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Characterization of an immunodeficiency mutant in *Drosophila*

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Abstract

Drosophila immunity and embryogenesis appear to be linked by an evolutionarily ancient signalling pathway, which includes the Rel-domain transcription factors Dif and *dorsal*, respectively, as well as a common inhibitor, *cactus*. Previous genetic screens have centered on maternal mutants that disrupt the *dorsal* pathway. In an effort to identify additional components that influence Rel-domain gene function we have conducted a search for immunodeficiency mutants in *Drosophila*. One such mutant, which maps near the *Black cells* (*Bc*) gene, causes a severe impairment of the normal immune response, including attenuated induction of several immunity genes. Survival assays indicate a positive correlation between the induction of these genes, particularly diptericin, and resistance to bacterial infection. These studies are consistent with the notion that insect anti-microbial peptides work synergistically by binding distinct targets within infecting pathogens. Evidence is also presented that non-specific acquired immunity results from the persistence of bacterial metabolites long after primary infection. We discuss the potential usefulness of this study with regard to the identification of conserved components of Rel signalling pathways.

Keywords: *Black cells*; *Drosophila*; Immunity; Insect

1. Introduction

Transcription factors containing the Rel domain have been implicated in a number of developmental and physiological processes, including dorsoventral patterning of the *Drosophila* embryo (Steward and Govind, 1993), insect immunity (Ip and Levine, 1994), the mammalian acute phase response (Hultmark, 1993), and lymphocyte differentiation (Sha et al., 1994). Each of these processes is controlled by a signalling pathway that modulates the release of Rel proteins from ankyrin-containing cytoplasmic inhibitors (Beg and Baldwin, 1993). Mounting evidence suggests that the inhibitors are phosphorylated and degraded in response to the induction of cytokine-like signalling pathways (Belvin et al., 1995). Once in the nucleus, Rel factors interact with target promoters that contain κB sequence motifs, as well as binding sites for unrelated transcriptional activators such as bHLH and bZIP proteins (Lenardo et al., 1987; Gonzalez-Crespo and Levine, 1994).

Previous efforts to identify the genetic components of these signalling pathways have centered on maternal mutant screens in *Drosophila*, which disrupt dorsoventral patterning of the early embryo (St. Johnston and Nusslein-Volhard, 1992). In an effort to identify additional components of Rel signalling pathways we have undertaken an analysis of immunodeficiency mutants.

A number of recent studies have emphasized the parallels between insect immunity and the acute phase response in mammals, in which the liver is induced to express proteins that promote wound healing, inhibit proteases, and scavenge free oxygen radicals (Gordon and Koji, 1985, Hoffmann et al., 1993). This induction in the liver is triggered by interleukin-1 (IL-1) and other cytokines that are secreted by macrophages and other hematopoietic cells. Activation of the IL-1 receptor results in the nuclear transport of NF-κB in the liver, and the induction of type I acute phase proteins (Gordon and Koji, 1985).

A number of immunity genes have been identified in *Drosophila*, including the cecropins (*cec*) (Kylsten et al., 1990) and diptericin (*dip*; abbreviated in previous work as dipt) (Wicker et al., 1990). The *cec* locus contains three closely linked genes. The *cec* genes and *dip* are all rapidly induced in the fat body and hemocytes upon infection or injury. Minimal regions of the *cec* and *dip* promoters

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have been identified that mediate tissue-specific expression in response to bacterial infection or LPS stimulation (Engstrom et al., 1993; Kappler et al., 1993; Meister et al., 1994). Both promoter regions contain essential regulatory elements that are related to κB sequence motifs, which are recognized by members of the Rel family of transcription factors, including NF-κB, Dif, and Dorsal. Multimerization of these κB-like sequence motifs is sufficient to induce expression from a naive promoter in response to LPS stimulation in transiently transfected *Drosophila* mbn-2 tissue culture cells (Engstrom et al., 1993). Previous studies suggest this induction is triggered by the nuclear transport of Dorsal and Dif in the fat body (Ip et al., 1993; Reichhart et al., 1993).

In an effort to identify additional genes essential for insect immunity, we have characterized mutants associated with melanotic pseudo-tumors (Sparrow, 1978; Watson et al., 1991). These pseudo-tumors are formed by the constitutive proliferation, aggregation, and subsequent melanization of hemocytes, and thus it seemed reasonable to suppose that such mutations might alter the presumptive 'signal' required for the induction of immunity genes. Here we present evidence that one of the mutations identified in this way, located near the *Black cells* (*Bc*) gene, results in a severe impairment of the humoral immune response. Mutant larvae fail to express *cec* and *dip* upon infection with *Enterobacter cloacae* (*E. cloacae*), a potent inducer of the immune response (e.g., Engstrom et al., 1993). The adult response is not as severely disrupted, in that *cec* induction is only slightly reduced while *dip* induction is delayed and fails to reach normal levels. Although *Bc* homozygotes are healthy under normal laboratory conditions, they are hypersensitive to bacterial infection and display a greater than 20-fold increase in mortality over wild-type strains. Evidence is presented that this immunodeficiency mutation maps near, but not within, the *Bc* gene. These results suggest a causal link between the induction of immunity genes and resistance to bacterial infection. We discuss the potential usefulness of this type of genetic screen for the identification of conserved components of Rel signalling pathways.

2. Results

2.1. Induction kinetics of immunity genes

The initial search for immunodeficiency mutants focused on the induction of *cec*A1(*cec*) and *dip* expression after infection with *E. cloacae*. The induction kinetics obtained for a wild-type strain (Canton-S) are presented in Fig. 1. Total RNA was extracted from groups of adult flies after different times following infection, fractionated on an agarose gel, and analyzed by Northern assay over a 3-day period. *cec* and *dip* exhibit different expression kinetics; neither gene is expressed prior to infection (time 0, lane 1). *cec* reaches peak levels earlier than *dip*, fades more rapidly between 4 and 16 h, then increases again between 16 and 24 h. This second peak of expression was observed in repeated experiments (see Fig. 2; data not shown). In contrast, *dip* expression steadily declines after reaching peak levels at 8 h following infection. The induction kinetics for *cec* and *dip* found in this study are in accordance with the results of Kylsten et al. (1990) for *cec* and Wicker et al. (1990) for *dip*.

2.2. Bc chromosome homozygotes exhibit an impaired immune response

The *cec* and *dip* expression patterns were compared in adults of two different strains, Canton-S (CS) and *Bc* chromosome homozygotes. The latter possess melanotic pseudo-tumors but are healthy under normal laboratory conditions. Such flies show abnormal kinetics of *dip* expression. The mutants virtually lack *dip* mRNA 2 h after infection (Fig. 2, compare lanes 2 and 8), and quantitation with a phosphoimager suggests that peak levels reach no more than one-fourth the maximal induction seen in the wild-type strain (lanes 3 and 9). Although peak levels of *cec* mRNA are only slightly reduced, the second peak observed at 24 h in the wild-type is absent in the mutant.

Mutant larvae exhibit a more severe immunodeficiency than adults. The normal *cec* and *dip* induction profiles are similar to the situation observed in adults (Fig. 3, lanes 1–4). However, neither mRNA is detected in mutant larvae, even 12 h after bacterial infection (lanes 5–8).

2.3. Attenuated cec and dip induction might not result from the Bc mutation

Previous studies have shown that one of the two classes of hemocytes in *Drosophila*, the crystal cells, form melanin aggregates in *Bc* mutants (Rizki et al., 1980). A potential explanation for the abnormal induction of *cec* and *dip* expression is that these melanized crystal cells are 'inert' and fail to relay a signal to the fat body upon infection.

This issue was investigated by analyzing *cec* and *dip* expression in *lozenge* (*lz*) mutants (Lindsley and Zimm, 1992). *lz* is X-linked, and males hemizygous for the strongest allele, *lz*[s], lack crystal cells (Peeples et al., 1969; Rizki and Rizki, 1981). Induction of both *cec* and *dip* appears normal in these mutants (Fig. 4, lanes 1–4), although both genes appear to be weakly expressed prior to infection (lane 3). These results suggest that normal crystal cells are not the source of an obligate signal required for the induction of immunity genes.

An alternative explanation for the immunodeficiency associated with *Bc* mutants is that the abnormal, melanized crystal cells generate a dominant inhibitory signal that blocks *cec* and *dip* expression in the fat body. A pre-

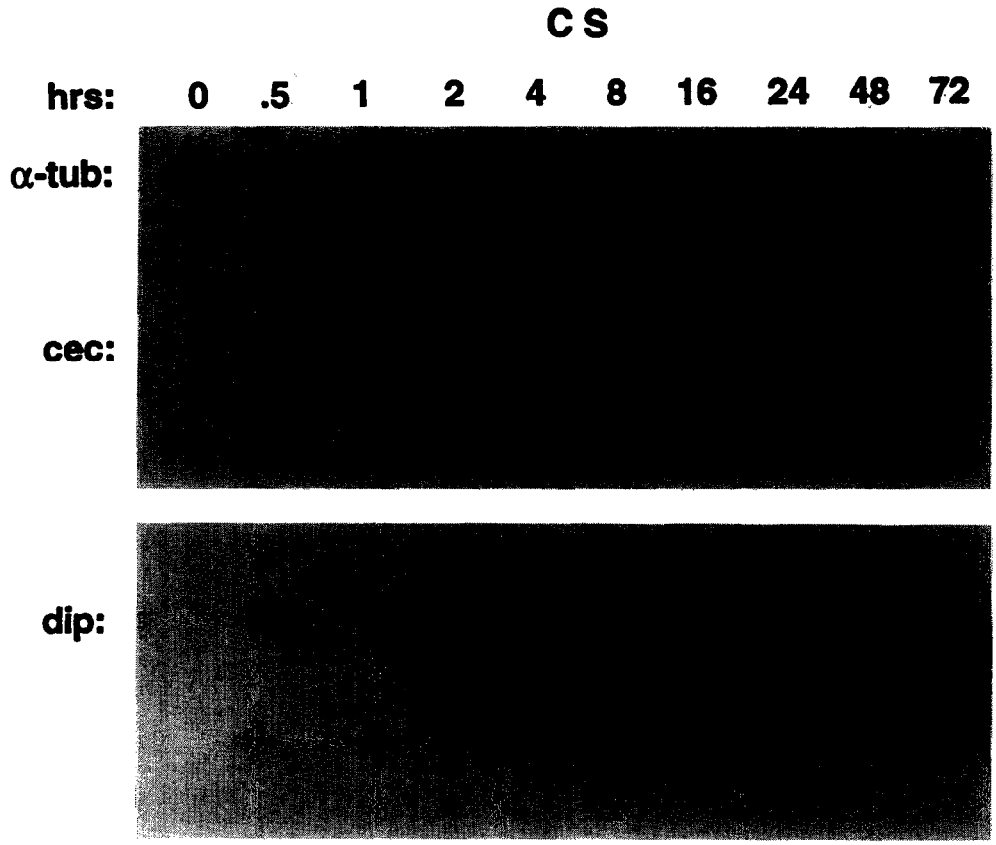

Fig. 1. Induction of *cec* and *dip* mRNAs in Canton-S adults. Total RNA was extracted from groups of adult flies after infection with *E. cloacae*. The RNA was fractionated on an agarose/formaldehyde gel, transferred to a solid matrix and hybridized with *cec*, *dip*, and α-tubulin probes (indicated to the left of the autoradiograms). The α-tubulin mRNA serves as an internal control to ensure that comparable amounts of RNA were loaded in all of the lanes. The numbers above the lanes indicate the number of hours after bacterial infection. For example, peak concentrations of the *cec*A1 mRNA are detected 4 h after infection (top panel, lane 5). CS (top) refers to the wild-type Canton-S strain.

diction of this model is that the immunodeficiency phenotype should be dominant. This does not appear to be the case since *Bc*/+ heterozygotes exhibit a normal immune response (data not shown; see below).

Further evidence that the melanized crystal cells do not block immunity was obtained by analyzing *lz*[s]/Y;*Bc*/*Bc* double mutants. Genetic experiments suggest that *lz* is epistatic over *Bc*, in that the double mutants completely lack crystal cells (both normal cells and the melanized aggregates) (Peeples et al., 1969). Despite this loss, the double mutants exhibit an immunodeficiency that is virtually identical to *Bc* chromosome homozygotes: early *dip* induction is lost, while *cec* expression is attenuated (Fig. 4, lanes 5–8). Taken together, these results suggest that the *Bc* phenotype (masses of aggregated, melanized crystal cells) is separable from the immunodeficiency phenotype.

2.4. The immunodeficiency mutation is separable from the Bc gene

Previous studies have mapped the *Bc* gene to the 55A region of chromosome 2 (Deng and Rizki, 1988). A number of chromosome deficiencies have been characterized in this region, including *Pcl*11B and PC4 (Lindsley and Zimm, 1992). The *Pcl*11B deficiency uncovers the region from 54F6;55A1 to 55C1–3, while PC4 removes 55A–F. Although both deletions remove the *Bc* gene (Deng and Rizki, 1988), only one, PC4, also manifests the immunodeficiency phenotype, as described below (see Fig. 6B for a summary of region 55).

Adults that are transheterozygous for the original *Bc* chromosome and the *Pcl*11B deletion exhibit a normal induction of *dip* expression (Fig. 5, upper panel, lanes 5–8). Strong expression is detected within 1 h after infection (lane 6) , and there is a progressive increase in mRNA levels from 1 to 4 h after infection (lanes 7 and 8). These results suggest that the *Bc* mutation is not responsible for the immunodeficiency phenotype.

Bc transheterozygotes carrying the PC4 deletion exhibit an immunodeficiency phenotype similar to that observed in *Bc* chromosome homozygotes (Fig. 5, lower panel). *dip* RNA is not induced above background levels, even 4 h after bacterial infection (lane 8). The simplest interpretation of these results is that the immunodeficiency disorder results from a second mutation, in the

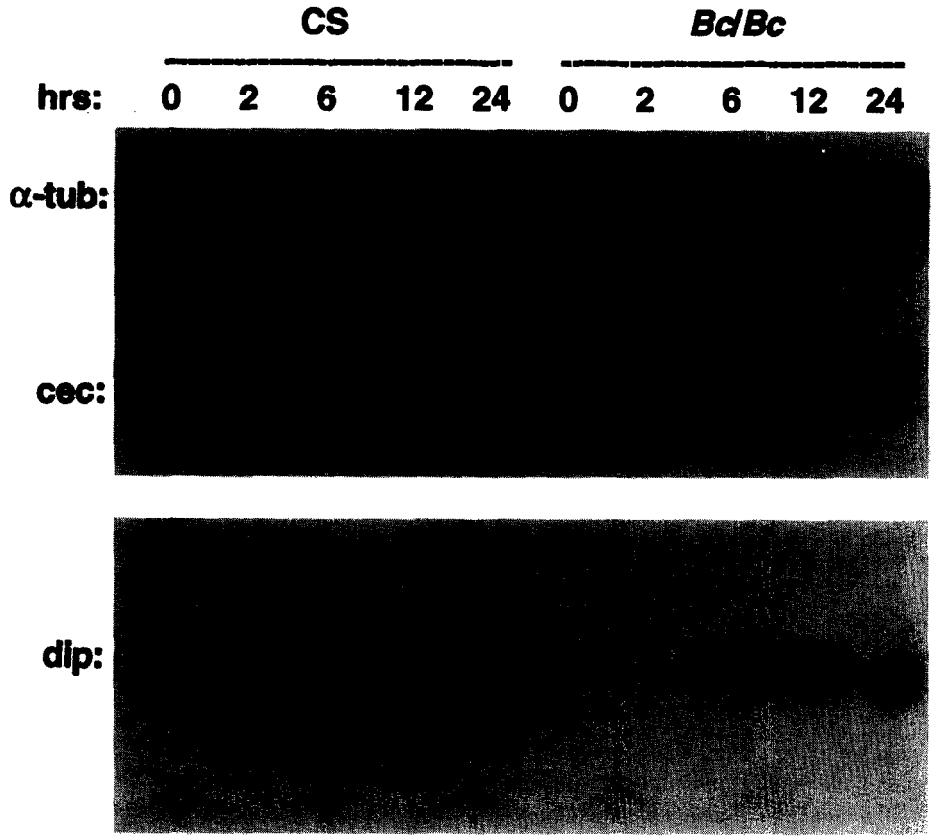

Fig. 2. *cec* and *dip* induction is disrupted in *Bc* adults. Northern hybridizations were done as described in Fig. 1 and Materials and methods. Lanes 1–5 show the induction of *cec* and *dip* mRNAs in wild-type adults (CS) between 0 and 24 h after bacterial infection. Lanes 6–10 show the induction kinetics in *Bc* chromosome homozygotes. *dip* induction is attenuated, while *cec* expression steadily declines between 6 and 24 h after infection. The numbers above the autoradiograms indicate the number of hours after infection. The use of the α-tubulin probe suggests that approximately equivalent amounts of RNA were loaded in each lane.

55C–F region, on the original *Bc*-bearing chromosome (see Section 3).

Further evidence that the *Bc* mutation itself is not responsible for the immunodeficient phenotype is presented in Fig. 6A. In this experiment a second strain is used (*Bc fj wt*) which is the product of recombination between the original *Bc*-bearing chromosome and another chromosome bearing the *four-jointed* (*fj*) and *welt* (*wt*) mutations (Rizki et al, 1980). In this strain the 55C–F region of the *Bc* chromosome has been replaced with the same region from a different chromosome. This strain exhibits normal induction of *dip* and *cec* (data not shown), even though it is homozygous for the *Bc* mutation. The preceding results provide strong evidence that the *Bc* mutation is not responsible for the deficient induction of immunity genes.

2.5. Immunodeficient flies are hypersensitive to bacterial infection

Survival assays were performed to determine whether there might be a causal link between the induction of immunity genes, such as *dip*, and resistance to bacterial infection. These experiments involved infecting groups of

Fig. 3. *cec* and *dip* induction is abolished in mutant larvae. Northern assays using total RNA extracted from groups of third-instar larvae that had been infected with *E. cloacae*. The wild-type strain (CS) shows robust induction of *cec* and *dip* mRNAs (lanes 1–4), similar to the kinetics observed in adults (see Figs. 1 and 2). In contrast, mutant larvae (*Bc* chromosome homozygotes) fail to induce either transcript. The numbers above the autoradiograms indicate hours after bacterial infection. The α-tubulin mRNA serves as an internal control.

Fig. 4. *cec* and *dip* mRNAs are induced in mutants lacking crystal cells. Northern assays using RNA extracted from either uninfected controls (lanes labeled 0) or adults that had been infected with *E. cloacae* 4 h earlier (lanes labeled 4). Four different strains of flies were examined, including wild-type (CS), *lozenge* mutants (lz[s]/Y), *Bc* chromosome homozygotes (*Bc/Bc*), and *lozenge;Bc* chromosome double mutants (*lz[s]/Y;Bc/Bc*). *cec* and *dip* mRNAs are strongly induced in wild-type and *lozenge* strains, but are attenuated (*cec*) or essentially absent (*dip*) in *Bc* chromosome mutants and *lz;Bc* double mutants. As in previous figures, the numbers above the autoradiograms indicate hours after bacterial infection. The α-tubulin mRNA serves as an internal control.

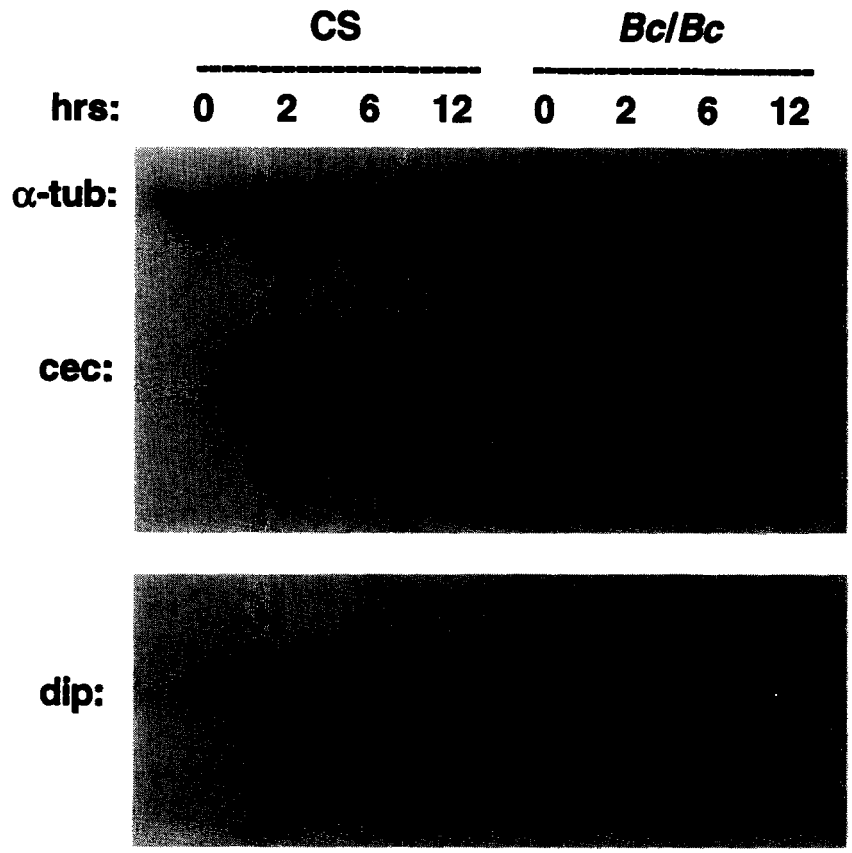
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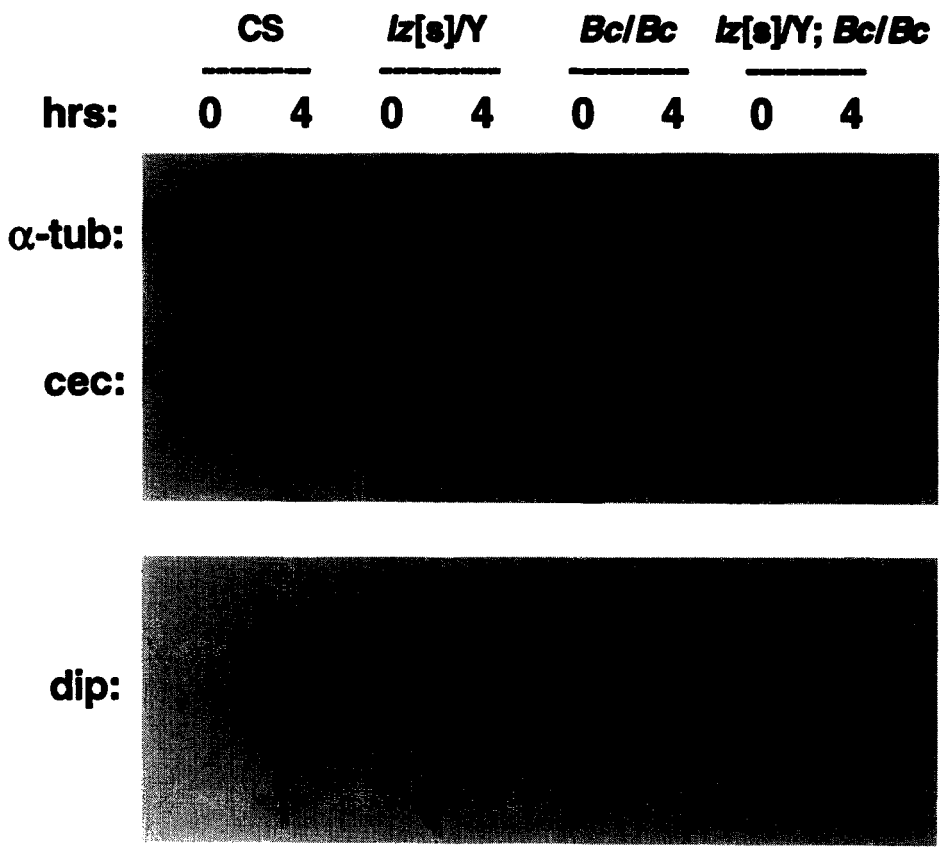
Fig. 3.


CS
lz[s]/Y
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hrs:
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α-tub:
cec:
dip:

Fig. 4.

adult flies (50 flies/group) with standardized concentrations of *E. cloacae*, and then monitoring mortality rates after 5 days (Fig. 7). The wild-type strain (CS) exhibited good resistance to infection, with less than 5% dying within the time frame of the experiment. This mortality is probably due to the damage caused by injection since similar numbers die after injection with a solution lacking *E. cloacae*. *Bc* chromosome homozygotes are highly sensitive to infection in that about 80% die within 5 days after infection. Uninfected control groups exhibit slightly elevated mortality as compared with the wild-type strain, suggesting that these mutants might be somewhat more susceptible to tissue damage resulting from injection. As discussed earlier, the *Bc* mutation does not exert a dominant effect on the immune response, and *Bc*/+ heterozygotes exhibit normal resistance to infection and injury.

The above results indicate a correlation between *dip* induction and resistance to infection. This correlation is further supported by additional survival assays (Fig. 7). For example, Df(*Pcl*11B)/*Bc* transheterozygotes display normal *dip* induction (Fig. 5A) and essentially normal mortality rates after infection. In contrast, *dip* is not induced above background levels in Df(PC4)/*Bc* transheterozygotes, and these flies are hypersensitive to bacterial infection (over 80% mortality; Fig. 7). Wild-type

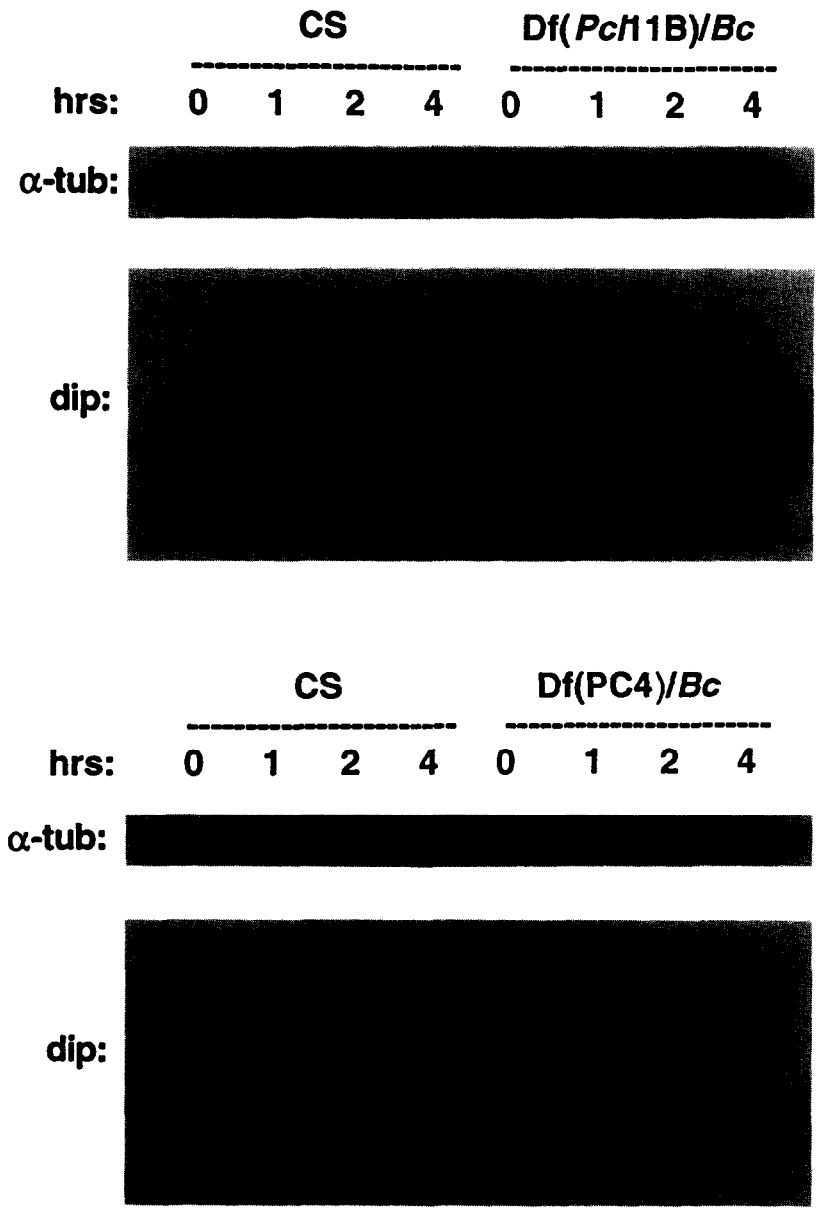

Fig. 5. The immunodeficiency mutation is linked to the *Bc* gene. Northern assays showing the induction profile of the *dip* mRNA in adult flies transheterozygous for the *Bc*-bearing chromosome and either the *Pcl*11B deficiency (upper panel) or the PC4 deficiency (lower panel). *dip* induction appears normal in Df(Pc/11B)/Bc flies, but is severely attenuated in Df(PC4)/Bc adults.

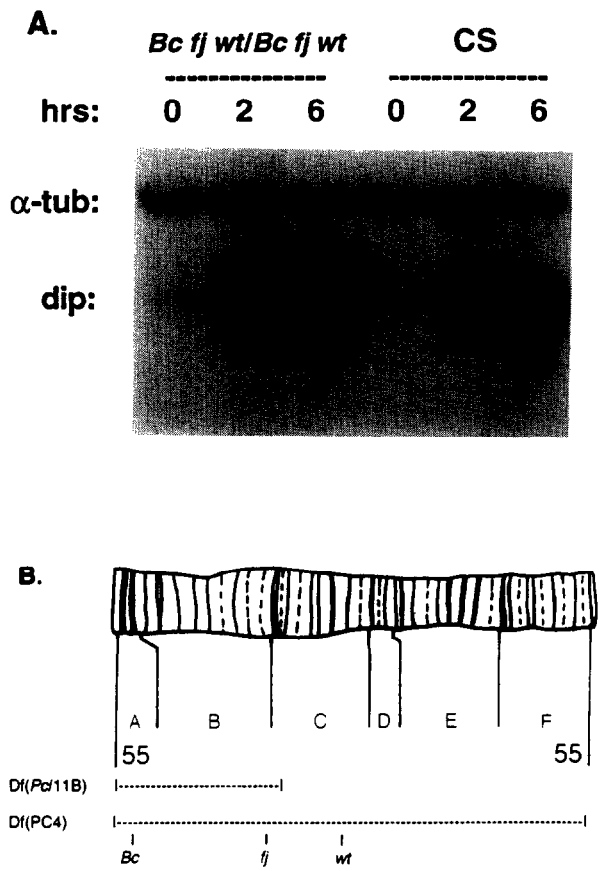

Fig. 6. (A). The *Bc* mutation alone does not affect induction of immunity genes. This figure shows the *dip* induction profile of the strain *Bc fj wt* compared with that of CS. The numbers above the autoradiograms indicates hours after infection. The α-tubulin mRNA serves as an internal control. (B). Summary of deficiencies and mutations in the region of cytological interval 55. The locations of the deficiency end-points and mutations are approximate and are based on information given in Deng and Rizki (1988), Lindsley and Zimm (1992) and Villano and Katz (1995). ((B) after Lefevre, 1976.)

levels of mortality were observed in *lz*[s]/Y males, whereas *lz*[s];*Bc*/*Bc* double mutants showed mortality levels similar to *Bc*/*Bc* alone (data not shown).

2.6. Hypersensitivity to infection correlates with enhanced bacterial growth

Living bacteria were measured in wild-type (CS) and *Bc* chromosome homozygous strains in order to determine whether there was a correlation between mortality and unrestricted growth of the pathogen (see Section 4). Groups of 50 adults were infected with the pathogen, and homogenates were plated in the presence of nalidixic acid. Wild-type flies contain an average of 6.0×10^3 bacteria/fly 24 h after infection (Fig. 8). In contrast, *Bc* homozygotes contain an average of greater than 100-fold more bacteria (6.6×10^5 bacteria/fly). These results suggest a positive correlation between *dip* induction, resistance to infection, and the suppression of bacterial growth.

3. Discussion

We have provided evidence that the induction of immunity genes such as diptericin is necessary for normal resistance to bacterial infection. Similar findings were recently reported by another group (Lemaitre et al., 1995a). *Bc* chromosome homozygotes (or Df(PC4)/*Bc* transheterozygotes) exhibit delayed and reduced induction of *dip* expression and are hypersensitive to bacterial infection. However, the physiological basis for resistance may be subtle. It would appear that healthy strains need not eliminate the bacterial infection but instead must restrict pathogen growth below a threshold level. Once induced, immunity genes remain activated for long periods after infection (at least several days), suggesting that resistance to secondary infections ('acquired immunity' in the sense of Boman et al., 1972) might result from persistence of living bacteria (or associated metabolites) from the primary infection and the consequent maintenance of anti-bacterial gene expression. The immunodeficiency mutation causes a more severe loss of induction in larvae as compared with adults, suggesting multiple, or alternate signalling pathways in adults.

Previous studies have demonstrated that insect antibacterial peptides possess potent bactericidal activities in cultured assays (reviewed by Boman and Hultmark, 1987). Moreover, different peptides may exhibit distinct activities by targeting separate sites of pathogenic bacteria. For example, it has been suggested that cecropins may target the inner membrane preferentially while diptericin affects the outer membrane (Hultmark, 1993). In this way different immunity genes may work synergistically to destroy invading bacteria. A prediction of this proposal is that different classes of immunity genes do not function redundantly, but instead, that the loss of just a single class of these genes might be sufficient to cause a substantial impairment in the immune response. The physiological functions of anti-bacterial peptides have remained somewhat elusive since none of the cloned genes in *Drosophila* correlate with known mutants. Thus, it has not been possible to determine whether the loss of individual immunity genes causes hypersensitivity to bacterial infection. The present study provides correlative evidence that insect immunity genes are indeed required for resistance to bacteria *in vivo*, and that the loss of just a subset of these genes is sufficient to cause hypersensitivity to infection.

Although this study was prompted by the analysis of mutants containing melanotic pseudo-tumors, the immunodeficiency phenotype appears to be associated with an unknown locus that maps in the 55C–F region, relatively far from the *Bc* gene (55A). Histochemical staining of uninfected and infected mutants with anti-Dif antibodies

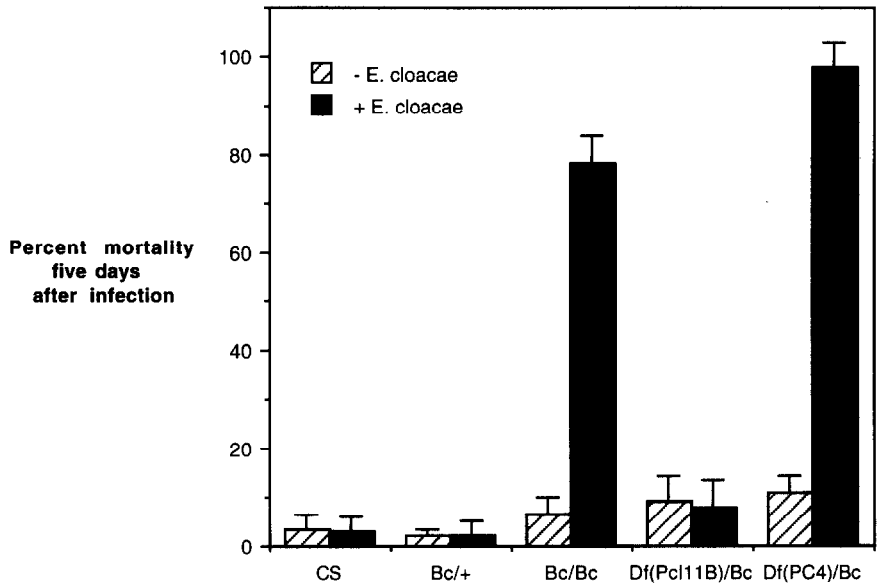

Fig. 7. Mutants are hypersensitive to bacterial infection. The bar graph presents the percentage mortality of various genotypes 5 days after infection. Each measurement represents the average of either ten (CS, Bc/+, Bc/Bc) or five (Df(Pcl11B/Bc), Df(PC4/Bc)) separate experiments in which groups of 50 adult flies were pricked with a clean needle (– *E. cloacae*) or with a needle dipped in bacteria (+ *E. cloacae*). Standard deviations among experiments are indicated.

suggests that the relocation of the Dif regulatory protein from the cytoplasm to the nucleus is probably not affected in this mutant (data not shown). Furthermore, Dif RNA is normally up-regulated upon infection in the immunodeficient strain (data not shown). It would appear that another pathway or regulatory factor, which might work in concert with Dif (and/or Dorsal), may be disrupted. Alternatively, perhaps the immunodeficiency mutation blocks a modification process, which influences Dif or Dorsal function but not nuclear transport.

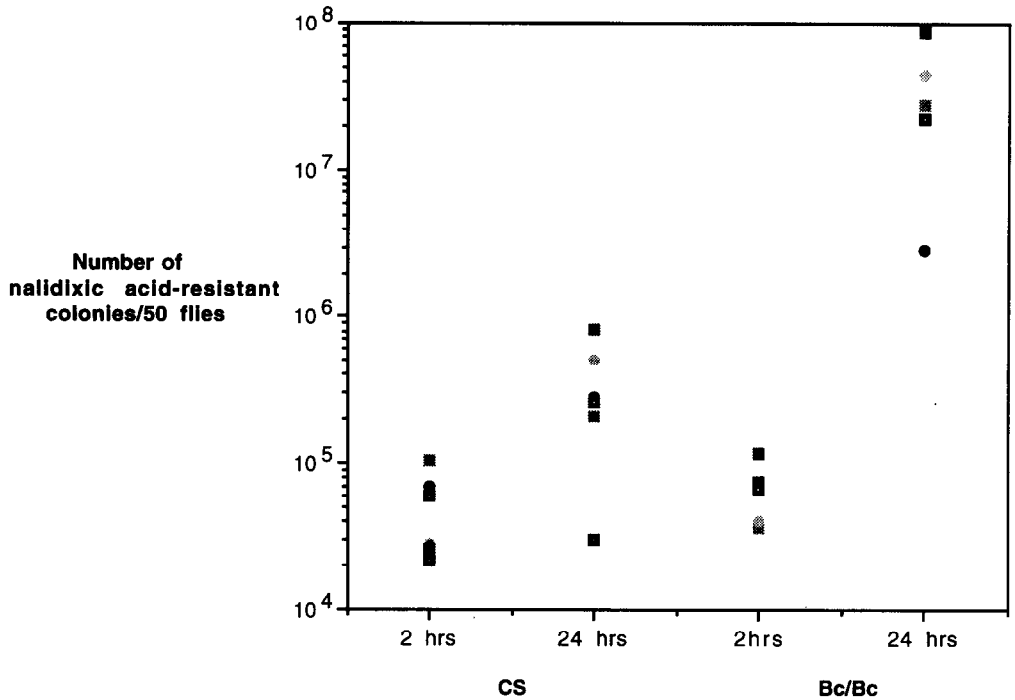

Fig 8. Mutants exhibit permissive growth of the pathogen. Titers of nalidixic acid-resistant bacteria recovered from *Bc* chromosome homozygotes versus wild type (CS) flies. Each column displays the results of six independent experiments in which groups of 50 adult flies were infected with a nalidixic acid-resistant strain of *E. cloacae*. Flies were homogenized either two or 24 h after infection, and lysates were cultured on plates containing nalidixic acid. Control cultures with lysates from uninfected flies showed no growth on nalidixic acid plates after 24 h (data not shown).

In this regard we note that the Dif/*dl* regulatory pathway is not sufficient for the immune response. For example, *Toll* dominant mutants and *cactus* mutants display constitutive nuclear expression of the Dorsal and Dif proteins, but do not constitutively express *cec* or *dip* in the absence of infection (Ip et al., 1993; Lemaitre et al., 1995b). The possibility of a second inductive pathway suggests additional similarities with the mammalian acute phase response, which depends on at least two different cytokines, IL-1 and IL-6 (Kishimoto et al., 1994). The molecular characterization of this immunodeficiency mutation could provide general insights concerning the regulation and activities of Rel-domain transcription factors.

4. Materials and methods

4.1. Fly stocks

The following fly strains were used in this study: (i) *Bc* (Bloomington stock 1046), (ii) *Bc fj wt* (Bloomington stock 1036), (iii) *lz*[s], (iv) Df(2R)*Pcl*11B/CyO (in this paper, Df(*Pcl*11B)), and (v) Df(2R)PC4/CyO (in this paper, Df(PC4)). These were all obtained from the Bloomington Stock Center (Indiana University) with the exception of *lz*[s] which was obtained from the Bowling Green Stock Center (Bowling Green State University, OH). The *lz*[s]; *Bc/Bc* double mutant was obtained by standard genetic crosses.

4.2. Northern blots

For each time point approximately 50 flies were pricked in the abdomen with a glass needle that had been dipped in a saturated culture of *E. cloacae* β 12 (kindly provided by Ylva Engstrom; see Kylsten et al., 1990). Infected flies were pulverized with a Dounce homogenizer in 50 mM Tris–HCl (pH 7.5), 50 mM NaCl, 10 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulfate (SDS), 250 μg/ml proteinase K, and then incubated at 37°C for 1 h. The sample was extracted twice with phenol/chloroform (1:1), once with chloroform alone, and then precipitated with ethanol. RNA samples were precipitated once with 2.5 M LiCl, and then resuspended in DEPC-treated water and stored at −70°C.

RNAs were fractionated on a denaturing formaldehyde/agarose gel, transferred to GeneScreen (DuPont), and the filter was then baked at 80°C under a vacuum for 1 h. Pre-hybridizations, hybridizations, and washes were performed essentially as described by Ausubel et al. (1994).

^{32}P-labeled hybridization probes for *cec*A1, diptericin, and α-tubulin were synthesized from full-length coding sequences using standard random priming procedures (Ausubel et al., 1994). *cec*A1 (Kylsten et al., 1990) and α-tubulin (see Azpiazu and Frasch, 1993) were kindly provided by Ylva Engstrom and Manfred Frasch, respectively. Diptericin was isolated by PCR using primers based on published sequences (Reichhart et al., 1992).

4.3. Survival assays

Groups of 50 flies, 3 days post-eclosion, were pricked in the abdomen with a clean glass needle or one that had been dipped in a saturated culture of *E. cloacae* β 12. The flies were then transferred daily to fresh food, and after 5 days the survivors were counted.

4.4. Bacterial proliferation assays

Groups of 50 flies were treated as described above and subsequently pulverized with a Dounce homogenizer in 10 mM $MgSO_4$. The homogenates were serially diluted and plated on agar containing LB (Ausubel et al., 1994) and nalidixic acid at a concentration of 25 μg/ml. These plates were incubated at 37°C overnight, and colonies were counted.

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References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (eds.) (1994) Current Protocols in Molecular Biology (Current Protocols).

Azpiazu, N and Frasch, M. (1993) Genes Dev. 7, 1325–1340.

Beg, A.A. and Baldwin, Jr., A.S. (1993) Genes Dev. 7, 2064–2070.

Belvin, M.P., Jin, Y. and Anderson, K.V. (1995) Genes Dev. 9, 783–793.

Boman, H.G. and Hultmark, D. (1987) Annu. Rev. Microbiol. 41, 103–126.

Boman, H.G., Nilsson, I. and Rasmuson B. (1972) Nature 237, 232–235.

Deng, Y. and Rizki, T.M. (1988) Genome 30 (Suppl. 1), 192.

Engstrom, Y., Kadalayil, L., Sun, S.-C., Samakovlis, S.G., Hultmark, D. and Faye, I. (1993) J. Mol. Biol. 232, 327–333.

Gonzalez-Crespo, S. and Levine, M. (1994) Genes Dev. 7, 1703–1713.

Gordon, A.H. and Koji, A. (eds.) (1985) Research Monographs in Cell and Tissue Physiology, Vol. 10, Elsevier, Amsterdam.

Hoffmann, J.A., Hetru, C. and Reichart, J.M. (1993) FEBS Lett., 325, 63–66.

Hultmark, D. (1993) Trends Genet. 9, 178–183.

Ip, Y.T. and Levine, M. (1994) Curr. Opin. Genet. Dev. 4, 672–677.

Ip, Y.T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., Gonzalez-Crespo, S., Tatei, K. and Levine, M. (1993) Cell 75, 753–763.

Kappler, C., Meister, M., Lagueux, M., Gateff, E., Hoffmann, J.A. and Reichhart, J.M. (1993) EMBO J. 12, 1561–1568.

Kishimoto, T., Taga, T. and Akira, S. (1994) Cell 76, 253–262.

Kylsten, P., Samakovlis, C. and Hultmark, D. (1990) EMBO J. 9, 217–224.

Lefevre, Jr., G. (1976) In Ashburner and Novitski (eds.), The Genetics and Biology of *Drosophila*, Vol. 1a, Academic Press, London.

Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J.M. and Hoffmann, J.A. (1995a) Proc. Nat. Acad. Sci. USA 92, 9465–9469.

Lemaitre, B., Meister, M., Govind, S., Georgel, P., Steward, R., Reichhart, J.M. and Hoffmann, J.A. (1995b) EMBO J. 14, 536–545.

Lenardo, M., Pierce, J.W. and Baltimore D. (1987) Science 236, 1573–1577.

Lindsley, D.L. and Zimm, G.G. (1992) The Genome of *Drosophila Melanogaster*. Academic Press, San Diego, CA.

Meister, M., Braun, A., Kappler, C., Reichhart, J.M. and Hoffmann, J.A. (1994) EMBO J. 13, 5958–5966.

Peeples, E.E., Geisler, A., Whitcraft, C.J. and Oliver, C.P. (1969) Genetics 62, 161–170.

Reichhart, J.M., Georgel, P., Meister, M., Lemaitre, B., Kappler, C. and Hoffmann, J.A. (1993) C. R. Acad. Sci. (Paris) 316, 1218–1224.

Reichhart, J.M., Meister, M., Dimarcq, J.L. Zachary, D., Hoffmann, D., Ruiz, C., Richards, G. and Hoffmann, J.A. (1992) EMBO J. 11, 1469–1477.

Rizki, T.M. and Rizki, R.M. (1981) Genetics 97, s90.

Rizki, T.M., Rizki, R.M. and Grell, E.H. (1980) Roux Arch. Dev. Biol. 188, 91–99.

Sha, W.C., Liou, H.C., Tuomanen, E.I. and Baltimore, D. (1995) Cell 80, 321–330.

Sparrow, J.C. (1978) In Ashburner, M. and Wright, T.R.F. (eds.), The Genetics and Biology of *Drosophila*, Vol. 2B, Academic Press, New York, pp. 277–313.

Steward, R. and Govind, S. (1993) Curr. Opin. Genet. Dev. 3, 556–561.

St. Johnston, D. and Nusslein-Volhard, C. (1992) Cell 68, 201–219.

Villano, J.L. and Katz, F.N. (1995) Development 121, 2767–2777.

Watson, K.L., Johnson, T.K. and Denell, R.E. (1991) Dev. Genet. 12, 173–187.

Wicker, C., Reichhart, J.M., Hoffmann, D., Hultmark, D., Samakovlis, C. and Hoffmann, J.A (1990) J. Biol. Chem. 265, 22493–22498.