Crystal Structure of Diedel, a Marker of the Immune Response of Drosophila melanogaster

Franck Coste¹, Cordula Kemp², Vanessa Bobezeau¹, Charles Hetru², Christine Kellenberger³, Jean-Luc Imler², Alain Roussel³*

¹ Centre de Biophysique Moléculaire, CNRS UPR4301, Orléans, France, ² Institut de Biologie Moléculaire et Cellulaire, UPR9022 CNRS, Université de Strasbourg, Strasbourg, France, ³ Architecture et Fonction des Macromolécules Biologiques, UMR6098 CNRS and Aix-Marseille Université, Marseille, France

Abstract

Background: The Drosophila melanogaster gene CG11501 is up regulated after a septic injury and was proposed to act as a negative regulator of the JAK/STAT signaling pathway. Diedel, the CG11501 gene product, is a small protein of 115 residues with 10 cysteines.

Methodology/Principal Findings: We have produced Diedel in Drosophila S2 cells as an extra cellular protein thanks to its own signal peptide and solved its crystal structure at 1.15 Å resolution by SIRAS using an iodo derivative. Diedel is composed of two sub domains SD1 and SD2. SD1 is made of an antiparallel β-sheet covered by an α-helix and displays a ferredoxin-like fold. SD2 reveals a new protein fold made of loops connected by four disulfide bridges. Further structural analysis identified conserved hydrophobic residues on the surface of Diedel that may constitute a potential binding site. The existence of two conformations, cis and trans, for the proline S2 may be of interest as prolyl peptidyl isomerisation has been shown to play a role in several physiological mechanisms. The genome of D. melanogaster contains two other genes coding for proteins homologous to Diedel, namely CG43228 and CG34329. Strikingly, apart from Drosophila and the pea aphid Acrythosiphon pisum, Diedel-related sequences were exclusively identified in a few insect DNA viruses of the Baculoviridae and Ascoviridae families.

Conclusion/Significance: Diedel, a marker of the Drosophila antimicrobial/antiviral response, is a member of a small family of proteins present in drosophilids, aphids and DNA viruses infecting lepidopterans. Diedel is an extracellular protein composed of two sub-domains. Two special structural features (hydrophobic surface patch and cis/trans conformation for proline S2) may indicate a putative interaction site, and support an extra cellular signaling function for Diedel, which is in accordance with its proposed role as negative regulator of the JAK/STAT signaling pathway.

Introduction

The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense. The innate immune system includes defenses that, for the most part, are constitutively present and ready to be mobilized upon infection. It is not antigen-specific and reacts equally well to a variety of organisms. Historically, the focus of most immunological studies has been on the adaptive response and its hallmarks, namely the generation of a large repertoire of antigen-recognizing receptors and immunological memory. Recently, however, more effort has been expended on understanding the innate immune system, as it became clear that innate immunity is an evolutionarily ancient defense mechanism, which governs the initial detection of pathogens and stimulates the first line of host defense. Invertebrates have proven to be a good model organism to study innate immunity as illustrated by the initial genetic identification of signaling pathways mediating antimicrobial peptide gene expression in Drosophila [1]. The induction of Toll and Imd pathways upon microbial detection leads to the activation of transcription factors of the NF-xb family [2] and then to the expression of hundreds of genes [3,4]. Ex vivo and in vivo studies have shown that the JAK/STAT pathway also contributes to inducible gene expression following infection, in particular in the case of viral infections [5–8]. The JAK/STAT signal transduction pathway is conserved from insects to mammals and is involved in a wide variety of biological processes such as the cellular proliferation, the stem cell maintenance, the haematopoiesis and the innate immunity responses [9]. This pleiotropic cascade is the principal signaling mechanism for a large array of cytokines and growth factors in mammals.

In Drosophila, this pathway is composed of the JAK kinase Hopscotch and the STAT factor STAT92E and is activated by the receptor Domeless, which is related to the gp130 subunit of the receptor for cytokines of the interleukin-6 family. Domeless is activated by cytokines of the Unpaired family (Upd, Upd2, Upd3).
These cytokines are expressed during development but also in response to stress, in particular during infections [9] [10].

In 2002, Perrimon and colleagues have used genome-wide expression profiling to analyze the contribution of different signaling pathways to the innate immune response. They reported that the *Drosophila melanogaster* gene *CG11501* is up-regulated after septic injury and that the JAK/STAT signaling pathway is involved in this induction [5]. This initial study was followed in the Boutros’ lab by a genome-wide RNA interference screen in *Drosophila* cells to identify novel genes involved in the regulation of the JAK/STAT pathway [11]. The *CG11501* gene was identified as a negative regulator of the JAK/STAT signaling pathway in this study, although its precise molecular function is still unknown [11].

The *CG11501* gene encodes a small cysteine-rich protein that we named Diedel. Diedel is 115 amino acids long and contains 10 cysteines. It displays a strong homology with the products of two other *Drosophila melanogaster* genes, *CG43229* and *CG43228*. Diedel does not seem to be conserved in living organisms outside drosophilids apart from the aphid *Acyrthosiphon pisum*. Curiously however, orthologues of this gene are present in the genome of insect DNA viruses of the *Baculoviridae* and *Ascoviridae* families.

In our effort to decipher the molecular mechanisms of the innate immune responses in *Drosophila* [12–15], we determined the crystal structure of the Diedel protein in two crystal forms at 1.15 Å and 1.45 Å resolution, respectively.

**Results**

**Structure determination**

The Diedel protein crystallized in two crystal forms. Form A crystals belonged to the orthorhombic space group *P*2₁2₁2₁, with unit-cell parameters *a* = 29.83 Å, *b* = 44.30 Å and *c* = 58.54 Å. Assuming the presence of one molecule in the asymmetric unit, the Matthews coefficient (*V*ₘ value) was calculated to be 1.83 Å³Da⁻¹ that gave an estimated solvent content of 33% [16]. The structure was solved by the SIRAS method using an iodo derivative and was refined to an R factor of 12.9% and an R-free factor of 15.1% to 1.15 Å resolution. Form B crystals belonged to the orthorhombic space group *P*2₁2₁2₂, with unit-cell parameters *a* = 49.43 Å, *b* = 78.29 Å and *c* = 21.72 Å. Assuming the presence of one Diedel molecule in the asymmetric unit, the *V*ₘ value was calculated to be 1.99 Å³Da⁻¹ with an estimated solvent content of 30% [16]. The structure in this crystal form was solved at 1.45 Å resolution by molecular replacement using the form A crystal structure as the starting model and was refined to an R factor of 16.0% and an R-free factor of 19.6%.

**Overall structure of Diedel**

The final model for form A includes the entire expressed protein from residue 25 to 115, the 24 first residues being the signal peptide that is cleaved upon exportation, two extra residues (116 and 117) being cloning artifacts, one thiocyanate molecule, one ethylene glycol molecule and 144 water molecules.

The overall structure shows a relatively elongated shape with approximate dimensions of 60×40×40 Å³ (Figure 1A), which can be divided into two sub-domains referred as SD1 and SD2 (Figure 1B). The SD1 sub-domain is made of two segments 29–59 and 90–117, and is composed of a four stranded anti parallel β-sheet (30–35, 48–49, 55–59, 109–113), one α-helix (92–99) and two 3₁₀ turns (43–46 and 100–103). The SD2 sub-domain is formed by the first four N-terminal residues (25–28) and by a long loop (residues 60 to 89) connecting the strand β3 and the α-helix of the sub-domain SD1. This domain is highly reticulated with four disulfide bridges; two of them tether the loop 60–89 to the N-terminal residues.

**Difference between Form A and Form B structures**

The structures solved with the crystal forms A and B have been superimposed with the program Turbo-Frodo. This superimposition indicates that the two structures are nearly identical with 76 Cz among the 92 (82%) of the model displaying equivalent positions in both molecules with distance between the superimposed Cz atoms less than 1 Å. As expected the differences occur in the N- and C-termini and in the tip of the loops. The main structural difference is located in the loop 51–54 with a distance between the Cz atoms of more than 5 Å for the residue 53. The change in the main chain trace is the result of a difference of peptide β-lization for the proline 52. Indeed this residue is in cis conformation in form A and in trans conformation in form B (Figure 2A). The residue P52 is not involved in crystal contact in both crystal forms A and B. Only one distance below 3.5 Å is found between Pro52 and the symmetry related molecules (3.41 Å between Pro52 CB and Gly53 C in form B). Nevertheless positioning the structure found in form A in the crystal packing of form B leads to several clashes (distance below 1.5 Å) between Pro52 and Gly53 of a symmetry related molecule. This is also the case, but in a lesser extend, when putting form B structure in form A crystal packing with few short contacts between Y51 and I48 of a symmetry related molecule. The remaining question is to know whether the different conformations are due to the crystal packing or alternatively if the crystallization leads to the separation of the two coexisting isomers. Unfortunately for the moment we have no data that allow us to answer this question.

**Structural comparisons**

A structural similarity search within the Protein Data Bank using the pairwise structural comparison server DALI [17] of the complete Diedel gave no statistically significant global similarities. Searching with the individual subdomains revealed that the overall architecture of SD1 is that of a ferredoxin-like fold (an antiparallel β-sheet covered on one side by two α-helices) with the highest Z-score of 5.4 for a domain of phosphomevalonate (PDB 1K47) with a core RMSD of 2.6 Å. The particularity of Diedel within the ferredoxin-like fold family is the presence of only one α-helix, the first α-helix of the standard fold being replaced by a short 3₁₀ helix. Therefore the secondary structure signature of Diedel SD1 domain is β₃α₁β₃β. A Dali search for similar structures to SD2 resulted in no hit.

**Phylogenetic analyses**

The genome of *D. melanogaster* contains two other genes coding proteins homologous to Diedel, namely *CG43228* (Diedel-2) and *CG34329* (Diedel-3). Diedel and *CG43228* are located very close to each other on the chromosome 3R whereas *CG34329* is located on the X chromosome. The synteny of these three genes is conserved among the melanogaster group (*D. melanogaster, D. simulans, D. sechellia, D. yakuba and D. erecta*) with some exceptions: (i) a fourth gene is present in *D. yakuba*; (ii) the gene located on the X chromosome in *D. sechellia* displays a mutation making it inoperative (unless it is a sequencing error); and (iii) only two genes have been retrieved in *D. erecta*, for which the annotation is not fully completed (Figure 3A). Interestingly, phylogenetic analyses indicate that these three genes originated between the speciation events in the melanogaster group (Figure 3B). A similar situation is found for the obscura, the replete and the virilis groups. This suggests that the common ancestor of drosophilids had a
single Diedel gene, which was subsequently duplicated independently in the different Drosophila groups.

Diedel molecules are not found in the sequenced genomes of other insects, with the exception of the pea aphid *Acyrthosiphon pisum*. Surprisingly, the two *A. pisum* genes encoding Diedel-like molecules are more closely related to the Diedel genes found in the melanogaster group than those found in the other drosophilids. Apart from Drosophila and pea aphid, Diedel related sequences were only identified in some insect DNA viruses of the Baculoviridae (*Pseudalata unipuncta* granulovirus, *Helicoverpa armigera* granulovirus and *Heliothis armigera* granulovirus) and Ascoviridae (*Spodoptera frugiperda* ascovirus) families (Figure 3B). Strikingly all these viruses infect lepidopterans and not dipterans. The independent acquisition of Diedel genes in two distinct families of DNA viruses [18] strengthens the connection between Diedel and the field of infectiology.

**Figure 1. Overall crystal structure of Diedel.** (A) Overall structure of Diedel. The structure is colored according to its secondary structure elements: α-helices (blue), 3_{10} helices (pink), β-strands (green), and loops (brown). The 10 cysteine residues involved in five disulfide bridges are displayed in yellow and labelled. The N and C terminus residues are mentioned. The figure was generated with PyMOL (http://www.pymol.org). (B) Topology diagram of Diedel. The color code for the secondary structure elements is similar to that in figure 1A. Diedel is composed of two sub domains named SD1 and SD2. While SD1 belongs to the ferredoxin-like fold family, SD2 display a quite original fold highly reticulated with four disulfide bridges. doi:10.1371/journal.pone.0033416.g001

**Discussion**

We have solved the crystal structure of Diedel in two crystal forms at 1.15 Å and 1.45 Å resolution, respectively. The structure is composed of two subdomains, one of these belonging to the ferredoxin family fold. The other one displays a particular fold highly reticulated by three disulfide bridges and not found in any other structure of the protein data bank. The name Diedel, which is the german translation of Tweedle, comes from the presence of two domains in such a little protein. Indeed, Tweedle-dum and Tweedle-dee are two little twins in Alice’s adventures in wonderland by Lewis Carroll. The lack of functional data for Diedel impedes a structure-function relationship analysis. However several molecular and structural features may be pointed out. Diedel is an extracellular protein, which presents a high level of stability certainly due to the presence of five disulfide bridges. Protein disulfide bonds are formed in the endoplasmic reticulum of eukaryotic cells upon exportation. All the sequences of Diedel-like proteins from Drosophila and aphids and most of viral sequences display a signal peptide for exportation. To the best of our knowledge, there is only one exception in the sequence from...
Xestia nigum granulovirus. In this case, the disulfide bonds may be formed in the intracellular medium upon the control of a sulfhydryl oxidase, an enzyme present in baculoviruses [19]. Diedel overall structure somewhat resembles that of certain cytokines of the CC or CXC chemokine families with a β-sheet covered by an α-helix. The conservation of several hydrophobic residues at the molecular surface may be the indication of a protein-protein interface. The existence of two conformations for the proline 52 may also have some significance. Indeed, an increasing number of reports indicate that peptidyl prolyl cis-trans isomerisation can play the role of a molecular switch in numerous physiological mechanisms [20]. In some proteins, proline isomerization may confer conformer-specific properties to a native protein fold by modulating the features its molecular surface [21]. All these features suggest an extra cellular signaling function for Diedel, which would be in accordance with its proposed role as negative regulator of the JAK/STAT signaling pathway. The determination of the tridimensional structure of Diedel paves the way for further studies at both the functional and structural levels to assess its role in the immune response of Drosophila.

Materials and Methods

Cloning, expression, and purification

Diedel overexpression and purification were performed as using protocols previously described [22]. Briefly, the amplified cDNA fragments of the CG11501 gene were subcloned into the SpeI/MseI site of the pMT/V5-His A plasmid (Invitrogen). The recombinant protein with its own signal peptide was overexpressed by induction of S2 cells with CuSO\textsubscript{4} at a cell density of 3.10\textsuperscript{6} cells ml\textsuperscript{-1}. After five days, cells were aseptically centrifuged, resuspended in fresh medium and induced again for five days. Up to ten inductions could be done using the same cells. The insoluble material was removed from the harvested medium by centrifugation at 4°C and 4,000 g for 5 min. The supernatant was clarified by filtering through 0.45 mm filter and loaded onto a chelating column (Chelating sepharose FF, GE Healthcare) equilibrated in 20 mM Na-phosphate pH 7.4 and 500 mM NaCl. Diedel was eluted with equilibration buffer containing 200 mM imidazole. Fractions containing Diedel were pooled, concentrated and buffer-exchanged into 20 mM Hepes pH 7.2 and 150 mM NaCl using a Millipore Ultrafree-15 spin concentrator with a 3 kDa molecular-weight cutoff. The V5-His tag used for purification was removed by an overnight trypsinolysis at 4°C using a trypsin:Diedel (w/w) ratio of 1:200. The cleavage product was then loaded onto a HiLoad 16/60 Superdex 75 gel-filtration column (GE Healthcare) equilibrated with 20 mM Hepes pH 7.2, 150 mM NaCl and 0.05% NaN\textsubscript{3}. The purified protein showed a single band with a molecular weight around 10,000 Da in SDS-PAGE as expected after cleavage. Maldi-Tof mass spectrometry analysis showed a main peak at 10,565 Da corresponding to a cleavage site between residues L119 and E120 of the V5-His tag.

Crystallization and data collection

The JCSG+ crystal screen (Molecular Dimensions) was used to search for the initial crystallization conditions using the hanging-
Crystal Structure of Diedel
The Diedel protein of Drosophila melanogaster was aligned to homologous gene products from Drosophila melanogaster (CG34329) and Pseudalatia unipuncta granulovirus (PuGV). The numbering is that of Diedel in this study. Secondary structure elements, i.e. strands and helices, are indicated below the sequences as arrows and rectangles, respectively. Conserved residues are boxed, and strictly conserved residues are shown in white with a red background. Note that the level of sequence identity is much more higher in the SD2 sub-domain than in the SD1 sub-domain. The figure was generated with ESPript [36].

doi:10.1371/journal.pone.0033416.g003

Crystal Structure of Diedel

Structure determination and refinement

To obtain experimental phases, we first considered using the 11 sulfur atoms present in the native crystals to conduct a sulphur SAD experiment. A highly redundant data set was collected in-house and show good statistics (table 1, Form A native) but was not suitable to give a clear solution to the phase problem. Therefore the quick-cryosocketing method was used. A crystal soaked in a solution containing 0.8 M NaI was collected in-house (table 1, NaI quick soak). The structure of Diedel was solved by SIRAS using SHELXD/E [27] as SAD on the NaI quick soak data set alone also failed to give a clear solution. As expected, phasing statistics were calculated at 2.3 Å resolution and improved by solvent flattening with DM [26]. Using the program ARP/wARP [26], the majority of the model was correctly automatically built. Incorrectly built remaining residues were manually modeled using Coot [29] and the model was refined in REFMAC [30].

The structure in the crystal form B was solved by molecular replacement with the program AMoRe [31] using the structure solved in crystal form A as starting model. Two strong peaks of electron density were found and were attributed to calcium ions due to the presence of 20 mM CaCl₂ in the cryocrystallization solution. The first calcium ion is coordinated by Asp 41 and Glu 49 as well as by Glu 25 of a symmetry related molecule. The second calcium ion stands on a crystallographic symmetry axis in the vicinity of Asp 91. None of these residues displays high level of conservation among the Diedel-related molecules.

The structures were analyzed with the program Turbo-Frodo [32]. The statistics on the structure refinement are summarized in Table 1.

Phylogenetic analyses

Sequences were retrieved from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) and Flybase (http://flybase.org/blast/) using the sequence

doi:10.1371/journal.pone.0033416.g004

Figure 3. Phylogenetic analysis of Diedel. (A) Genome localization of Diedel and related genes in drosophilids. Genes are named according to Flybase. The asterisk indicates that sequence has been re-analysed and differs from the annotated one. For each Drosophila species indicated the orientation and the position of the gene on the chromosome (3R, X, 2) or on a scaffold. The precise position is indicated by the number at the beginning and the end of each base on the sequence given on flybase. When the distances are not too long, the position respect a scale (for pseudoobscura, persimilis and virilis). In the case of virilis, the gene G11856 is duplicated. No new ID has been proposed. (B) Phylogeny of Diedel-related molecules. The names are proposed according to Flybase for Drosophila species. IDs of the viral molecules can be found in Table S1. In red the melanogaster subgroup, in green the obscura group, in blue the repleta and the virilis groups, in purple the sequences from viruses. The sequences are listed in Text S1.

doi:10.1371/journal.pone.0033416.g003

Figure 4. Amino acid sequence alignment of Diedel and other homologous proteins. The Diedel protein of Drosophila melanogaster was aligned to homologous gene products from Drosophila melanogaster (CG34329) and Pseudalatia unipuncta granulovirus (PuGV). The numbering is that of Diedel in this study. Secondary structure elements, i.e. strands and helices, are indicated below the sequences as arrows and rectangles, respectively. Conserved residues are boxed, and strictly conserved residues are shown in white with a red background. Note that the level of sequence identity is much more higher in the SD2 sub-domain than in the SD1 sub-domain. The figure was generated with ESPript [36].

doi:10.1371/journal.pone.0033416.g004
retrieval system or/and basic local alignment search tool (BLAST) [33]. Not annotated sequences were found by similarity search and predicted using the gene prediction tools ‘‘GENSCAN’’ (http://genes.mit.edu/GENSCAN.html) and ‘‘Eukaryotic Gene-Mark.hmm’’ (http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi) and by a manual and careful analysis. Alignments were carried out using clustalW (ref), MUSCLE [34] (www.phylogeny.fr) or COnstraint-Based multiple Alignment Tool (COBALT) [35] (www.ncbi.nlm.nih.gov/tools/cobalt).

Phylogenetic trees were constructed on the basis of amino acid differences using PhyML [34] (www.phylogeny.fr), Fast Minimum Evolution, Neighbor joining and Cobalt Tree [35] (www.ncbi.nlm.nih.gov/blast/treeview). Reliability of the trees was assessed by bootstrapping and comparison between the methods. The number of bootstraps cycles performed for the analysis was 100. The median bootstrap values for the phylogenetic trees were not less than 98%.

Data deposition

Atomic coordinates and structure factors have been deposited in the RSCB Protein Data Bank under the accession codes 3ZZO (Form A) and 3ZZR (Form B).

### Table 1. Data collection and refinement statistics.

| Data collection statistics | Form A (Native) | Form A (NaI quick soak) | Form A (HR) | Form B |
|----------------------------|------------------|------------------------|-------------|--------|
| Radiation source           | In-house         | In-house               | ESRF ID23-EH1 | ESRF ID14-EH1 |
| Wavelength (Å)             | 1.5418           | 1.5418                 | 0.9834       | 0.9340 |
| Spacegroup                 | P2₁2₁2₁          | P2₁2₁2₁                | P2₁2₁2₁      | P2₁2₁2₁ |
| Cell dimensions a, b, c (Å) | 29.83, 44.30, 58.54 | 29.82, 44.58, 59.03 | 29.92, 44.58, 59.01 | 49.43, 78.29, 21.72 |
| Resolution range (Å)       | 26.58–1.90 (2.00–1.90) | 22.85–2.30 (2.42–2.30) | 19.00–1.15 (1.21–1.15) | 49.43, 78.29, 21.72 |
| Total observations          | 87402 (12028)    | 31543 (4535)           | 92621 (11406) | 105291 (12056) |
| Unique reflections          | 6485 (907)       | 3811 (536)             | 28072 (3995) | 15572 (2147) |
| Completeness (%)            | 99.5 (98.6)      | 99.9 (100.0)           | 98.0 (97.6)  | 99.5 (97.8) |
| Redundancy                  | 13.5 (13.3)      | 7.8 (7.9)              | 3.3 (2.9)    | 6.8 (5.6) |
| Rmerge²                    | 6.7 (20.6)       | 3.7 (6.3)              | 8.6 (26.3)   | 6.8 (24.1) |
| Average I/σ(I)             | 10.2 (3.5)       | 15.4 (10.4)            | 9.8 (3.1)    | 19 (6.4) |

| Refinement and model statistics | Form A (HR) | Form B |
|---------------------------------|-------------|--------|
| Resolution range (Å)            | 18.00–1.15  | 20.00–1.45 |
| Number of reflections used      | 26611       | 14757  |
| Rwork (%)¹ / Rfree (%)²         | 12.89 / 15.13 | 15.97 / 19.57 |
| Average B values                |             |        |
| All atoms (Å²)                  | 12.34       | 14.78  |
| Protein atoms (Å²)              | 10.37       | 12.48  |
| Thiocyanate atoms (Å²)          | 7.73        | -      |
| Ethylene glycol atoms (Å²)      | 12.82       | -      |
| MPD atoms (Å²)                  | -           | 24.76  |
| Ca atoms (Å²)                   | -           | 15.69  |
| Water atoms (Å²)                | 22.42       | 23.90  |
| Root mean square deviation from ideality |        |
| Bond lengths (Å)                | 0.018       | 0.021  |
| Bond angles (°)                 | 1.595       | 1.988  |
| Torsion angles (°)              | 5.869       | 6.820  |
| Ramachandran analysis           |             |        |
| Favoured regions/ Allowed regions / Outliers (% of residues) | 96.7 / 3.3 / 0.0 | 94.6 / 4.3 / 1.1 |
| No. of atoms                    |             |        |
| Protein                        | 731         | 732    |
| Thiocyanate                    | 3           | -      |
| Ethylene glycol                 | 4           | -      |
| MPD                            | -           | 32     |
| Ca                             | -           | 2      |
| Water                          | 144         | 150    |

²Rmerge = ΣhΣ|<|Ih,i>|Ih,i|^2|/ΣhΣ|<|Ih,i>| where <|Ih,i>| is the mean intensity of the symmetry-equivalent reflections.

¹Rwork = Σh|F_o| - |F_c| / |Σh|F_o| where |F_o| and |F_c| are the observed and calculated structure factor amplitudes, respectively, for reflection h.

²Rfree is the R value for a subset of 5% of the reflection data, which were not included in the crystallographic refinement.

doi:10.1371/journal.pone.0033416.t001
Supporting Information

Text S1 Protein sequences of analogues of CG11501.

Acknowledgments

We thank the European Synchrotron Radiation Facility (ESRF) at Grenoble and in particular the beamline ID14 staff for their assistance. We also thank Estelle Santiago for expert technical assistance.

References

1. Lemaitre B, Hoffmann J (2007) The host defense of Drosophila melanogaster. Annu Rev Immunol 25: 697–743.
2. Hedengren M, Alding B, Dushay MS, Ando I, Eguren S, et al. (1999) Relish, a central factor in the control of humoral but not cellular immunity in Drosophila. Mol Cell 4: 827–837.
3. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86: 973–983.
4. Lemaitre B, Kromer-Metzger E, Michaut L, Nicolas E, Meister M, et al. (1995) A recessive mutation, immune deficiency (imd), defines two distinct control pathways in the Drosophila host defense. Proc Natl Acad Sci U S A 92: 9465–9469.
5. Boutros M, Agaisse H, Perrimon N (2002) Sequential activation of signaling pathways during innate immune responses in Drosophila. Dev Cell 3: 711–722.
6. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N (2003) Signaling role of hemoocytes in Drosophila JAK/STAT-dependent response to septic injury. Dev Cell 5: 441–450.
7. Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, et al. (2005) The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nat Immunol 6: 946–953.
8. Souza-Neto JA, Sim S, Dimopoulos G (2009) An evolutionary conserved function of the JAK-STAT pathway in anti-dergene defense. Proc Natl Acad Sci U S A 106: 17841–17846.
9. Arbouzova NI, Zeidler MP (2006) JAK/STAT signalling in Drosophila: insights into conserved regulatory and cellular functions. Development 133: 2605–2616.
10. Agaisse H, Perrimon N (2004) The roles of JAK/STAT signaling in Drosophila immune responses. Immunol Rev 198: 72–92.
11. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N (2003) Signaling role of hemoocytes in Drosophila JAK/STAT-dependent response to septic injury. Dev Cell 5: 441–450.
12. Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, et al. (2005) The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of drosophila. Nat Immunol 6: 946–953.
13. Mishima Y, Coste F, Herveau V, Hervouet N, Kellenberger C, et al. (2009) Expression, purification, crystallization and preliminary X-ray analysis of the N-terminal domain of GNBP3 from Drosophila melanogaster. Acta Crystallogr Sect F Struct Biol Cryst Commun 65: 876–873.
14. Dauter Z, Dauter M, Rajashankar KR (2000) Novel approach to phasing proteins: derivatization by short cryo-soaking with halides. Acta Crystallogr D Biol Crystallogr 56: 232–237.
15. Kabsch W (2010) Xds. Acta Crystallogr D Biol Crystallogr 66: 125–132.
16. Evans P (2006) Scaling and assessment of data quality. Acta Crystallogr D Biol Crystallogr 62: 72–82.
17. CCP4 (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr D Biol Crystallogr 50: 760–763.
18. Sheldrick GM (2008) A short history of SHELX. Acta Crystallogr A 64: 112–122.
19. Perekatsa A, Morris R, Lamzin VS (1996) Automated protein model building combined with iterative structure refinement. Nat Struct Biol 6: 438–463.
20. Emaley P, Costant K (2004) Cosnt: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 60: 2126–2132.
21. Murshudov GN, Vagin AA, Dodson EJ (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr D Biol Crystallogr 53: 240–255.
22. Navaza J (2001) Implementation of molecular replacement in AMoRe. Acta Crystallogr D Biol Crystallogr 57: 1367–1372.
23. Ronsel A, Camilliau C (1991) Turbo-Frodo. In: Graphics S, ed. Silicon Graphics Geometry Partners Directory Mountain View, CA. 85 p.
24. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.
25. Oury J, Desnoyers G (1994) Molecular replacement calculation. Acta Crystallogr D Biol Crystallogr 50: 760–763.
26. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410.
27. Durepere A, Guignion V, Blance G, Audic S, Buffet S, et al. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36: W465–469.
28. Papadopoulos JS, Aragawala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences. Bioinformatics 23: 1073–1079.
29. Goertzen P, Robert X, Courcelle E (2003) ESPript/ENDscript: Extracing and rendering sequence and 3D information from atomic structures of proteins. Nucleic Acids Res 31: 3320–3323.