Expression and nuclear translocation of the *rel*/NF-κB-related morphogen *dorsal* during the immune response of *Drosophila*

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Reprints : J. A. Hoffmann

Expression et translocation nucléaire du morphogène dorsal pendant la réponse immunitaire chez la drosophile

ABSTRACT

The *rel*/NF-κB-related morphogen *dorsal* is a maternally expressed gene which is involved in the control of the dorso-ventral axis during early embryogenesis of *Drosophila*. We show that this gene is also expressed in the fat body of larvae and adults of *Drosophila* as well as in a tumorous blood cell line : its expression is noticeably enhanced upon bacterial (or lipopolysaccharide) challenge. This challenge also induces within 15-30 min a nuclear translocation of the dorsal protein. The genes encoding inducible antibacterial peptides in *Drosophila* contain κB-related nucleotide sequences and we show that the dorsal protein can bind to such motifs and sequence-specifically transactivate a reporter gene in co-transfection experiments with a *Drosophila* cell line. However, in *dl¹* mutants, in the absence of dorsal protein, the genes encoding antibacterial peptides retain their inducibility, suggesting a multifactorial control. The results indicate that in addition to its role in embryogenesis, *dorsal* is involved in the immune response of *Drosophila*. They also strengthen the analogy between mammalian acute phase response and the insect immune response. ▲

RÉSUMÉ

Le morphogène dorsal *est un gène à expression maternelle impliqué dans l'organisation de l'axe dorso-ventral de la drosophile. La protéine* dorsal *fait partie de la famille des activateurs transcriptionnels* rel/NF-κB. *Nous montrons que le gène* dorsal *est également exprimé dans le corps gras de larves et d'adultes de drosophile ainsi que dans des cellules sanguines tumorales : son expression est fortement augmentée par piqûre septique, qui induit également une translocation nucléaire de la protéine* dorsal. *Les gènes codant pour les peptides antibactériens inductibles de la drosophile contiennent dans leur promoteur des séquences nucléotidiques homologues aux sites de fixation de l'activateur transcriptionnel NF-κB des mammifères. Nous montrons que la protéine* dorsal *peut se fixer sur de telles séquences. En cotransfection de cellules de drosophile,* dorsal *peut transactiver de façon séquence-spécifique l'expression d'un gène rapporteur par l'intermédiaire de motifs de type κB. Nos résultats révèlent que le morphogène* dorsal, *en plus de sa fonction bien établie au cours du développement embryonnaire, est réutilisé au cours de la réponse immunitaire. Ils apportent d'autre part de nouveaux arguments en faveur de l'analogie entre la réponse immunitaire de la drosophile et la réponse de phase aiguë des mammifères.* ▲

VERSION ABRÉGÉE

L e morphogène *dorsal* est un gène à expression maternelle qui intervient dans l'organisation de l'axe dorso-ventral de la drosophile. Il agit comme activateur ou répresseur de plusieurs gènes ; sa translocation nucléaire est sous le contrôle d'une cascade complexe dans laquelle interviennent les produits de 11 gènes (le

groupe dorsal). On sait depuis peu que *dorsal* fait partie d'une famille d'activateurs transcriptionnels à laquelle appartiennent également *rel* et NF-κB. Le rôle de NF-κB dans la réponse de phase aiguë des mammifères a été établi par de nombreux travaux récents. La réponse immunitaire de la drosophile présente certaines analogies avec la réponse de phase aiguë des mammifères : en particulier, on trouve dans les promoteurs de nombreux gènes induits au cours de la réponse immunitaire des insectes des séquences nucléotidiques homologues aux sites de fixation de NF-

κB dans les gènes de réponse de phase aiguë des mammifères. Nous avons recherché si le morphogène *dorsal*, en plus de son rôle dans le développement embryonnaire, pouvait être impliqué dans la réponse immunitaire de la drosophile. Nous montrons que, contrairement à ce qui était admis jusqu'ici, ce gène est effectivement transcrit dans le corps gras de larves et d'adultes de drosophile ainsi que dans des cellules sanguines tumorales. L'expression est significativement augmentée par des stimuli immuns (blessure septique, addition de lipopolysaccharides). Point essentiel : de telles blessures induisent en 15-30 min la translocation nucléaire de la protéine dorsal, ce qui est en accord avec l'hypothèse d'un rôle d'activateur transcriptionnel inductible. Nous montrons également que la protéine dorsal se lie à des motifs de type κB et

peut transactiver de façon séquence-spécifique la transcription d'un gène rapporteur lors de cotransfections dans des lignées cellulaires de drosophile. L'ensemble des résultats présentés dans cette note montrent que *dorsal*, en plus de son rôle dans l'établissement de l'axe dorso-ventral, est effectivement réutilisé par la drosophile au cours de sa réponse immunitaire. Il est possible, mais non démontré, qu'une des fonctions de la protéine dorsal soit la transactivation *via* des sites *cis*-régulateurs de type κB de gènes codant pour les peptides antibactériens inductibles. L'analyse de mutants devrait permettre de clarifier les fonctions de *dorsal* au cours de la réponse immunitaire. Nos résultats soulignent également les analogies qui existent entre la réponse de phase aiguë des mammifères et les défenses antibactériennes des insectes. ▲

The *Drosophila* morphogen *dorsal* is a maternal effect gene which is essential for the establishment of the dorso-ventral polarity in the developing embryo [1, 2]. The dorsal protein is initially uniformly distributed in the egg cytoplasm. Prior to gastrulation, eleven genes, referred to as the dorsal group, function to direct its spatially regulated nuclear import, ultimately forming a dorso-ventral nuclear gradient of the transcription factor *dorsal* during the syncytial blastoderm stage of the embryo [3-5]. At that time, peak levels are attained in ventral regions and progressively lower levels in lateral and dorsal regions. Peak levels activate the expression of two regulatory genes, *twist* and *snail*, which initiate the differentiation of the mesoderm. *Dorsal* also functions as a repressor and restricts the activity of certain regulatory genes, *zerknüllt* and *decapentaplegic*, to dorsal regions where they are important for the differentiation of the amnioserosa and the dorsal epidermis [5-8]. The cloning of *dorsal* cDNA by Steward [9] in 1987 showed that the deduced protein is almost 50 percent identical over an extensive region, to the protein encoded by the avian oncogene v-*rel*, which is highly oncogenic in avian lymphoid, spleen and bone-marrow cells, as well as to its cellular homolog c-*rel*. It became apparent in 1990, with the cloning and sequencing of the cDNAs encoding the DNA binding subunits of the transcription factor NF-κB [10, 11], that dorsal, rel and NF-κB belong to a common family of proteins that function as transcriptional regulators [12]. NF-κB was originally described as a factor that binds to a short nucleotide sequence in the enhancer of the κ light chain immunoglobulin gene [13] and it is now clear that it is active on many promoters and namely is involved in immediate early gene activation during the mammalian acute phase response [14]. As is the case for the regulated nuclear import of dorsal, a complex pathway has been worked out which leads to the nuclear translocation of NF-κB. Substantial parallels exist between the *Drosophila* dorso-ventral pathway and the mammalian NF-κB pathway [15]. In particular, a marked sequence conservation is noted between functionally equivalent proteins in both pathways (e. g. cactus and I-κB which bind to dorsal, respectively to NF-κB, maintaining their cytoplasmic localisation) [16-19].

We are interested in the control of immune gene expression during the antibacterial response of *Drosophila*. This response is strongly evocative of the acute phase response of mammals (reviewed in [20]). Strikingly, the promoters of

the insect genes encoding inducible antibacterial peptides contain several copies of nucleotide motifs similar to *cis*-regulatory elements binding NF-κB and NF-IL6 in the promoters of most acute phase reactant genes of mammals. At least some of these insect motifs function as regulators, as recently illustrated for the κB-related sequences of the gene encoding the inducible antibacterial polypeptide diptericin in *Drosophila* [21]. Experiments based on the establishment of transgenic fly lines and on transfections of *Drosophila* tumorous blood cells have indeed shown that the presence of the κB-related motifs is mandatory for bacteria-inducibility of this gene, which contains several copies of these motifs in its promoter [22].

Intrigued by the parallels between the *Drosophila* dorsal system and the mammalian NF-κB system, we have asked whether *dorsal*, in addition to its role in early embryonic development, was involved in the immune response in larvae and adults. So far, zygotic expression of the *dorsal* gene had not been reported. As a first step to provide an answer to the above question, we have now studied the expression of the gene in bacteria-challenged larvae and adults, as well as in an immune-responsive tumorous blood cell line. Our data indicate that the morphogen *dorsal* is actually involved in the immune response of *Drosophila*.

Materials and methods

Drosophila stocks

Oregon® flies were used as a standard wild type strain. The *dorsal* allele used in these experiments was *dl¹*. The *dorsal* chromosome was further marked with *cn* and *sca*. Homozygous *dl¹ cn sca* females were obtained from a *dl¹ cn sca/CyO* stock from the Bloomington *Drosophila* Center. 2-5 day-old adults or a mix of male and female larvae were used in the RNA and protein extracts analysed in Northern blot, Western blot and gel shift experiments.

Northern blot analysis

Larvae and adults of *Drosophila* were challenged by pricking with a needle which had been dipped into a concentrated bacterial culture (mixture of *Escherichia coli* and *Micrococcus luteus*). Tumorous *mbn-2* blood cells [23] were treated with 10 μg/ml of lipopolysaccharide (Difco, *E. coli* 55:B5). Poly(A)-enriched RNA was extracted and separated as described [24] on a 1% agarose-formaldehyde gel, blotted onto a nylon membrane (Hybond N, Amersham) and hybridised first to a nick-translated 654 bp *dorsal* cDNA probe corresponding to

amino acids 61 to 279, then to a rp49 cDNA probe. Hybridisation was in 6X SSC, 0.1 % SDS, 50 % formamide at 42 °C and washing in 0.2X SSC, 0.1 % SDS at 65 °C.

In situ hybridisation

Female adults of *Drosophila* were fixed, embedded, sectioned and hybridised with a digoxigenin-labelled 654 bp *dorsal* cDNA probe (see above), using a DIG DNA labelling and detection kit (Boehringer, Mannheim). The protocol of Tautz and Pfeiffle [25] was modified as follows : fixation was performed in Carnoy's fix ; prehybridisation and hybridisation were carried out at 48 °C in 50 % formamide ; an amplification step (Vectastain ABC Kit, Vector) was added after the first antibody ; the sections were subsequently counterstained with acridine orange.

Immunolocalisation and Western blot analysis

Larval fat bodies were dissected in PBS and fixed in 4 % paraformaldehyde, 2 mM MgSO$_4$, 1 mM EGTA and 0.1 M PIPES buffer for 15 min. They were washed three times for 5 min in PBS and permeabilised by a 2 h incubation in PBT A (1 % BSA, 0.1 % Triton X-100 in PBS). An anti-dorsal monoclonal antibody (gift of Pr. R. Steward) was applied to the fat bodies at a 1 : 50 dilution in PBT A and incubated overnight at 4 °C. The preparation was then washed three times for 30 min in PBT B (0.1 % BSA, 0.1 % Triton X-100 in PBS) with 2 % sheep serum. The second antibody was an alkaline phosphatase-linked sheep anti-mouse-IgG (Boehringer, Mannheim). It was first preabsorbed on fixed fat body and then diluted 1 : 500 and applied for 4 h to fat body in PBT B at room temperature. The preparation was fixed for 10 min in 0.5 % glutaraldehyde in PBS, washed three times in AP-Sol (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM MgCl$_2$, 0.1 % Triton X-100) and incubated for 2 h in the staining solution (0.34 mg/ml NBT, 0.17 mg/ml X-Phosphate in AP-Sol). The fat bodies were subsequently mounted in glycerol.

For Western blot experiments, 20 μg of protein extracts were resolved under denaturing conditions by SDS-PAGE, transferred to nitrocellulose and probed with the monoclonal antibody. Second antibody and staining solutions were as above.

Gel shift experiments

10 μg of proteins were extracted [22] from bacteria-challenged adult males and incubated for 6 h in ice either in the absence of antibody or with : (1) monoclonal anti-dorsal antibody ; or (2) control hybridoma supernatant. 20 000 cpm (2 fmol) of a labelled 16 bp double stranded probe containing a κB-related motif (5' ATCGGGGATTCCTTTT 3') [22] were added and after incubation for 15 min at room temperature, complexes were analysed in a standard gel shift assay (as described in [22]).

Cell culture and transfection experiments

Schneider S2 cells were grown to 80 % confluent monolayers for 24 h prior to transfection in Schneider's medium (Gibco BRL) supplemented with 10 % fetal calf serum (Gibco BRL), 10^5 U/l penicillin and 100 mg/l streptomycin. The cells were co-transfected using the transfection reagent DOTAP (Boehringer Mannheim) with

3 μg of *cat* reporter plasmid, 1 μg of the β-galactosidase expression vector pACH [26] as an internal control for the transfection efficiencies and 0.1, 0.5, 1 and 3 μg of the pPAC expression vector containing the *dorsal* cDNA with the actin 5C promoter and polyadenylation site [26, 27]. Reporter *cat* plasmids were derived from pBLCAT5 [28] containing the *E. coli cat* gene under the control of the thymidilate kinase promoter. The construction of D6, D8, D9 and D11 has been described in a previous study [22]. 24 h after transfection, the cells were harvested, lysed by the freeze-thaw procedure and analysed for β-galactosidase and CAT activities as described [22].

Results

Zygotic expression of the *dorsal* gene

Poly(A)-enriched RNA was prepared from the following sources : (1) wandering third-instar larvae ; (2) adult males ; (3) adult females ; (4) a tumorous blood cell line, *mbn-2* : these cells can be induced by addition of bacteria or lipopolysaccharides (LPS) to the culture medium to synthesize antibacterial peptides [22, 29]. Samples were prepared separately from untreated and bacteria- (or LPS-) challenged insects and cells. In Northern blot experiments, a partial *dorsal* cDNA (corresponding to the N-terminal half of the open reading frame, see *Materials and methods*) was hybridised under conditions of high stringency to the poly(A)-enriched RNA. As shown in *Figure 1a*, all samples contained a hybridisation positive band at 2.8 kb which corresponds to the size reported for *dorsal* transcripts [9]. In normal adult males (lane 8), the amount of transcripts hybridising to the *dorsal* cDNA was faint, but was dramatically enhanced upon bacterial challenge (lane 10). In larvae and *mbn-2* cells, the level was already significant before challenge (lanes 5 and 1 respectively) and was enhanced upon challenge (lanes 6 and 2-4 respectively). In adult females (lanes 7, 9 and 11) ovarian expression of *dorsal* probably masked the response, although the hybridisation positive band at 2.8 kb appears somewhat stronger 1 h after challenge (lane 9) than in untreated females (lane 7).

In addition to the 2.8 kb transcript, a larger band at 4.4 kb was observed in most samples and its intensity was clearly enhanced upon challenge in the four biological systems under investigation. A smaller band at <2 kb was observed in our conditions of high stringency only in bacteria-challenged larvae.

We have also investigated the presence of *dorsal* transcripts by *in situ* hybridisation in female adults. Positive results were obtained with fat body and ovaries of unchallenged females. In the fat body of these insects, the level of *dorsal* transcripts was strongly enhanced after bacterial challenge as shown in *Figure 2 (a, b)*.

The *dorsal* mRNA reportedly contains an open reading frame of 2 206 nucleotides [3, 9]. To confirm the presence of *dorsal* transcripts in our experiments, we have used primers corresponding to the 5' and 3' extremities of this open reading frame and have performed PCR with reverse-transcribed poly(A)-enriched RNA from bacteria-challenged adult males and from LPS-induced tumorous blood cells. In both cases, we obtained a 2.2 kb band of DNA which we have fully sequenced. The nucleotide
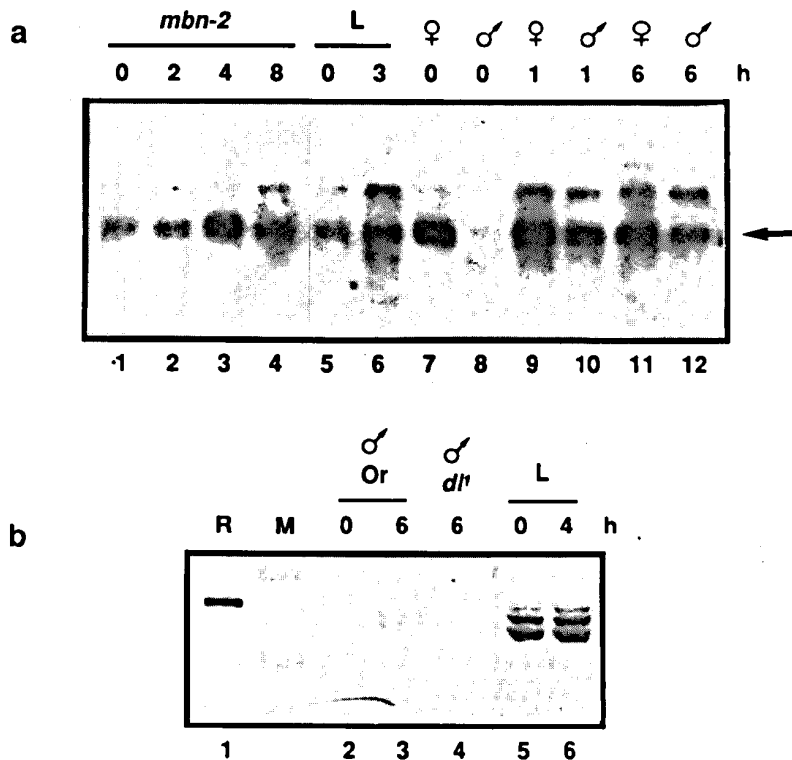
a

mbn-2				L		♀	♂	♀	♂	♀	♂	
0	2	4	8	0	3	0	0	1	1	6	6	h

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

b

		♂ Or	♂ dl¹		L		
R	M	0	6	6	0	4	h

| 1 | | 2 | 3 | 4 | 5 | 6 |

Figure 1. **Induction of dorsal expression in Drosophila.** (a) Northern blot analysis of dorsal transcripts. 5 µg poly(A) RNA from control (lane 1) and 2, 4 or 8 h LPS-treated (lanes 2-4) mbn-2 cells, from control (lane 5) and 3 h bacteria-challenged (lane 6) larvae, from control, 1 h or 6 h bacteria-challenged female (lanes 7, 9, 11) and male adults (lanes 8, 10, 12) were hybridised with a dorsal cDNA probe. The arrow indicates the position of the 2.8 kb dorsal transcript. Note the presence of additional bands in the positive lanes, the identity of which is under investigation. The same blot was subsequently hybridised with a ribosomal protein (rp49) cDNA probe as a control and showed comparable levels of transcripts in the various RNA samples (data not shown). (b) Western blot analysis of dorsal protein. Lane 1, recombinant dorsal protein (R). Protein extracts were obtained from control (lane 2) and 6 h bacteria-challenged (lane 3) adult Oregon® (Or) males, 6 h bacteria-challenged dl¹ mutant males (lane 4), fat body from control (lane 5) and 4 h bacteria-challenged (lane 6) larvae. The Western blot was incubated with a monoclonal antibody directed against the C-terminal domain of the dorsal protein (gift of Pr R. Steward). M, two molecular weight markers : 119 kDa (upper band) and 75 kDa (lower band).

sequences were identical to the sequence published for dorsal mRNA [3, 9] (EMBL data bank).

Presence of the dorsal protein in larvae and adults

We next used a monoclonal antibody (gift of Pr R. Steward, Princeton) directed against the C-terminal domain of the dorsal protein to investigate the presence of this protein in normal and bacteria-challenged adult males and in fat body of wandering third instar larvae. As shown in Figure 1b, in adult males, immunoreactive proteins (lane 3) were observed after bacterial challenge which show a relative size close to that of recombinant dorsal protein (lane 1). We interpret the presence of two immunoreactive bands as representing different states of modifications (e. g. phosphorylation) of the dorsal protein. In larval fat body, a strong signal (in the form of a triplet) was observed before challenge (lane 5), which was not markedly enhanced thereafter (lane 6). No immunoreactive bands were detected with protein extracts from bacteria-challenged dl¹ mutants (lane 4) which reportedly do not produce the dorsal protein [5]. This result strengthens the interpretation that the immunoreactive bands detected by the monoclonal antibody correspond to the dorsal protein (and isoforms).

Nuclear translocation of the dorsal protein after bacterial challenge

Using the same monoclonal antibody as above, we have undertaken an immunocytochemical localisation of the dorsal protein in the large polytenic fat body cells of wandering third-instar larvae. As illustrated in

Figure 2 (c, d), in untreated cells the protein is uniformly distributed. However, bacterial challenge induces within 15-30 min a marked nuclear localisation of the protein. No staining was observed with this monoclonal antibody, neither in the cytoplasm nor in the nuclei of mutant dl¹ larvae, from which the dorsal protein is reportedly absent [5].

Binding of the dorsal protein to κB-related motifs and sequence-specific activation of a reporter gene via κB-related motifs

The results presented so far are clearly indicative of an involvement of dorsal in the immune response of Drosophila (see Discussion). Given the parallels between the dorsal and the NF-κB systems outlined in the introduction, we have probed whether insect κB-related nucleotide motifs which serve as cis-regulatory sequences, bind the dorsal protein in the immune response of Drosophila. The binding motifs which mediate the activity of the dorsal protein in the promoters of target genes in early development (e. g. twist, zerknüllt) have been investigated in some detail and they differ significantly in sequence from the κB-related motifs of the immune response genes [30, 31]. To clarify this problem, we have chosen the native κB-related sequence of the proximal promoter region of the diptericin gene, which is present in two copies nested each within a 17-bp repeat closely upstream of the TATA box [21]. As indicated above, the presence of these sequences is mandatory for bacteria-inducibility of the diptericin gene of Drosophila [22]. We have incubated oligonucleotides corresponding to these
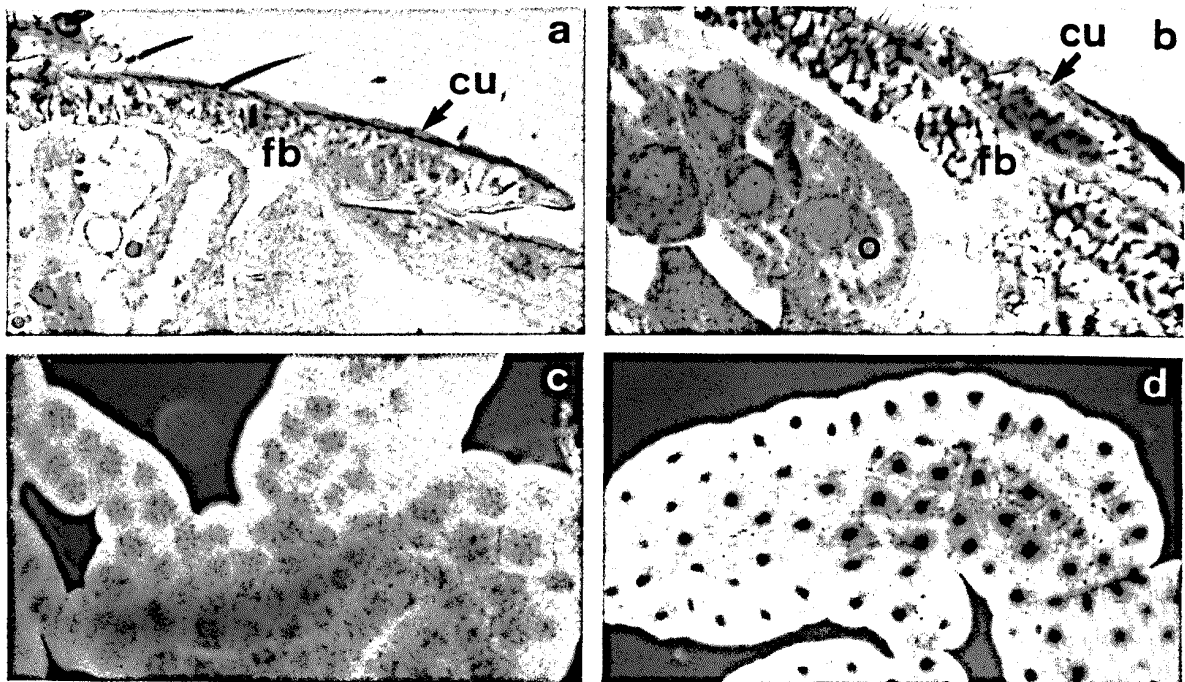
Figure 2. **Detection of** dorsal **transcripts and dorsal protein during the immune response of** Drosophila. (a, b) *Localisation of* dorsal *transcripts in a) control and b) 4 h bacteria-challenged female adults by* in situ *hybridisation as described in* Materials and methods. *cu : cuticle ; fb : fat body ; o : ovary.* (c, d) *Nuclear translocation of the dorsal protein after bacterial challenge in larval fat body cells. c) Control third instar wandering larva ; d) 20 min bacteria-challenged larva. As a control, the same experiment was performed with* dl¹ *larvae (progeny from* ♀ dl¹/+ x ♂ dl¹/dl¹ *), which showed no specific staining (not shown).*

sequences with protein extracts of bacteria-challenged adult males. A DNA-protein complex was observed in electrophoretic mobility shift assays (*Fig. 3*) confirming our previous results [22]. Interestingly in the context of the present study, the addition of the monoclonal antibody directed against the C-terminal domain of dorsal, induced a

Figure 3. *DNA-protein complex formed between proteins extracted from* Drosophila *adult males and the diptericin κB-related motif. 10 μg of proteins extracted from bacteria-challenged adult males of* Drosophila *were incubated with 20 000 cpm (2 fmol) of radioactive probe (lane 1), and after a 6 h preincubation with a control hybridoma supernatant (lane 2) or with a monoclonal anti-dorsal antibody (lane 3).*
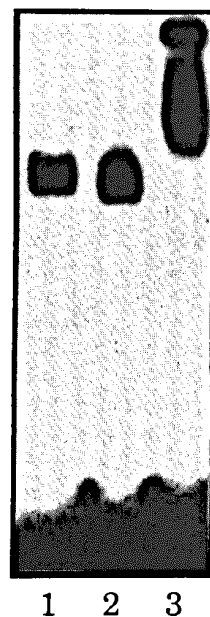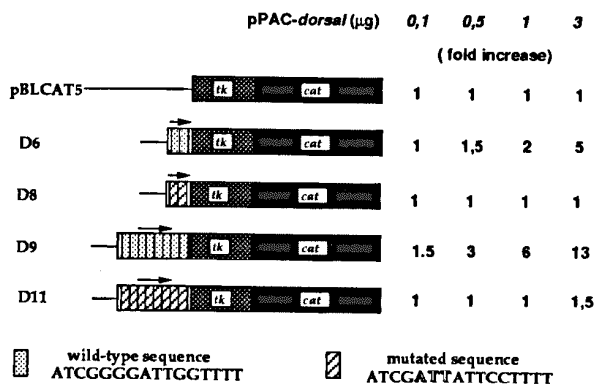
supershift. Addition of an irrelevant hybridoma supernatant did not affect the migration of the DNA-protein complex.

Finally to see whether dorsal can activate a reporter gene *via* κB-related motifs, we have co-transfected Schneider S2 cells with two constructs (see *Materials and methods*) : (1) a *dorsal* expression vector ; (2) reporter plasmids in which two or eight copies of wild-type or mutated 17-bp motifs containing κB-related sequences were placed upstream of a minimal thymidilate kinase promoter fused to the *cat* gene (*Fig. 4*). When transfected alone, the reporter plasmid generated only background CAT activity, whereas co-transfection with the *dorsal* expression vector induced a significant level of activation which was proportional between 0.1 μg and 3 μg of *dorsal* vector. When the κB sites were mutated in the reporter plasmid, the level of CAT activity was not increased by the *dorsal* expression vector over background values. These results indicate that the dorsal protein can sequence-specifically transactivate a reporter gene *via* the κB-related motifs.

Discussion

Our data show in the first place that the expression of the *dorsal* gene is not restricted to the ovaries and early embryos of *Drosophila*. The presence of *dorsal* transcripts is observed in the fat body of untreated third instar larvae and male adults, as well as in tumorous blood cells. Our assumption that we are actually in the presence of *dorsal* transcripts in these systems is based on the following points : (1) the size of the major transcript hybridising to the partial *dorsal* cDNA has the same length as that

Figure 4. **Specific transactivation by dorsal of a reporter cat gene under the control of κB-related motifs.** Schneider S2 cells were co-transfected with the indicated cat reporter plasmid, the β-galactosidase expression vector pACH as an internal control and 0.1 to 3 μg of a dorsal expression vector, as described in Materials and methods. The cat plasmids contained 2 or 8 copies of the wild-type (D6 and D9) or mutated (D8 and D11) κB motif. Results were normalised for β-galactosidase activity and expressed as the mean fold increase in CAT activity for three independent experiments.

reported for dorsal transcripts (2.8 kb) ; (2) high stringency conditions were used for these Northern blottings ; (3) the PCR experiments in which the 5' and 3' extremities of the open reading frame of dorsal were used as primers in the presence of poly(A) enriched RNA yielded DNA bands of the expected length (2.2 kb) with a nucleotide sequence identical to that reported for dorsal. Several other hybridisation-positive bands were observed in the Northern blot experiments and it is therefore likely that the tissues which we have examined contain, in addition to dorsal transcripts several dorsal-related transcripts which remain to be characterized.

Of major importance in the context of the control of the immune response of Drosophila is the observation that the expression of the dorsal gene is enhanced by bacterial (or LPS) challenge. This is particularly striking in the case of male adults which show only a faint signal for dorsal transcripts on Northern blots before challenge. It should be noted that the challenge also enhances the intensity of the signals obtained for dorsal-related transcripts in the biological systems investigated here.

With the use of a monoclonal antibody, we have detected several immunoreactive bands in larval fat body, in induced male adults (total) and in tumorous blood cells (not shown). The sizes of these bands are within the range of that of recombinant dorsal protein and we believe that the various bands (doublets or triplets) correspond to dorsal isoforms (see above). In dl¹ mutants, which reportedly lack dorsal protein, no immunoreactive bands are detected in larval fat body (not shown) or in male adults, which lends full credit to our interpretation that the bands recognized by the monoclonal antibody actually correspond to dorsal. This method has not shown a clearcut difference between the intensities of the immunoreactive bands in larval fat body or in tumorous blood cells (not shown) before and after bacterial challenge ; in contrast, in adult males, dorsal-immunoreactive bands can only be detected after bacterial challenge.

From these experiments it can be concluded that, in addition to its expression in early development, transcription of dorsal occurs under apparently normal conditions in larval and adult fat body cells and that this transcription is enhanced by bacterial challenge. The same conclusion is valid for the tumorous blood cells mbn-2. The mechanism by which bacterial challenge upregulates the transcription of the dorsal gene remains to be elucidated.

Our observation that the dorsal protein is translocated into the nuclei within 15-30 min after bacterial challenge is in full agreement with the idea that during the immune response dorsal acts as a transactivator. Although the mechanism by which the bacterial challenge induces the nuclear translocation of the dorsal protein is not yet fully understood, preliminary results suggest that significant parallels exist with the control of nuclear translocation of dorsal in the preblastoderm embryo (Lemaitre et al., in preparation).

One of the best established consequences of bacterial (or LPS) challenge in Drosophila is the rapid (1 to 2 h) transcription of a battery of antibacterial genes. As pointed out above, the promoters of the genes encoding these peptides contain κB-related motifs which appear at first sight as candidate binding and regulatory sites for the NF-κB related dorsal protein. Our results indeed show that protein(s) present in extracts of bacteria-challenged adults bind to these motifs (no binding occurs in extracts of unchallenged insects) and that dorsal is present in the DNA-protein complex formed under in vitro conditions. The co-transfection of Schneider S2 cells with a dorsal expression vector and a reporter gene fused to κB-related motifs demontrates that the dorsal protein can transactivate the reporter gene via a wild-type but not a mutated κB-related motif. These results are compatible with the idea that one of the roles of dorsal in the immune response is linked to the control of the expression of the gene encoding diptericin. However, they do not unequivocally prove that this is the case under in vivo conditions. In dorsal mutants, the diptericin gene retains its bacteria-inducibility (Lemaitre et al., in preparation), suggesting the existence of other control mechanisms. Genetic analysis should clarify the roles of the dorsal protein in the immune response.

It is now well established that several genes involved in early development of the Drosophila embryo are reexpressed at later stages, e. g. in differentiating imaginal discs and during neurogenesis (see [32]). Our results show for the first time the enhanced expression during the immune response of a gene known to play an essential role in early development. These results also strengthen the analogy between the mammalian acute phase reaction and the Drosophila immune response by pointing to the involvement of the NF-κB-related dorsal protein in this response. Strikingly, dorsal expression is predominantly observed in fat body cells, a functional equivalent of

the mammalian hepatocytes which are a major site of acute phase response, and in certain blood cells. With the exceptionally favourable possibilities of genetic analysis in *Drosophila*, the immune response of this insect may represent an excellent model system for the study of innate immunity. ▼

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