The *Drosophila* homolog of NTF-2, the nuclear transport factor-2, is essential for immune response

Ananya Bhattacharya & Ruth Steward⁺

Waksman Institute, Department of Molecular Biology and Biochemistry, Cancer Institute of New Jersey, Rutgers University, 190 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA

Received October 11, 2001; revised and accepted February 21, 2002

Nuclear transport factor-2 (NTF-2) functions in yeast and mammalian cell culture in targeting proteins into the nucleus. The *Drosophila* homolog, DNTF-2, is an essential component of the nuclear import machinery, since *ntf* mutants are lethal. Interestingly, hypomorphic alleles show specific phenotypes. Some are viable, but the number of ommatidia in the eye is severely reduced. The immune response in the *Drosophila* larval fat body is also affected; the three NF- κ B/Rel proteins Dorsal, Dif and Relish do not target to the nucleus after infection, and, consequently, the expression of the anti-microbial peptide genes drosomycin, attacin and drosocin is severely impaired. Hence, in spite of its general requirement in many developmental processes, DNTF-2 has a higher specific requirement in the development of the eye and in the immune response. We also found that DNTF-2 interacts directly with Mbo/DNup88, which does not contain phenylalanine-glycine-rich repeats, but has been shown to function in the import of Rel proteins.

INTRODUCTION

The innate immune systems of insects and mammals recognize distinct classes of microbes and activate effector genes through conserved pathways. The Toll signaling cascade upon immune challenge culminates in the activation of the NF- κ B/Rel family of transcription factors. In *Drosophila*, a septic wound induces rapid transcriptional induction of a battery of anti-microbial peptide genes including attacin, cecropin, defensin, metchnikowin, diptericin and drosomycin (Hultmark, 1993; Dushay and Eldon, 1998), which are synthesized mostly in the fat body. Metchnikowin and cecropin have both anti-bacterial and anti-fungal properties, whereas drosomycin acts specifically

against fungi (Levashina *et al.*, 1998; Ekengren and Hultmark, 1999; for reviews, see Hoffmann *et al.*, 1999; Khush and Lemaitre, 2000). The activation of the peptides is regulated by the three Rel proteins, Dif, Dorsal and Relish, all of which are activated in response to immune challenges in the fat body (Ip *et al.*, 1993; Reichhart *et al.*, 1993; Lemaitre *et al.*, 1995; Stoven *et al.*, 2000). Dorsal and Dif are normally retained in the cytoplasm bound to Cactus, the I κ B homolog, but are imported to the nucleus upon microbial challenge (for a review, see Govind, 1999). Adults mutant for Dif fail to show induction of drosomycin and defensin, which is active against Gram-positive bacteria (Manfruelli *et al.*, 1999; Meng *et al.*, 1999; Rutschmann *et al.*, 2000), whereas adults mutant for Dorsal show normal induction of the anti-microbial peptide genes in response to infection (Lemaitre *et al.*, 1995). However, in larvae, Dorsal acts redundantly with Dif to induce expression of drosomycin (Manfruelli *et al.*, 1999; Rutschmann *et al.*, 2000). Relish, a compound Rel protein with an N-terminal Rel homology domain and a C-terminal I κ B-like domain, similar to mammalian p100 and p105 (Dushay *et al.*, 1996), is activated by signal-dependent proteolysis. This liberates the N-terminal Rel domain, allowing it to translocate into nuclei (Stoven *et al.*, 2000). Adults that lack Relish completely fail to induce the anti-bacterial peptides diptericin and cecropin and show reduced induction of the other anti-microbial peptides (Hedengren *et al.*, 1999). Thus, the nuclear import of these Rel proteins is a key step in the manifestation of an immune response.

Proteins destined for nuclear import containing the nuclear localization sequence (NLS) are transported across the double membrane of the nuclear envelope through nuclear pore complexes (NPCs) in a complex multi-step process (for reviews, see Jans *et al.*, 2000, and references therein). The NLS of Dorsal

⁺Corresponding author. Tel: +1 732 445 3917; Fax: +1 732 445 5735; E-mail: steward@mbcl.rutgers.edu

has been shown to be essential and sufficient for nuclear import (Govind *et al.*, 1996). The recognition of the NLS-containing protein by the transporter complex is a key event in the pathway leading to nuclear import. The NPC, a well-ordered structure containing 50–100 different proteins and located at distinct regions of the nuclear membrane, has been shown to interact with transport receptors. Recent studies have identified a nucleoporin (Nup88 or *mbo*) that is involved in the import of a subset of proteins, among them the Rel proteins (Uv *et al.*, 2000). This suggests that the presence of individual nucleoporins, showing substrate specificity within the NPC, is required for distinct import pathways.

Nuclear transport factor-2 (NTF-2) is an essential gene in both yeast and *Caenorhabditis elegans* (Corbett and Silver, 1996; Quimby *et al.*, 2000). We investigated the function of NTF-2 in *Drosophila*—in particular, whether import of Rel proteins after an immune challenge is mediated through the basic cellular machinery and whether it involves NTF-2. In this paper, we characterize the *Drosophila* homolog of NTF-2. We observe that DNTF-2 is an essential component of the nuclear import machinery, since mutants in *ntf* are lethal. Hypomorphic, viable alleles show a reduced eye phenotype, indicating that a higher level of NTF-2 is required for eye development than for viability. In these mutants, nuclear translocation of Dorsal, Dif and Relish is also impaired in response to bacterial infection, and, consequently, induction of the anti-microbial peptides drosomycin, attacin and drosocin is severely reduced. We also find that DNTF-2 is associated with Mbo/DNup88, which has been shown to function in the import of Rel proteins. Thus, DNTF-2 plays an essential role in the pathway leading to immune response.

RESULTS AND DISCUSSION

Isolation of the *Drosophila* homolog of NTF-2

After searching for genes that may control nuclear targeting of Rel proteins, we found that the *Drosophila* homolog of NTF-2 maps to the 19E region of the X chromosome. A P-element enhancer trap line *l(1)G0428* was mapped between the break-points of *Df(1)T2-14A* (19E5-19E7,8, Flybase Personal Communications; see <http://flybase.bio.indiana.edu/bin/fbpcq.html>). We cloned the genomic DNA flanking the enhancer trap by plasmid rescue, and sequence analysis of this genomic fragment revealed that the P element was inserted 5 bases upstream of the annotated gene corresponding to NTF-2. The organization of the *ntf-2* gene is shown in Figure 1B. The sequence of a 1.3 kb cDNA (GM06333, obtained from Research Genetics) identified an open reading frame of 130 amino acids. Protein database searches yielded a family of *ntf-2* genes cloned from various species. The predicted *Drosophila* protein was 42% identical to the yeast protein, 39% identical to the human protein and 47% identical to the *C. elegans* protein (Figure 1A).

Using the cDNA as a probe, the expression profile of *ntf* was determined. Two transcripts of 3.0 and 2.0 kb were present throughout development in roughly equal amounts (Figure 1C). The highest amount of mRNA was seen in ovaries and early embryos and was present at lower levels during larval and pupal stages. These transcripts were uniformly distributed throughout oogenesis and early embryogenesis.

Fig. 1. (A) Amino-acid sequence of DNTF-2. The sequence is aligned with human, *C. elegans*, *Xenopus laevis* and *Saccharomyces cerevisiae* NTF-2 sequences. Residues that are identical in all known NTF-2 proteins are shown in red. (B) Organization of the DNTF-2 genomic region. The solid blue boxes represent exons, empty boxes represent introns. The position of the P element *l(1)G0428*, a few bases upstream from the start of the cDNA, is shown as a red triangle. (C) RNA expression profile of the *ntf-2* gene. In the upper panel, a blot of poly(A)⁺ RNA is probed with the *ntf-2* cDNA. E refers to the RNA from the three embryonic stages, 0–4 h, 4–8 h and 8–16 h. L1–3 represent the three larval stages; P, pupal stage; O, ovaries; F, females and M, male RNA. The same blot was probed with *RpS5*, encoding the ribosomal protein (lower panel). (D) Hypomorphic mutants in the *ntf-2* gene show abnormalities in eye development. (a) Mutants have small eyes with a strongly reduced number of ommatidia. (b) The wild-type eye.

NTF-2 function is required throughout development

Homozygous *l(1)G0428* mutants die as late third instar larvae. To confirm that the lethality in *l(1)G0428* was due to the insertion of the enhancer trap into the *ntf* gene only, we isolated wild-type revertants that had lost the P element. In this screen, we also isolated several hypomorphic alleles in which the males were viable and fertile. All lines showed an eye phenotype that ranged from the virtual absence of all ommatidia to eyes that were reduced in size (see, for example, Figure 1D). The nuclear import of Rel proteins (see below) was also rescued in these hypomorphic alleles and in the wild-type revertants (data not shown). This result shows that the P-element mutation was responsible for both the lethal and immune-response phenotypes.

A. Bhattacharya & R. Steward

To confirm that the mutation affects only the *ntf-2* gene, we established transgenic lines. The UAS–cDNA transgene, expressed under the control of the *armadillo* driver, could rescue the lethality of the P element. The rescued males showed an eye phenotype similar to that seen in some hypomorphic alleles. This phenotype indicates that eye development is particularly sensitive to levels of NTF-2.

The immune response is affected in *P[ntf]* mutants

Infection of larvae or adults with bacterial suspension results in the rapid transcriptional activation of genes encoding anti-microbial peptides (Lemaître *et al.*, 1997; Hoffmann *et al.*, 1999). We examined whether this immune response is dependent on DNTF-2 function by assaying the induction of some of the immune peptide genes in *ntf* mutant larvae. The P-element insertion in the *ntf* gene caused the mutants to die as late third instar larvae. We administered the immune challenge at least 24 h before they died. Heterozygous and homozygous female larvae were morphologically indistinguishable and were sorted based on the green fluorescent protein (GFP)-expressing balancer chromosome. To examine the response to the immune challenge, we used a GFP-tagged drosomycin reporter construct (Ferrandon *et al.*, 1998) and compared the levels of induction between the wild-type larvae and *ntf-2* mutant larvae 6 h after induction (Figure 2A). The induction of drosomycin expression was not observed in immune-challenged homozygous mutant larvae (bottom panel C), while wild-type larvae showed strong induction after an immune challenge (top panel C). Both wild-type and *ntf-2* larvae that were not challenged showed no expression of GFP (top and bottom panels U).

In a parallel approach, we compared the induction of transcription of the immune peptide genes attacin, drosocin and drosomycin between heterozygous and homozygous *P[ntf]* third instar larvae. Northern blots with total RNA from non-challenged and induced larvae were probed with drosomycin, attacin and drosocin cDNA probes (Figure 2B). Both wild-type and heterozygous mutant larvae showed induction of these three peptide genes, whereas all three genes were induced only slightly (~20% of wild-type levels) in homozygous mutant larvae (Figure 2C). Wild-type and *ntf* heterozygous larvae that were not immune challenged do not show any induction of the peptide genes.

DNTF-2 is required for nuclear translocation of the Rel proteins Dorsal, Dif and Relish

During early embryogenesis, the *Drosophila* NF- κ B protein Dorsal is released from the I κ B homolog Cactus in response to signaling from the Toll receptor and becomes nuclear on the ventral side of the embryo. The same pathway also functions later in development. Upon infection with a bacterial suspension, Dorsal, Dif and Relish target rapidly to the nucleus of larval fat bodies (Reichhart *et al.*, 1993; for a review, see Govind, 1999). We induced an immune response in homozygous *ntf* female larvae and their heterozygous siblings and analyzed localization of the Rel proteins in fat bodies. Instead of looking at the endogenous Dorsal protein distribution, we used a GFP-tagged *dorsal* transgene, expressed under the control of the *dorsal* promoter. This transgene behaves like the endogenous

Fig. 2. *P[ntf]* mutant larvae show low induction of the anti-microbial peptides when challenged with a bacterial solution. (A) Comparison of the induction of the GFP-tagged drosomycin reporter gene between wild-type and *l(1)G0428/l(1)G0428* larvae. U represents bacterially unchallenged larvae; C, challenged larvae. (B) Northern blots of total RNA samples from wild-type, *l(1)G0428/+* and *l(1)G0428/l(1)G0428* larvae probed with cDNA probes corresponding to drosomycin, attacin and drosocin genes. The *RpS5* loading control is shown at the bottom. (C) Profiles of anti-microbial gene induction. The data from the northern analyses shown in (B) were quantitated by phosphorimager analysis, and the amount of induction is calculated as a percentage of *RpS5* RNA.

gene in early embryos (data not shown) as well as in the immune response (see Methods). The wild-type and homozygous *ntf* mutant larvae, also homozygous for the *dl-GFP* transgene, were reared under identical conditions. In non-challenged larvae of all genotypes, expression of the transgene is very faint and predominantly cytoplasmic (Figure 3B and H). Upon infection with the bacterial suspension, *dl-GFP* is clearly visible in the fat

Fig. 3. The Rel proteins Dorsal, Dif and Relish do not target to the nuclei of fat body cells in response to bacterial challenge in *ntf* mutants. The distribution of Dorsal, Dif and Relish proteins in unchallenged wild-type (A–C, M and N), challenged wild-type (D–F, O and P), unchallenged larvae from *l(1)G0428/l(1)G0428* (G–I, Q and R) and challenged larvae from *l(1)G0428/l(1)G0428* (J–L, S and T) are shown. The nuclei stained with Hoechst are shown in (A, D, G, J, M, O, Q and S). In the homozygous mutant fat bodies, none of the Rel proteins are seen in the nuclei after bacterial infection (K, L and T), in contrast to the wild type, where all three Rel proteins are translocated to nuclei after bacterial infection (E, F and P).

body nuclei in wild-type larvae 2 h after infection (Figure 3E), whereas *dl-GFP* remained faint and cytoplasmic in *ntf* mutants (Figure 3K). Both Dif and Relish target to the fat body nuclei 2 h or 30 min after infection (Figure 3F and P). Both these Rel proteins failed to target to the nucleus in immune-challenged *ntf* mutant larvae (Figure 3L and T). Thus, higher levels of DNTF-2 are essential in targeting all three Rel proteins to the nuclei upon an immune challenge than are required for viability.

Detailed analysis of the requirements for high levels of nuclear import of Dorsal in embryonic development has revealed both the presence of its NLS and phosphorylation to be essential (Govind *et al.*, 1996; Drier *et al.*, 1999, 2000). Thus, the NLS of Dorsal in conjunction with its signal-dependent phosphorylation could provide a substrate for a distinct complex of transport factors. Less is known about the sequences essential for nuclear targeting of Dif and Relish. Both have an NLS, and also the closely linked Serine residue, which plays an important role in the control of nuclear import in Dorsal. Thus, the three proteins could be recognized by different NLS receptors, since their NLS is not identical, but still DNTF-2 is an essential part of the import machinery of all three proteins.

DNTF-2 participates in a multi-protein complex to target Dorsal to the nucleus

Studies from other systems show that NTF-2 interacts physically with Ran, a small GTPase (Clarkson *et al.*, 1996), and nucleoporin

p62 via the phenylalanine-glycine (XFXFG) repeats (Paschal and Gerace, 1995). Mbo, a homolog of the mammalian Nup88, is known to function in the nuclear import of Rel proteins (Uv *et al.*, 2000). Our experiments point to DNTF-2 playing a key role in nuclear targeting of Rel proteins. We investigated whether NTF-2 forms part of a multi-protein complex by performing glutathione *S*-transferase (GST) pull-down assays using *Drosophila* embryonic extracts. DNTF-2 was expressed as a GST-tagged protein and immobilized on glutathione-Sepharose beads. These beads were incubated with extracts from 0–4-h-old embryos, followed by western analysis. GST–DNTF-2 binds DNup88/Mbo (Figure 4A, lane 7) but not Dorsal (lane 3). As a positive control, we immobilized GST-tagged full-length Cactus and, as expected, Dorsal bound strongly to the Cactus beads (lane 4). Further, GST–Dorsal beads also pulled down DNup88 from the embryonic extracts (lane 6). Neither Mbo nor Dorsal bound to GST alone (lanes 8 and 2).

To investigate whether the association of DNTF-2 with DNup88 is direct, we used the same fusion proteins, GST, GST–DNTF-2 and GST–Cactus and *in vitro* translated ³⁵S-labeled Dorsal and DNup88 proteins. We observed that GST–DNTF-2 bound only DNup88 (Figure 4B, lane 7) and not Dorsal (lane 3). Cactus beads bound Dorsal strongly (lane 4). GST alone did not show any binding (lanes 2 and 6). The binding of DNup88 to DNTF-2 was somewhat surprising because Nup88 does not contain any XFXFG repeats; these repeats have been shown to be essential for the interaction

Fig. 4. GST-DNTF-2 associates with the *Drosophila* homolog of Nup88 (DNup88)/Mbo. (A) Western blot probed with anti-Mbo antibody show binding of DNup88 from embryonic extracts with GST-DNTF-2 (lane 7) and weaker interaction with Dorsal (lane 6) but not with GST alone (lane 8). In contrast, Dorsal does not associate with GST-DNTF-2 (lane 3) and GST alone (lane 2) but associates with GST-Cactus (lane 4), the positive control. (B) DNup88 directly binds to DNTF-2. cDNA clones of DNup88 and Dorsal were *in vitro* translated with [³⁵S]met and tested for binding with GST-DNTF-2, GST-Cactus and GST alone. Only DNup88 shows direct binding with GST-DNTF-2 (lane 7), whereas Dorsal (lane 3) shows no association with DNTF-2. As a positive control, Dorsal strongly binds to GST-Cactus (lane 4). As a negative control, all the translated proteins were tested for their binding with GST alone (lanes 2 and 6), which shows no binding.

between NTF-2 and Nup62 (Clarkson *et al.*, 1996). Bayliss *et al.* (1999) showed that an NTF-2 protein lacking the XFXFG-binding domain still bound to the cytoplasmic and nucleoplasmic sides of the nuclear pore, but not to the central channel, like the wild type. This residual binding might be due, at least in part, to the interaction of NTF-2 with Nup88 and also with other Nups.

NTF-2 interacts with Nup62 in *Drosophila* (data not shown), as it does in vertebrate cells. We found that NTF-2 also interacts

directly with Nup88. Nup88 is found complexed with Dorsal, albeit indirectly (Uv *et al.*, 2000). The current model for nuclear import predicts the presence of two separate complexes. Complex formation between NTF-2 and Ran-GDP is necessary to import Ran into the nucleus and generate a nucleocytoplasmic Ran gradient. This in turn regulates the import of the cargo-importin- α -importin- β complex. According to this model, the two complexes do not need to interact (Quimby *et al.*, 2000; Ström and Weis, 2001). However, our results indicate that the complexes may interact at the nuclear pore when they bind Nup88, since NTF-2 is probably associated with Ran, and Dorsal is probably part of the importin complex. This binding could represent a pivotal event in the nuclear import process.

CONCLUSION

We found that DNTF-2 levels higher than that required for survival are essential for eye development and controlled nuclear import of the NF- κ B/Rel proteins in immune responses in *Drosophila*. We also found that NTF-2 interacts with Nup88/Mbo, which forms a complex with Dorsal and is essential for nuclear import of Rel proteins (Uv *et al.*, 2000). This suggests that Nup88 forms a link between the Ran-NTF-2 and Rel-importin complexes. The NF- κ B/Rel pathway is highly conserved in vertebrates, and it is likely that there is the same requirement both for higher levels of NTF-2 and for the function of Nup88 in the vertebrate immune response. Misregulation of NF- κ B/Rel proteins in vertebrates has been shown with a variety of hematopoietic tumors (Luque and Gelinas, 1997). Interestingly, chromosome translocations or inversions that result in gene fusions between NUP98, another nucleoporin, and several homeobox genes are found in human acute myelogenous leukemia (Arai *et al.*, 1997; Nakamura *et al.*, 1999). It remains to be seen whether the nuclear targeting of NF- κ B/Rel proteins is affected by these chromosomal rearrangements.

METHODS

Flies. The *l(1)G0428* stock was obtained from the Bloomington Stock Center (Bloomington, IN). The P element was mobilized, and several wild-type and other excision strains were obtained.

pUASP(w⁺)-GFP-NTF-2 cDNA fusion was expressed under the control of the *armadillo*-Gal4 driver and checked for the ability to rescue *l(1)G0428*. GFP was not expressed in any of the lines. Flies expressing Dorsal GFP carried a transgene expressing Dorsal tagged at the C-terminus with GFP under its own promoter.

Immune response. *ntf* mutant larvae were distinguished from their heterozygous siblings using a Balancer chromosome expressing GFP under the actin promoter. The immune response was assayed as described by Lemaitre *et al.* (1995).

Immunohistochemistry. Third instar larvae were dissected inside-out in phosphate-buffered saline, fixed and stained as described by Stoven *et al.* (2000). For localization of Dif, polyclonal anti-Dif antibody (a gift from D. Ferrandon, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France) was used at a dilution of 1:500. For localization of Relish, polyclonal anti-relish (RHD) antibody (a gift from D. Hultmark, Umea University, Umea, Sweden; Stoven *et al.*, 2000) was used at 1:1000 dilution.

In vitro translation and GST-fusion protein binding assay. Bacterially expressed GST-fusion proteins were synthesized and purified as described previously (Smith and Johnson, 1988). Embryonic extracts were made from ~100 µl of dechorionated, 0–4-h-old embryos. *In vitro* translation of *dorsal* and *mbo* cDNAs were carried out as described by Lin *et al.* (2000). The incorporation of [³⁵S]met to each of the reactions was quantitated by the ImageQuant software, and equivalent amounts of labeled proteins were used for binding to the beads.

ACKNOWLEDGEMENTS

We thank Dominique Ferrandon for anti-Dif, Dan Hultmark for anti-Relish and Christos Samakovlis for anti-Mbo antibodies. We also thank Ursula Stochaj, Celine Gelinis, Svetlana Minakhina and Cordelia Rauskolb for helpful comments on the manuscript, Leslie H. Huang and Lynn Donnelly for technical assistance, and Le Nguyen for the fly food. This work was supported by grants from the National Institute of Health (PHS HD 18055) and the Horace W. Goldsmith Foundation.

REFERENCES

- Arai, Y., Hosoda, F., Kobayashi, H., Arai, K., Hayashi, Y., Kamada, N., Kaneko, Y. and Okhi, M. (1997) The inv(11)(p15q22) chromosome translocation of *de novo* and therapy-related myeloid malignancies result in fusion of the nucleoporin gene, NUP98, with the putative RNA helicase gene DDX10. *Blood*, **89**, 3936–3944.
- Bayliss, R., Ribbeck, K., Akin, D., Kent, H.M., Feldherr, C., Görlich, D. and Stewart, M. (1999) Interaction between NTF2 and xFG-containing nucleoporins is required to mediate nuclear import of RanGDP. *J. Mol. Biol.*, **293**, 579–593.
- Clarkson, W.D., Kent, H.M. and Stewart, M. (1996) Separate binding sites on nuclear transport factor 2 for GDP-Ran and the phenylalanine-rich repeat regions of nucleoporins p62 and Nsp1p. *J. Mol. Biol.*, **263**, 517–524.
- Corbett, A.H. and Silver, P.A. (1996) The NTF2 gene encodes an essential, highly conserved protein that functions in nuclear transport *in vivo*. *J. Biol. Chem.*, **271**, 18477–18484.
- Drier, E.A., Huang, L.H. and Stewart, R. (1999) Nuclear import of the *Drosophila* Rel protein Dorsal is regulated by phosphorylation. *Genes Dev.*, **13**, 556–568.
- Drier, E.A., Govind, S. and Stewart, R. (2000) Cactus-independent regulation of Dorsal nuclear import by the ventral signal. *Curr. Biol.*, **10**, 23–26.
- Dushay, M.S. and Eldon, E.D. (1998) *Drosophila* immune responses as models for human immunity. *Am. J. Hum. Genet.*, **62**, 10–14.
- Dushay, M.S., Asling, B. and Hultmark, D. (1996) Origins of immunity: Relish, a compound Rel-like gene in the antibacterial defense of *Drosophila*. *Proc. Natl Acad. Sci. USA*, **93**, 10343–10347.
- Ekengren, S. and Hultmark, D. (1999) *Drosophila* cecropin as an antifungal agent. *Insect Biochem. Mol. Biol.*, **29**, 965–972.
- Ferrandon, D., Jung, A.C., Cricui, M.-C., Lemaitre, B., Uttenweiler-Joseph, S., Michaut, L., Reichart, J.-M. and Hoffmann, J.A. (1998) A drosomycin-GFP reporter transgene reveals a local immune response in *Drosophila* that is not dependent on the Toll pathway. *EMBO J.*, **17**, 1217–1227.
- Govind, S. (1999) Control of development and immunity by rel transcription factors in *Drosophila*. *Oncogene*, **18**, 6875–6887.
- Govind, S., Drier, E., Huang, L.H. and Stewart, R. (1996) Regulated nuclear import of the *Drosophila* rel protein dorsal: structure–function analysis. *Mol. Cell. Biol.*, **16**, 1103–1114.
- Hedengren, M., Asling, B., Dushay, M.S., Ando, I., Ekengren, S., Wihlborg, M. and Hultmark, D. (1999) Relish, a central factor in the control of humoral but not cellular immunity in *Drosophila*. *Mol. Cell*, **4**, 827–837.
- Hoffmann, J.A., Kafatos, F.C., Janeway, C.A. and Ezekowitz, R.A. (1999) Phylogenetic perspectives in innate immunity. *Science*, **284**, 1313–1318.
- Hultmark, D. (1993) Immune reactions in *Drosophila* and other insects: a model for innate immunity. *Trends Genet.*, **9**, 178–183.
- Ip, Y.T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., Gonzalez-Crespo, S., Tatei, K. and Levine, M. (1993) Dif, a dorsal-related gene that mediates an immune response in *Drosophila*. *Cell*, **75**, 753–763.
- Jans, D.A., Xiao, C.-Y. and Lam, M. (2000) Nuclear targeting signal recognition: a key control point in nuclear transport? *BioEssays*, **22**, 532–544.
- Khush, R.S. and Lemaitre, B. (2000) Genes that fight infection: what the *Drosophila* genome says about animal immunity. *Trends Genet.*, **16**, 442–449.
- Lemaitre, B., Meister, M., Govind, S., Georgel, P., Steward, R., Reichhart, J.M. and Hoffmann, J.A. (1995) Functional analysis and regulation of nuclear import of dorsal during the immune response in *Drosophila*. *EMBO J.*, **14**, 536–545.
- Lemaitre, B., Reichhart, J.M. and Hoffmann, J.A. (1997) *Drosophila* host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc. Natl Acad. Sci. USA*, **94**, 14614–14619.
- Levashina, E.A., Ohresser, S., Lemaitre, B. and Imler, J.L. (1998) Two distinct pathways can control expression of the gene encoding the *Drosophila* antimicrobial peptide metchnikowin. *J. Mol. Biol.*, **278**, 515–527.
- Lin, P.-H., Huang, L.H. and Stewart, R. (2000) Cactin, a conserved protein that interacts with the *Drosophila* IκB protein Cactus and modulates its function. *Mech. Dev.*, **94**, 57–65.
- Luque, I. and Gelinis, C. (1997) Rel/NF-κB and IκB factors in oncogenesis. *Semin. Cancer Biol.*, **8**, 103–111.
- Manfruell, P., Reichhart, J.M., Steward, R., Hoffmann, J.A. and Lemaitre, B. (1999) A mosaic analysis in *Drosophila* fat body cells of the control of antimicrobial peptide genes by the Rel proteins Dorsal and Dif. *EMBO J.*, **18**, 3380–3391.
- Meng, X., Khanuja, B.S. and Ip, Y.T. (1999) Toll receptor-mediated *Drosophila* immune response requires Dif, an NF-κB factor. *Genes Dev.*, **13**, 792–797.
- Nakamura, T., Yamazaki, Y., Hatano, Y. and Miura, I. (1999) NUP 98 is fused to PMX1 homeobox gene in human acute myelogenous leukemia with chromosome translocation t(1;11)(q23;p15). *Blood*, **94**, 741–747.
- Paschal, B.M. and Gerace, L. (1995) Identification of NTF-2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. *J. Cell Biol.*, **129**, 925–937.
- Quimby, B.B., Lamitina, T., L'Hernault, S.W. and Corbett, A.H. (2000) The mechanism of ran import into the nucleus by nuclear transport factor 2. *J. Biol. Chem.*, **275**, 28575–28582.
- Reichhart, J.M., Georgel, P., Meister, M., Lemaitre, B., Kappler, C. and Hoffmann, J.A. (1993) Expression and nuclear translocation of the rel/NF-κB-related morphogen dorsal during the immune response of *Drosophila*. *C R Acad. Sci. III*, **316**, 1218–1224.
- Rutschmann, S., Jung, A.C., Hetru, C., Reichhart, J.M., Hoffmann, J.A. and Ferrandon, D. (2000) The Rel protein Dif mediates the antifungal but not the antibacterial host defense in *Drosophila*. *Immunity*, **12**, 569–580.
- Smith, D.B. and Johnson, K.S. (1988) Single step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene*, **67**, 31–40.
- Stoven, S., Ando, I., Kadalayil, L., Engstrom, Y. and Hultmark, D. (2000) Activation of the *Drosophila* NF-κB factor Relish by rapid endoproteolytic cleavage. *EMBO rep.*, **1**, 347–352.
- Ström, A.-C. and Weis, K. (2001). Importin-β-like nuclear transport receptors. *Genome Biol.*, **2**, reviews 3008.
- Uv, A.E., Roth, P., Xylourgidis, N., Wickberg, A., Cantera, R. and Samakovlis, C. (2000) Members only encodes a *Drosophila* nucleoporin required for rel protein import and immune response activation. *Genes Dev.*, **14**, 1945–1957.

DOI: 10.1093/embo-reports/kvf072