVPA----------------------------- 345
C. familiaris        PGPPPATSALSTTPGNNGASTPAAPTSALGPKASPAP------------- 362
B. taurus            PGPPPAASALSATPGSNGAPAAAAPASALGAKASPAP------------- 361
D. rerio             PGGGTGESGLGGNGSSSGVSG----------------------------- 307
C. elegans           VTPASAAPPPPGIPYNSVAAG----------------------------- 356
A. thaliana          GGRPSLNVPAGNVSN---------TSVTLSTSIPTQTSIESMG------- 351
O. sativa japonica   TSTAAVSVSAETISSPVRPIVPTTTAAVLPASVTARSAPENIP------- 364
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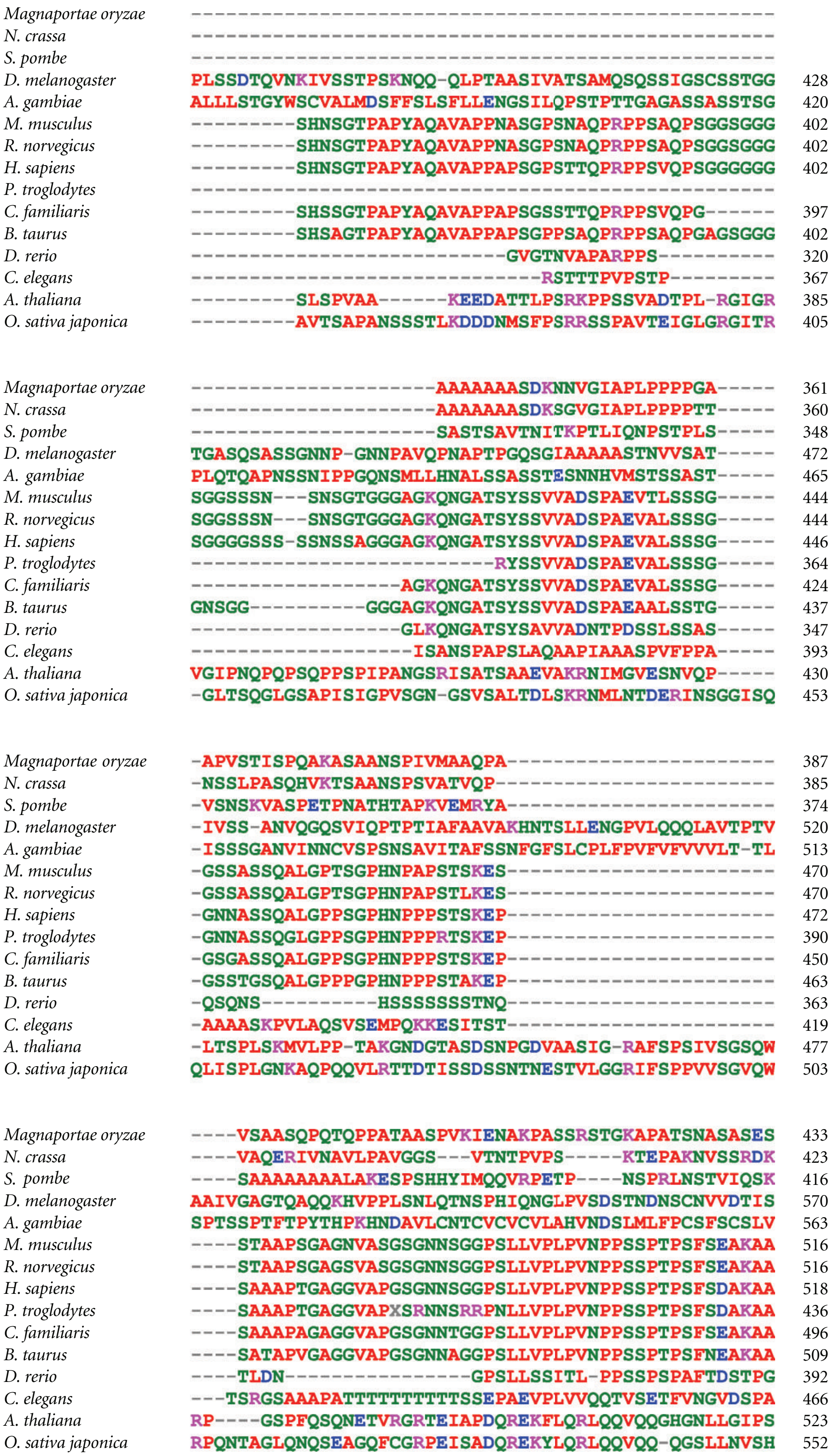
Figure 4: Continued.



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Magnaportae oryzae    --------------------------------------------------
N. crassa             --------------------------------------------------
S. pombe              --------------------------------------------------
D. melanogaster       PLSSDTQVNKIVSSTPSKNQQ-QLPTAASIVATSAMQSQSSIGSCSSTGG   428
A. gambiae            ALLLSTGYWSCVALMDSFFSLSFLLENGSILQPSTPTTGAGASSASSTSG   420
M. musculus           ---------SHNSGTPAPYAQAVAPPNASGPSNAQPRPPSAQPSGGSGGG   402
R. norvegicus         ---------SHNSGTPAPYAQAVAPPNASGPSNAQPRPPSAQPSGGSGGG   402
H. sapiens            ---------SHNSGTPAPYAQAVAPPAPSGPSTTQPRPPSVQPSGGGGGG   402
P. troglodytes        --------------------------------------------------
C. familiaris         ---------SHSSGTPAPYAQAVAPPAPSGSSTTQPRPPSVQPG------   397
B. taurus             ---------SHSAGTPAPYAQAVAPPAPSGPPSAQPRPPSAQPGAGSGGG   402
D. rerio              ---------------------------GVGTNVAPARPPS----------   320
C. elegans            ------------------------------RSTTTPVPSTP---------   367
A. thaliana           ---------SLSPVAA------KEEDATTLPSRKPPSSVADTPL-RGIGR   385
O. sativa japonica    ---------AVTSAPANSSSTLKDDDNMSFPSRRSSPAVTEIGLGRGITR   405

Magnaportae oryzae    ---------------------AAAAAAASDKNNVGIAPLPPPPGA-----   361
N. crassa             ---------------------AAAAAAASDKSGVGIAPLPPPPTT-----   360
S. pombe              ---------------------SASTSAVTNITKPTLIQNPSTPLS-----   348
D. melanogaster       TGASQSASSGNNP-GNNPAVQPNAPTPGQSGIAAAAASTNVVSAT-----   472
A. gambiae            PLQTQAPNSSNIPPGQNSMLLHNALSSASSTESNNHVMSTSSAST-----   465
M. musculus           SGGSSSN---SNSGTGGGAGKQNGATSYSSVVADSPAEVTLSSSG-----   444
R. norvegicus         SGGSSSN---SNSGTGGGAGKQNGATSYSSVVADSPAEVALSSSG-----   444
H. sapiens            SGGGGSSS-SSNSSAGGGAGKQNGATSYSSVVADSPAEVALSSSG-----   446
P. troglodytes        --------------------------RYSSVVADSPAEVALSSSG-----   364
C. familiaris         ------------------AGKQNGATSYSSVVADSPAEVALSSSG-----   424
B. taurus             GNSGG----------GGGAGKQNGATSYSSVVADSPAEAALSSTG-----   437
D. rerio              ------------------GLKQNGATSYSAVVADNTPDSSLSSAS-----   347
C. elegans            -------------------ISANSPAPSLAQAAPIAAASPVFPPA-----   393
A. thaliana           VGIPNQPQPSQPPSPIPANGSRISATSAAEVAKRNIMGVESNVQP-----   430
O. sativa japonica    -GLTSQGLGSAPISIGPVSGN-GSVSALTDLSKRNMLNTDERINSGGISQ   453

Magnaportae oryzae    -APVSTISPQAKASAANSPIVMAAQPA-----------------------   387
N. crassa             -NSSLPASQHVKTSAANSPSVATVQP------------------------   385
S. pombe              -VSNSKVASPETPNATHTAPKVEMRYA-----------------------   374
D. melanogaster       -IVSS-ANVQGQSVIQPTPTIAFAAVAKHNTSLLENGPVLQQQLAVTPTV   520
A. gambiae            -ISSSGANVINNCVSPSNSAVITAFSSNFGFSLCPLFPVFVFVVVLT-TL   513
M. musculus           -GSSASSQALGPTSGPHNPAPSTSKES-----------------------   470
R. norvegicus         -GSSASSQALGPTSGPHNPAPSTLKES-----------------------   470
H. sapiens            -GNNASSQALGPPSGPHNPPPSTSKEP-----------------------   472
P. troglodytes        -GNNASSQGLGPPSGPHNPPPRTSKEP-----------------------   390
C. familiaris         -GSGASSQALGPPSGPHNPPPSTSKEP-----------------------   450
B. taurus             -GSSTGSQALGPPPGPHNPPPSTAKEP-----------------------   463
D. rerio              -QSQNS----------HSSSSSSSTNQ-----------------------   363
C. elegans            -AAAASKPVLAQSVSEMPQKKESITST-----------------------   419
A. thaliana           -LTSPLSKMVLPP-TAKGNDGTASDSNPGDVAASIG-RAFSPSIVSGSQW   477
O. sativa japonica    QLISPLGNKAQPQQVLRTTDTISSDSSNTNESTVLGGRIFSPPVVSGVQW   503

Magnaportae oryzae    ----VSAASQPQTQPPATAASPVKIENAKPASSRSTGKAPATSNASASES   433
N. crassa             ----VAQERIVNAVLPAVGGS---VTNTPVPS-----KTEPAKNVSSRDK   423
S. pombe              ----SAAAAAAAALAKESPSHHYIMQQVRPETP----NSPRLNSTVIQSK   416
D. melanogaster       AAIVGAGTQAQQKHVPPLSNLQTNSPHIQNGLPVSDSTNDNSCNVVDTIS   570
A. gambiae            SPTSSPTFTPYTHPKHNDAVLCNTCVCVCVLAHVNDSLMLFPCSFSCSLV   563
M. musculus           ----STAAPSGAGNVASGSGNNSGGPSLLVPLPVNPPSSPTPSFSEAKAA   516
R. norvegicus         ----STAAPSGAGSVASGSGNNSGGPSLLVPLPVNPPSSPTPSFSEAKAA   516
H. sapiens            ----SAAAPTGAGGVAPGSGNNSGGPSLLVPLPVNPPSSPTPSFSDAKAA   518
P. troglodytes        ----SAAAPTGAGGVAPXSRNNSRRPNLLVPLPVNPPSSPTPSFSDAKAA   436
C. familiaris         ----SAAAPAGAGGVAPGSGNNTGGPSLLVPLPVNPPSSPTPSFSEAKAA   496
B. taurus             ----SATAPVGAGGVAPGSGNNAGGPSLLVPLPVNPPSSPTPSFNEAKAA   509
D. rerio              ----TLDN----------------GPSLLSSITL-PPSSPSPAFTDSTPG   392
C. elegans            ---TSRGSAAAPATTTTTTTTTTSSEPAEVPLVVQQTVSETFVNGVDSPA   466
A. thaliana           RP----GSPFQSQNETVRGRTEIAPDQREKFLQRLQQVQQGHGNLLGIPS   523
O. sativa japonica    RPQNTAGLQNQSEAGQFCGRPEISADQREKYLQRLQQVQQ-QGSLLNVSH   552
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Figure 4: Continued.

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Magnaportae oryzae      SEAAGKASSSKSRKGALGEASNQSSA------------------------   459
N. crassa               ASAPVPAATATTSKATPEPEAVKTQP------------------------   449
S. pombe                WDSLGHTASPKMQTQPVR--SVSQSS------------------------   440
D. melanogaster         LKTMAQDAINRSAIDPNSLNQQQTSSIDL---------------------   599
A. gambiae              HLGVAQEAG----PVPSNQTPQPQSGGGG---------------------   588
M. musculus             G-TLLNGPPQFS-TTPEIKAPEPLSS------------------------   540
R. norvegicus           G-TLLNGPPQFS-TTPEIKAPEPLSS------------------------   540
H. sapiens              G-ALLNGPPQFS-TAPEIKAPEPLSS------------------------   542
P. troglodytes          G-ALLNGPPQFS-TAPEIKAPEPLSS------------------------   460
C. familiaris           G-ALLNGPPQFS-TAPEIKAPEPLSS------------------------   520
B. taurus               G-SLLNGPPQFS-AAPEIKAPEPLSS------------------------   533
D. rerio                GGSLLNGPHSYTPNTEAIKAPEPPSS------------------------   418
C. elegans              AATRLTQQERQQ----QLQQQHHHQS------------------------   488
A. thaliana             LSGGNEKQFSSQQQNPLLQQSSSISPHGSLGIGVQAPGFNVMSSASLQQQ   573
O. sativa japonica      ITGISQKQFPSQQPNPLLQQFNSQSSS-------------ISSQAGIG--   587
                                                 .

Magnaportae oryzae      ------------TSSHTNGVTNGVKSK-----------AKGKSGKGQPQ-   485
N. crassa               ------------QVPQTNGATNGIKP------------------------   463
S. pombe                ------------ATTETN-----VKP------------------------   449
D. melanogaster         -----RQPQSQKSLLQHFNSETNTNQQQLTSQQQQQLQNNSLAATTGSN-   643
A.  gambiae             -----GGGAGQSLMVDASGVPAGAN----AGNNLLPTSSATAAITNGPN-   628
M. musculus             ----------LKSMAERAAISSGIED-----------PVPTLHLTDRD--   567
R. norvegicus           ----------LKSMAERAAISSGIED-----------PVPTLHLTDRD--   567
H. sapiens              ----------LKSMAERAAISSGIED-----------PVPTLHLTERD--   569
P. troglodytes          ----------LKSMAERAAISSGIED-----------PVPTLHLTERD--   487
C. familiaris           ----------LKSMAERAAISSGIED-----------PVPTLHLTERD--   547
B. taurus               ----------LKSMAERAAISSGIED-----------PVPTLHLTERD--   560
D. rerio                ----------LKAMAERAALGLALDG-----------EIPSLHLTDRDS-   446
C. elegans              -----------TIIPTTPTTTTTSSS-----------MLGGMMSTDDPA-   515
A. thaliana             SNAMSQQLGQQPSVADVDHVRNDDQSQ---QNLPDDSASIAASKAIQSED   620
O. sativa japonica      -------LG-QVQVPESGHTKSEEQQQSFAEDVSVESVATAGANKHMSED   629
                                                .

Magnaportae oryzae      -------------VQAQEEPAEEESIYHLPA------------------S   504
N. crassa               ----------------IEEVEEEESIYHLPA------------------S   479
S. pombe                -----------------TKEENADVPVSSPD------------------Y   464
D. melanogaster         -------------NGTSTGSGLMNVANATGQPISGNAKTHACQPQQMATT   680
A. gambiae              -------------TIINTNSSISNAANVNSAGGGGGGGPGGMKP-SAGTH   664
M. musculus             --------------IILSSTSAPP-TSSQPP---------------LQLS   587
R. norvegicus           --------------IILSSTSAPP-TSSQPP---------------LQLS   587
H. sapiens              --------------IILSSTSAPP-ASAQPP---------------LQLS   589
P. troglodytes          --------------IILSSTSAPP-ASAQPP---------------LQLS   507
C. familiaris           --------------IILSSTSAPP-ASAQPP---------------LQLS   567
B. taurus               --------------ILLSSTSAPP-ASAQPP---------------LQLS   580
D. rerio                -------------LELFSGSSAPPGPTTAPQ---------------PAVS   468
C. elegans              -------------AALQAALNMAAASQQAVA---------------TGPK   537
A. thaliana             DSKVLFDTPSGMPSYMLDPVQVSSGPDFSPGQPIQPGQSSSSLGVIGRRS   670
O. sativa japonica      DTKIPFSNPS---ASITEGTQLSRDPDLPAGQPLQPGMSSSGVGVIGRRS   676

Magnaportae oryzae      LQDLVDSYEMSK--KRPAQANSTSTLRAMSHSQANLPDLTDAEAPSSYQP   552
N. crassa               LQDLVESYEVTK--KCPASVDALATQRMHAVAVANKPSALDTELPRPYYP   527
S.  pombe               LKDLVNALNTSKE-QHKGAIDKEKLTEALNISCVYVPDATDAAKPQYYIP   513
D. melanogaster         EAHIPTLLGVTPLGPTPLQKEHQMQFQMMEAAYYHLPQPMDTEKLQTYFH   730
A. gambiae              EACIPPLLGVAPLGTSKLQKEHQIQFQLMEAAYYHLPTPSDSERLRPYLQ   714
M. musculus             EVNIPLSLGVCPLGPVSLTKEQLYQQAMEEAAWHHMPHPSDSERIRQYLP   637
R. norvegicus           EVNIPLSLGVCPLGPVSLTKEQLYQQAMEEAAWHHMPHPSDSERIRQYLP   637
H. sapiens              EVNIPLSLGVCPLGPVPLTKEQLYQQAMEEAAWHHMPHPSDSERIRQYLP   639
P. troglodytes          EVNIPLSLGVCPLGPVPLTKEQLYQQAMEEAAWHHMPHPSDSERIRQYLP   557
C. familiaris           EVNIPLSLGVCPLGPVPLTKEQLYQQAMEEAAWHHMPHPSDSERIRT-FP   616
B. taurus               EVNIPLSLGVCPLGPVPLTKEQLYQQAMEEAAWHHMPHPSDSERIRQYLP   630
D. rerio                EVSLPPSLGACPLGPTPLTKEQLYQQAMQEAAWTHMPHPSDSERIRQYLM   518
C. elegans              RAHIPAWLGASPLGRTSMTQEFDGQLAALELACAKATFPLDSEKPRNYLS   587
A. thaliana             NSELGAIGDPSAVG---PMHDQMHNLQMLEAAFYKRPQPSDSERPRPYSP   717
O. sativa japonica      VSDLGAIGDNLSVASASTSHDLLYNLQMLEAAFHRLPQPKDSERVKNYIP   726
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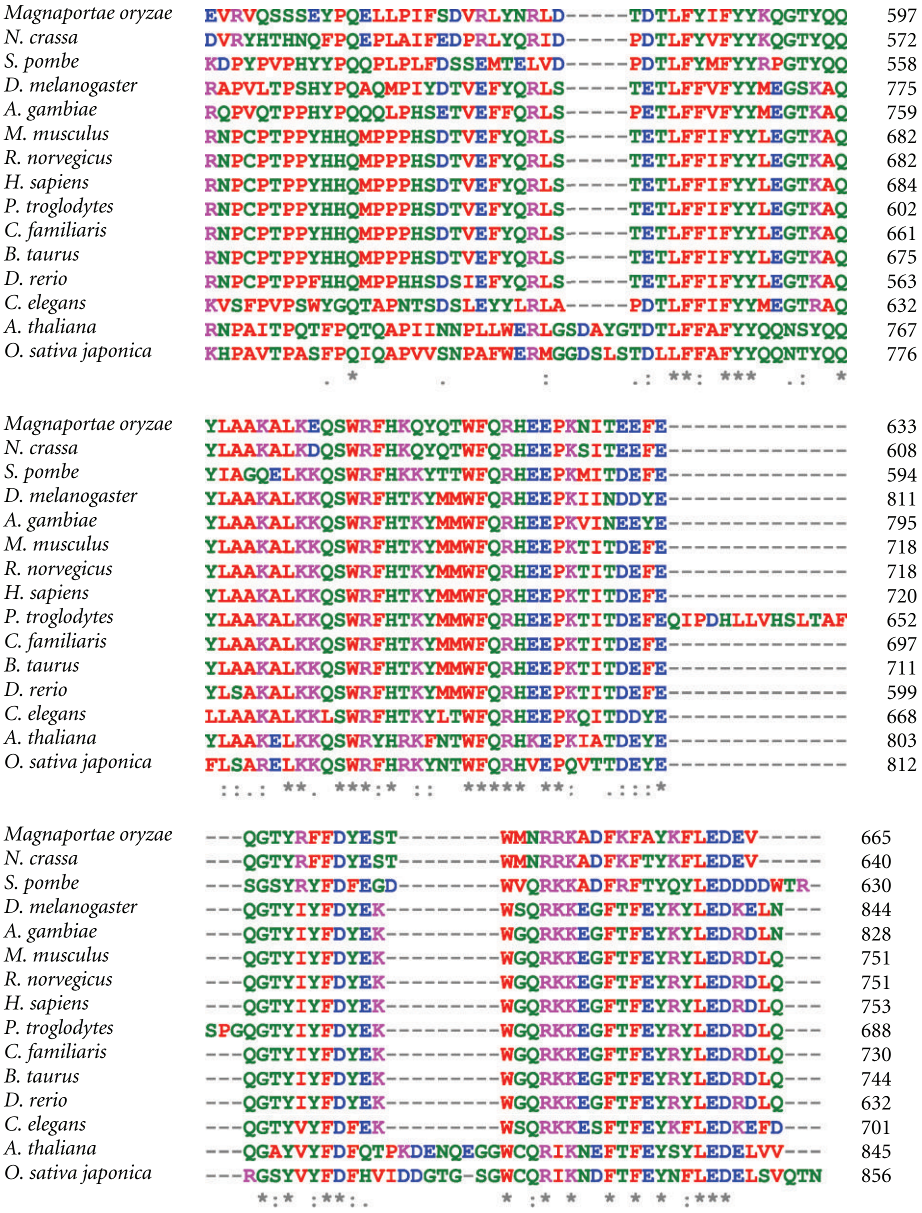
Figure 4: Continued.



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Magnaportae oryzae  EVRVQSSSEYPQELLPIFSDVRLYNRLD-----TDTLFYIFYYKQGTYQQ  597
N. crassa           DVRYHTHNQFPQEPLAIFEDPRLYQRID-----PDTLFYVFYYKQGTYQQ  572
S. pombe            KDPYPVPHYYPQQPLPLFDSSEMTELVD-----PDTLFYMFYYRPGTYQQ  558
D. melanogaster     RAPVLTPSHYPQAQMPIYDTVEFYQRLS-----TETLFFVFYYMEGSKAQ  775
A. gambiae          RQPVQTPPHYPQQQLPHSETVEFFQRLS-----PETLFFVFYYMEGTKAQ  759
M. musculus         RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  682
R. norvegicus       RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  682
H. sapiens          RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  684
P. troglodytes      RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  602
C. familiaris       RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  661
B. taurus           RNPCPTPPYHHQMPPPHSDTVEFYQRLS-----TETLFFIFYYLEGTKAQ  675
D. rerio            RNPCPTPPFHHQMPPHHSDSIEFYQRLS-----TETLFFIFYYLEGTKAQ  563
C. elegans          KVSFPVPSWYGQTAPNTSDSLEYYLRLA-----PDTLFFIFYYMEGTRAQ  632
A. thaliana         RNPAITPQTFPQTQAPIINNPLLWERLGSDAYGTDTLFFAFYYQQNSYQQ  767
O. sativa japonica  KHPAVTPASFPQIQAPVVSNPAFWERMGGDSLSTDLLFFAFYYQQNTYQQ  776
                              .*      .       :      .: **: ***  .:  *

Magnaportae oryzae  YLAAKALKEQSWRFHKQYQTWFQRHEEPKNITEEFE--------------  633
N. crassa           YLAAKALKDQSWRFHKQYQTWFQRHEEPKSITEEFE--------------  608
S. pombe            YIAGQELKKQSWRFHKKYTTWFQRHEEPKMITDEFE--------------  594
D. melanogaster     YLAAKALKKQSWRFHTKYMMWFQRHEEPKIINDDYE--------------  811
A. gambiae          YLAAKALKKQSWRFHTKYMMWFQRHEEPKVINEEYE--------------  795
M. musculus         YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  718
R. norvegicus       YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  718
H. sapiens          YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  720
P. troglodytes      YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFEQIPDHLLVHSLTAF  652
C. familiaris       YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  697
B. taurus           YLAAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  711
D. rerio            YLSAKALKKQSWRFHTKYMMWFQRHEEPKTITDEFE--------------  599
C. elegans          LLAAKALKKLSWRFHTKYLTWFQRHEEPKQITDDYE--------------  668
A. thaliana         YLAAKELKKQSWRYHRKFNTWFQRHKEPKIATDEYE--------------  803
O. sativa japonica  FLSARELKKQSWRFHRKYNTWFQRHVEPQVTTDEYE--------------  812
                     ::.: **. ***:* ::  ***** **:  .:::*

Magnaportae oryzae  ---QGTYRFFDYEST--------WMNRRKADFKFAYKFLEDEV-----  665
N. crassa           ---QGTYRFFDYEST--------WMNRRKADFKFTYKFLEDEV-----  640
S. pombe            ---SGSYRYFDFEGD--------WVQRKKADFRFTYQYLEDDDDWTR-  630
D. melanogaster     ---QGTYIYFDYEK---------WSQRKKEGFTFEYKYLEDKELN---  844
A. gambiae          ---QGTYIYFDYEK---------WGQRKKEGFTFEYKYLEDRDLN---  828
M. musculus         ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  751
R. norvegicus       ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  751
H. sapiens          ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  753
P. troglodytes      SPGQGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  688
C. familiaris       ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  730
B. taurus           ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  744
D. rerio            ---QGTYIYFDYEK---------WGQRKKEGFTFEYRYLEDRDLQ---  632
C. elegans          ---QGTYVYFDFEK---------WSQRKKESFTFEYKFLEDKEFD---  701
A. thaliana         ---QGAYVYFDFQTPKDENQEGGWCQRIKNEFTFEYSYLEDELVV---  845
O. sativa japonica  ---RGSYVYFDFHVIDDGTG-SGWCQRIKNDFTFEYNFLEDELSVQTN  856
                        *:* :**:.          * :* *  * * * :***
```

Figure 4: Not3/5 orthologs. Clustal W [1, 2] was used to align sequences extracted from the NCBI database. Amino acid (aa) color-coding is from Clustal W: red, small aliphatic, hydrophobic, and aromatics; blue, acidic; magenta, basic; green, hydroxyl, sulphydryl, amine, and glycine; grey, unusual aa. Symbols for aa conservation are from Clustal W: (asterisk ∗): positions with a single, fully conserved residue. (Colon :): conservation between groups of strongly similar properties-scoring >0.5 in the Gonnet PAM 250 matrix. (Period .): conservation between groups of weakly similar properties-scoring ≤0.5 in the Gonnet PAM 250 matrix.

3.6. Bic-C and Polycystic Kidney Disease. In humans, two polycystic kidney disease (PKD) forms are caused by mutations in the *PKD1* and *PKD2* genes (autosomal, dominant [49–55]) or in PKHD1 (autosomal recessive, [52–55]). The link between Bic-C malfunction and PKD is compelling: two mouse models developing polycystic kidneys harbor mutations of the *Bicc1* gene [56]; Bic-C inactivation in *Xenopus* induces cystic kidneys [27, 57]; recently, a zebrafish model of PKD was validated that inhibits the Bicc1 function [58]. Finally, human studies on patients with renal disorders identified two mutations associated with the *BICC1* gene: one affecting the first KH domain and the other affecting the SAM domain [48], proving the relevance of the *Bic-C* animal models for understanding the etiology of this incurable disease.

In 3D cultures of mouse IMCD cells, depleting Bicc1 disrupts cadherin-mediated cell adhesion, normal epithelial polarization, proliferation, and apoptosis that prevent tubulomorphogenesis *in vitro* [59]. Interestingly, aspects of the *Drosophila* phenotype also affect cell migration and may influence cell-cell interaction and polarization. For example, migration of the follicle cells (FCs) in the ovary is defective in Bic-C mutant [3], resulting in eggs that remain open at the anterior end. This defect may occur because of inefficient communication between germ line and somatic cells, although to date we do not know the molecular pathway underlying this phenomenon (for an alternate possibility, see also Section 3.7).

In a recent paper [27] Tran and colleagues report that in a novel *Bicc1*$^{-/-}$ mutant mice and in *Xenopus* depleted for Bicc1 the *Pkd2* mRNA and its cognate protein are downregulated (29 and 54%, resp.), while both *Pkd1* and *Pdhd1* levels are unaffected. In the mouse these effects are clearest specifically at stage E18.5. The regulation appears to be mediated via a cellular microRNA, *miR-17* [27] that is also amplified in certain cancers [60]. Here Bicc1 may relieve the *miR-17*-mediated repression via a mechanism that does not involve regulation of the polyadenylation state of at least the mRNAs tested and may mildly impact mRNA stability [27]. The fact that the Bicc1 protein may bind multiple mRNAs and that it may be involved in the possible antagonistic regulation of the *miR-17* complexes, also assembled on multiple mRNAs, reinforces the view that the Bic-C orthologs are central to the regulation of many cellular processes and that many more aspects of their function await elucidation.

3.7. Other Bic-C Functions. Another hint to Bic-C function comes again from *Drosophila*, where the *Bic-C* mutants exhibit disrupted pattern of the cortical filamentous actin in the growing oocyte and abnormal actin-containing structures in the ooplasm that trap both the dorsal fate determinant Gurken [61–63] and other proteins that would normally be secreted [31, 32]. This function requires Trailer hitch, a protein originally identified in a screen for mutants for axial polarity that may regulate expression of endoplasmic reticulum (ER) exit site components on the ER surface. A malfunctioning secretory pathway could affect communication between the oocyte and the overlying FC and may affect their migration. Since many mRNAs involved in vesicular trafficking and/or organization of the actin cytoskeleton were also recovered in Bic-C immunoprecipitates [15], it is possible that their posttranscriptional control may contribute to the observed Bic-C defects. Lastly, and not mutually exclusive, the altered actin dynamics exhibited by the *Bic-C* and *Tral* mutants must also add to the observed inhibition of the normal dumping of nurse cell contents into the nascent oocyte during late oogenesis.

4. Concluding Remarks

Bic-C is an ancient protein conserved from *Drosophila* to man. Its mutation induces a pleiotropic phenotype. In fruit flies the Bic-C protein binds to RNAs involved in establishing the embryonic polarity, the Wnt pathway, actin dynamics and results in many observed defects, including abnormal development. In the vertebrates the better characterized aspect of lack of Bic-C function is the induction of cystic kidneys and the alteration of cell proliferation and three dimensional organization; however, defects in pancreatic and liver function and heterotaxia (i.e., randomization of the left-right symmetry) of the visceral organs have also been observed [26, 27]. Further, effects on the Wnt pathway have also been reported in human patients with renal displasia [48], as well as in mice and frogs [26]. Bicc1 is also expressed in the nervous system [58] which suggests that there may be novel aspects of its function ready to be discovered and that Bic-C homologs may be involved in fundamental, evolutionarily conserved mechanisms of determination of polarity, from establishment of the body axes to planar cell polarity.

The experimental evidence so far also suggests that Bic-C function may also be required at specific times of development in many species. Since Bic-C is a negative regulator of translation, we can expect at least part of the mutant phenotypes to be linked with inappropriate spatial and/or temporal regulation of gene expression. Further, Bic-C has multiple mRNA targets, and it exists in multiple isoforms in many organisms. At least in the case of one of the Bic-C interacting partners, the CCR4 deadenylase, it is proposed that multiple forms of this complex exist in higher vertebrates [47], as there are documented isoforms for a few of the complex subunits. Therefore, it is possible that the Bic-C-CCR4-dependent regulation acts via and is regulated by combinatorial mechanisms, with variant complexes having partially redundant function. This could also explain why all the individual molecular effects/phenotypes described for Bic-C tend to be mild and why years of concerted experimental efforts have yielded only a few proven targets for this gene, since many of the real targets would presumably not have been highly enriched compared to the controls.

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