Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 10343–10347, September 1996
Immunology

Origins of immunity: *Relish*, a compound Rel-like gene in the antibacterial defense of *Drosophila*

MITCHELL S. DUSHAY*†, BENGT ÅSLING*, AND DAN HULTMARK‡

Departments of Developmental and Molecular Biology, Stockholm University, S-106 91 Stockholm, Sweden

Communicated by Thomas Maniatis, Harvard University, Cambridge, MA, June 25, 1996 (received for review April 15, 1996)

ABSTRACT **NF-κB/Rel transcription factors are central regulators of mammalian immunity and are also implicated in the induction of cecropins and other antibacterial peptides in insects. We identified the gene for Relish, a compound *Drosophila* protein that, like mammalian p105 and p100, contains both a Rel homology domain and an IκB-like domain. Relish is strongly induced in infected flies, and it can activate transcription from the *Cecropin A1* promoter. A *Relish* transcript is also detected in early embryos, suggesting that it acts in both immunity and embryogenesis. The presence of a compound Rel protein in *Drosophila* indicates that similar proteins were likely present in primordial immune systems and may serve unique signaling functions.**

Unexpected similarities have been found between the immune systems of insects and mammals. In particular, the Rel family of transcription factors play an important role in both groups (1–3). Five different Rel proteins have been found in mammals, and they combine to form different homo- or heterodimers, collectively referred to as nuclear factor (NF)-κB. They are normally kept inactive in the cytoplasm, in complex with an inhibitor (I)κB. In response to different pathogenic signals, IκB is degraded and NF-κB is translocated to the nucleus to activate genes important for the immune response (1, 2). Two of the mammalian Rel proteins, p105 (4–7) and p100 (8–11), have a compound structure; they contain an IκB-like domain in addition to the Rel homology domain, and they can act as NF-κB inhibitors. Their IκB-like domains may also be removed by proteolytic processing, and the remaining Rel proteins become integral parts of the NF-κB complex (reviewed in refs. 1 and 2). In *Drosophila*, two Rel proteins, dorsal-related immunity factor (Dif) (12) and Dorsal (13, 14), may mediate the induction of *Cecropin* and other genes following injury and infection (reviewed in refs. 15 and 16). However, neither of them carries IκB-like domains.

We are screening for genes involved in the *Drosophila* immune system by using PCR differential display (17, 18) to identify genes induced in infected flies. Among the induced genes we expect to find regulators as well as effectors of the antibacterial response. Here we describe a compound Rel protein found in this screen, Relish, which is involved in *Drosophila* immune responses and probably in embryogenesis as well.

MATERIALS AND METHODS

cDNA Libraries. We generated a cDNA library from mbn-2 cells (19), taken 1 h after the addition of 100 μg/ml lipopolysaccharide and 100 μg/ml laminarin. RNA was purified as described (20), and 5 μg of poly(A)$^+$ RNA was prepared using Hybond paper (Amersham). cDNA was synthesized and directionally cloned between the *Eco*RI and *Xho*I sites in λ ZAP II (21) using the ZAP-cDNA synthesis kit (Stratagene). A second library from immunostimulated adult flies was described previously (22).

Isolation of cDNA Clones. The differential display screen (23), comparing flies 0, 6, and 16 h after the injection of *Enterobacter cloacae* β12, has been described previously (18). One induced PCR band, cut from the differential display gel, was cloned using a TA cloning kit (Invitrogen) and then used as a probe to screen the two induced cDNA libraries. Twenty clones of different lengths were isolated. The longest clone, called 5.3, was isolated from the mbn-2 cell library. It was sequenced on both strands, using the Sequenase kit (United States Biochemical) and synthetic oligonucleotide primers. The other clones were sequenced from the ends, and all sequences were superimposable.

RNA and Northern Blots. Adult wild-type Canton-S *Drosophila melanogaster*, >1-day-old males and females, were injected with a stationary phase culture of *E. cloacae* β12 diluted 1:10 in Ringer's solution (24). Surviving flies were collected at different times postinjection and frozen in liquid N_2. RNA was prepared from these flies and from matched control flies by hot phenol extraction (25). For the developmental study, staged eggs were collected on apple juice agar plates on which females were permitted to lay eggs for 2 h. The plates were then aged by incubating at 25°C. Adults were injected with bacteria as described above. Samples of 6-μg total RNA were separated on a formaldehyde gel and blotted (26). The same filters were probed with *Relish* and *Rp49* (27) probes, generated with the Rediprime kit (Amersham).

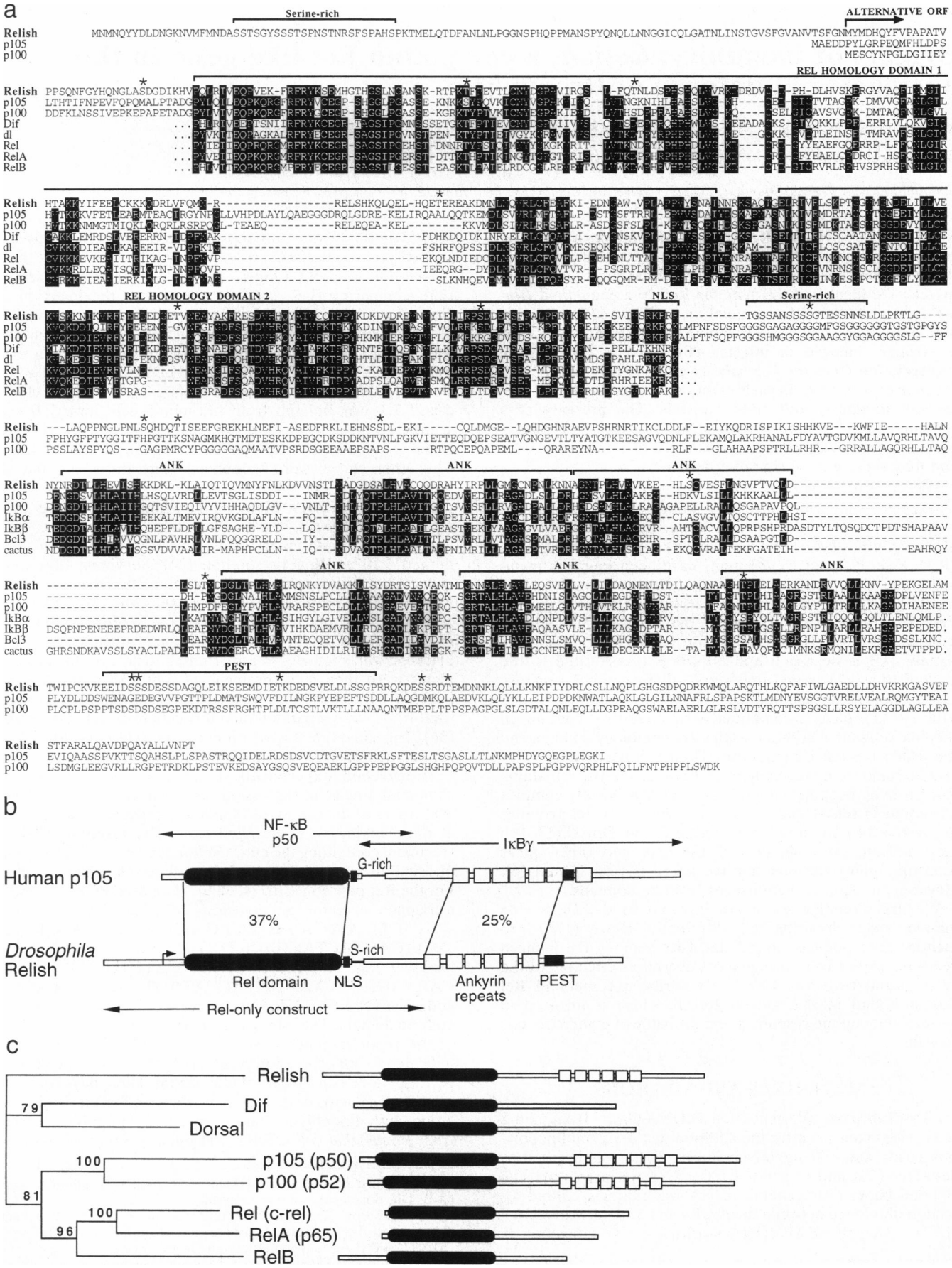
Transfection Experiments. Full-length *Relish* cDNA was cloned into the actin expression vector pPacPL (28) as follows. The insert of the largest cDNA clone, called 5.3, was cut out at the flanking *Spe*I and *Kpn*I sites. The resulting 3387-bp fragment, containing the entire *Relish* open reading frame, was purified and ligated into *Spe*I–*Kpn*I-digested pPacPL vector. For the Rel-only construct (see Fig. 1) we used PCR to amplify a fragment encoding amino acids 4-600. The 5′ primer GACTAGTTCAACATGCATCACCATCACCATCACAATCAGTACTACGACCTGG adds a *Spe*I site and a hexahistidine tag, and the 3′ primer GCTCTGAATAACTACAATAGAGACTAAGGTACCATAGT adds a stop codon and a *Kpn*I site. The 3′ Rel primer was put just upstream of the ankyrin domain. The mbn-2 cells were cotransfected with 1 μg of the reporter gene construct pA10 (30) and 1 μg of the indicated *Relish* expression construct (or the empty expression vector pPacPL), in a total volume of 5 ml. Three days later, the relative β-galactosidase activity was assayed, after 4 h stimulation with lipopolysaccharide (purified lipopolysaccharide from *Escherichia coli* 055:B5, 100 μg/ml). Protocols were as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NF-κB, nuclear factor kappa-B; IκB, inhibitor kappa-B; Dif, dorsal-related immunity factor.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U62005).

*M.S.D. and B.Å. contributed equally to this work.
†Present address: Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.
‡To whom reprint requests should be addressed. e-mail: dan@ molbio.su.se.


a
Serine-rich
ALTERNATIVE ORF
REL HOMOLOGY DOMAIN 1
REL HOMOLOGY DOMAIN 2
NLS
Serine-rich
ANK
ANK
ANK
ANK
ANK
ANK
PEST
Relish
p105
p100
Dif
dl
Rel
RelA
RelB
IκBα
IκBβ
Bcl3
cactus
b
NF-κB p50
IκBγ
Human p105
G-rich
37%
25%
Drosophila Relish
S-rich
Rel domain
NLS
Ankyrin repeats
PEST
Rel-only construct
c
Relish
Dif
Dorsal
p105 (p50)
p100 (p52)
Rel (c-rel)
RelA (p65)
RelB
79
100
81
100
96

described by Petersen *et al.* (31), except that transfection efficiency, measured by chloramphenicol acetyltransferase expression from a cotransfected plasmid, was assayed by enzyme-linked immunosorbent assay (Boehringer Mannheim).

***In Situ* Hybridization.** *Relish*'s cytological localization was determined by *in situ* hybridization to salivary gland chromosomes as described by Pardue (32) at 58°C. Digoxigenin-labeled probe was generated with a DNA labeling and alkaline phosphatase detection kit (Boehringer Mannheim) using an *Xho*I–*Not*I fragment of cDNA clone 5.3. Chromosomal maps in Lindsley and Zimm (33) were used as reference.

RESULTS

Cloning and Sequence of *Relish* cDNA. One PCR product that was differentially expressed in flies after a bacterial injection included several ankyrin repeats, similar to those seen in the IκB proteins. We used this PCR fragment as a probe to screen cDNA libraries from immunostimulated adult flies and mbn-2 cells, a hemocyte cell line (19). Twenty overlapping cDNA clones that differed only by length were isolated from the two libraries. This and genomic Southern blot results (data not shown) suggest the presence of a single copy gene.

The conceptual protein translated from the 3334-bp sequence of the longest cDNA clone contains both a Rel homology domain (hence the name Relish) and an IκB-like domain with six ankyrin repeats (Fig. 1). In this respect Relish is similar to the compound mammalian NF-κB precursors p100 and p105, although no obvious similarity is seen outside the two conserved domains. In fact, Relish is not closely related to any other known Rel protein. A phylogenetic reconstruction of the possible relationship between the different Rel homology domains (Fig. 1*c*) shows that Relish may have branched off from other Rel proteins at a very early stage. The sequences of the ankyrin repeats are also quite different from those of other IκB-like proteins, and they are about equally close to the ankyrin repeats in the Notch, ankyrin and IκB families. Like in other Rel proteins, a putative nuclear localization sequence is found at the C-terminal end of the Rel homology domain, and the ankyrin repeats are followed by an acidic, PEST-like sequence. PEST sequences are rich in proline, glutamic acid, serine and threonine residues, and they have been implicated in protein turnover (34).

Interesting features are noted in the regions outside the conserved domains (Fig. 1). Like RelB, Relish has an unusually long region N-terminal of the Rel homology domain. Furthermore, just downstream of the Rel homology domain there is a serine-rich stretch, corresponding to the position where p100 and p105 have a glycine-rich region that serves as a processing signal for the generation of p50 (35). The serine-rich sequence in Relish may serve a similar function. Another serine-rich region is found in the N-terminal region of Relish. Finally, there are several potential target sites for phosphorylation by casein kinase II; including four in the spacer between the Rel and ankyrin domains, and five in, or near the PEST region. Casein kinase II has been implicated in the constitutive phosphorylation of the PEST region in IκBα (36), and the signal-induced phosphorylation of the same protein is mediated by a kinase with similar target sites (37–39).

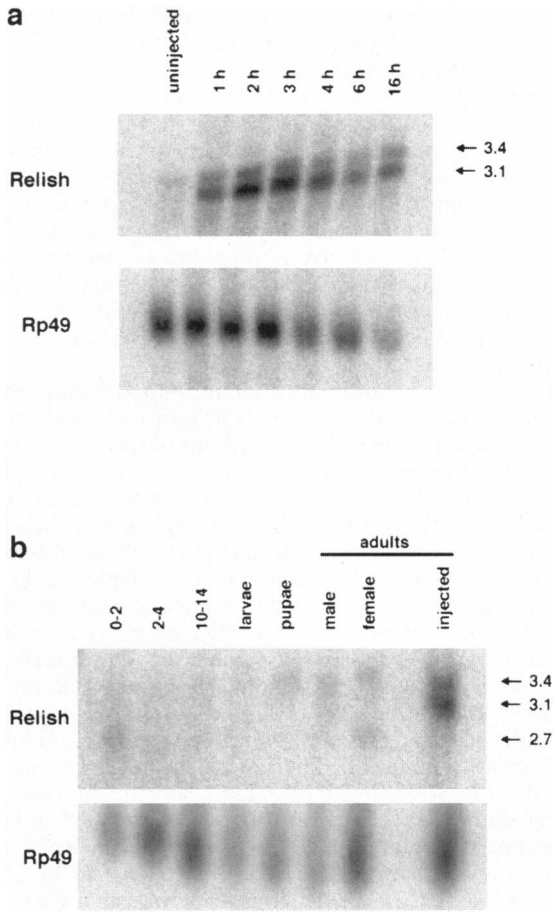


FIG. 2. Relish expression in immunity and development, detected on Northern blots, with the *Rp49* gene as an RNA loading control. (*a*) Induction of *Relish* mRNA following bacterial injection. Lane numbering indicates the number of hours postinjection. The 3.4-kb *Relish* transcript is detectable at all times, while the 3.1 kb transcript is not seen in uninjected adults. (*b*) *Relish* mRNA expression in embryos at different stages of development. Lane numbering indicates the number of hours after oviposition. The 3.4-kb constitutive, 3.1-kb induced, and 2.7-kb maternal *Relish* transcripts are seen. The constitutive 3.4 *Relish* transcript is detectable in all stages, although its expression is not marked before 10–14 h. The same pattern was seen when filters were hybridized with a 5′ probe covering the Rel domain, or a 3′ probe containing the ankyrin-repeat region, indicating that all three transcripts contain both of these domains.

Different Embryonic and Inducible Transcripts. Two major *Relish* transcripts are seen in Northern blots of adult flies (Fig. 2*a*). A 3.4-kb transcript is expressed constitutively and is further induced about 15-fold after infection. In contrast, a 3.1-kb transcript is undetectable in untreated animals, but is strongly induced in infected flies (greater than 50-fold) with induction kinetics similar to the *Cecropin A1* gene (data not shown). *Relish* is thus induced much more strongly than either of the two other *Drosophila* Rel protein genes, *Dif* and *dorsal*, which are only induced about 3-fold under these conditions (data not shown). The *dorsal* gene is known for its role in early embryogenesis (reviewed in ref. 40). *Dif*, on the other hand, is

FIG. 1. A comparison of Relish to other members of the Rel and IκB families. (*a*) Comparison of amino acid sequences. Alignments were aided by the GENEJOCKEY II program (Biosoft, Milltown, NJ) and manually adjusted. Except for Relish, p105 and p100, only the conserved regions are shown. Potential casein kinase II phosphorylation sites in Relish are indicated with stars. Besides Relish, Dif, and Dorsal, all sequences are for the human homologs. The full DNA sequence for *Relish* has been deposited in the EMBL/GenBank/DDBJ databases, accession number U62005. Accession numbers for the other sequences shown are: L29015 (EMBL/GenBank/DDBJ), and P15330, P19838, Q00653, Q04864, Q04206, and Q01201 (Swissprot). (*b*) Domain structure of the Relish and p105 proteins, showing percent amino acid sequence identity. The part of *Relish* expressed in the Rel-only transfection construct is indicated. (*c*) Phylogenetic comparison with other Rel proteins. The maximum parsimony analysis (29) is based on the Rel homology domains only. Numbers indicate the percentage of bootstrap replications that support each branch.

only expressed late in embryogenesis and probably serves no developmental function (12). To test if *Relish* is expressed during development, Northern blots were prepared with RNA from different staged embryos, larvae, pupae, and adults (Fig. 2*b*). A 2.7-kb *Relish* transcript is present in 0–2 h embryos and uninjected adult females. This transcript is present at low levels in 2–4 h embryos and is not detectable in embryos after 8 h, or in larvae, pupae, or adult males. This pattern of expression is similar to *dorsal* and other maternally transcribed genes, and suggests that *Relish* mRNA is provided to the egg by the mother. While we have not strictly proven that this *Relish* transcript is maternally expressed (by *in situ* hybridization or mutant analysis), we call this the maternal *Relish* transcript for convenience.

All three *Relish* transcripts contain both Rel and ankyrin domains, since hybridization of Northern blots with probes from both domains show identical results (data not shown). To further characterize the maternal transcript, we isolated five cDNA clones from an ovarian library (kindly provided by Peter Tolias, Public Health Research Institute). These clones were identical to those from the adult and mbn-2 cell libraries except for various degrees of truncation at the 5′ end. The longest clone had an insert of 2.7 kb and thus must be near full-length. 5′-rapid amplification of cDNA ends products from 0- to 2-h-old embryos show the same sequence and terminate near the 5′ end of this clone (B.Å., unpublished data). We conclude that the maternal transcript differs from the other two *Relish* transcripts at the 5′ end only, and that if there is a unique 5′ exon it must be very short. The maternal transcript is too short to encode the N-terminal part of the open reading frame, and a likely alternative translation start site is indicated in Fig. 1.

Genomic Localization. *Relish* was cytologically localized to 85C on the right arm of the third chromosome by *in situ* hybridization (Fig. 3). We sometimes saw the signal as a double band, and also saw faint hybridizations to other chromosomal regions. This is probably due to artifacts of our *in situ* procedure, since no evidence of cross-hybridizing genes was found on genomic Southern blots hybridized at low stringency using separate probes covering the Rel and Ankyrin domains of the *Relish* cDNA (data not shown).

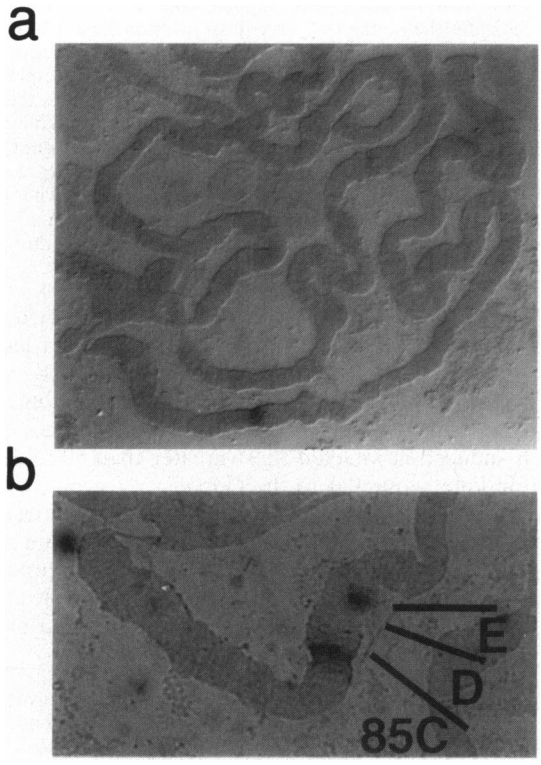


FIG. 3. Cytological localization of *Relish*. Polytene chromosomes were hybridized with digoxigenin-labeled cDNA as described in *Materials and Methods*. (*a*) Photomicrograph showing entire chromosome arm 3R. (*b*) Higher resolution view of division 85.

Stimulation of the *Cecropin A1* Promoter by Overexpression of *Relish*. The *Cecropin A1* gene promoter is known to have a functional κB-site (30), and is thus a potential target for Relish regulation. We tested the effect of *Relish* overexpression on a *CecA1–lacZ* reporter gene construct (30) after cotransfection of the mbn-2 blood cell line. In addition to a full-length *Relish* cDNA, we also investigated the effect of a truncated "Rel-only" construct that lacks the IκB-like domain (Fig. 1*b*). Mammalian p50 is produced by the proteolytic degradation of the ankyrin domain of p105. We designed the Rel-only construct to be similar to a p105 fragment that produced a stable p50 when transfected into mammalian cells (41). Fig. 3 shows that overexpression of the full-length *Relish* gene stimulates expression from the *CecA1-lacZ* fusion reporter 3-fold over the maximally lipopolysaccharide-induced control, while the Rel-only construct increased expression as much as 10-fold (Fig. 4). Relish can thus stimulate cecropin transcription, directly or indirectly, and the sequences present in the Rel-only construct are sufficient for this effect. The lesser effect seen with the full-length construct may be due to the presence of an inhibitory IκB-like domain. Alternatively, this construct may be less efficiently translated, as the cDNA clone contains a short open reading frame 5′ of the start site that is likely to reduce the level of protein expression (42). Similar false starts are found at the 5′ end of *Dif* cDNA clones, and these have been suggested to possibly serve a translational regulation function (12).

DISCUSSION

Relish has the capacity to activate cecropin gene transcription, as do Dorsal and Dif (31), and the *Relish* gene is itself very strongly induced after infection, much more than either *dorsal* or *Dif*. These observations strongly suggest that *Relish* is involved in the regulation of the immune response in *Drosophila*. Moreover, if protein levels parallel RNA levels, Relish may be the predominant Rel protein in flies following infection. A maternal *Relish* transcript is detectable in early embryos, so like *dorsal*, *Relish* may also serve a function in early embryogenesis. Although the exact role of *Relish* in development is unknown, the timing of expression and the ability of Rel proteins to form heterodimers (2) suggest that Relish may interact with Dorsal. A residual dorsal-ventral polarity is seen

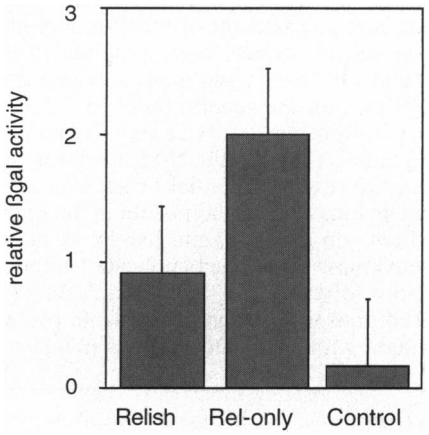


FIG. 4. Relish activation of the *Cecropin A1* promoter. We assayed β-galactosidase expression from pA10, a *CecA1-LacZ* fusion construct (30) cotransfected into mbn-2 cells with plasmids that express Relish, Rel-only, or the empty expression vector alone. Each bar represents the average of four transfections, from two independent experiments, and the standard error is indicated.

in strong *cactus* mutants (43), which implies that there could be another factor with inhibitor activity in the *dorsal* signaling pathway. Relish protein, with its ankyrin repeats, could serve that role. Alternatively, Relish could function in a completely independent pathway. We are now characterizing antibodies generated against Relish and isolating *Relish* mutants to study Relish's interactions with Dif, Dorsal, and other proteins, and to better characterize its role in immunity and development.

It will be important to determine whether Relish is proteolytically processed like p105 and p100. The fact that the p50-like Rel-only construct is biologically active indicates that this could indeed be the case. In the mouse, the NF-κB inhibitor IκBγ is formed by alternative splicing of the p105 gene (44). We have not detected a corresponding splice form of Relish, but it is possible that a similar protein could also be produced by processing of Relish protein.

The discovery of a compound Rel protein in flies strengthens the similarity between the mechanisms used by insects and vertebrates to regulate their immune responses (3). If *Relish* separated very early from other Rel protein genes as suggested by the phylogenetic analysis (Fig. 1*c*), it implies that early Rel proteins were compound, and that proteins like Dorsal and Dif later lost their ankyrin repeats. In this context it is interesting to note that the *Dif* and *dorsal* genes are closely linked on the second chromosome to the IκB homolog *cactus*, which could reflect the common origin of these genes from an ancestral compound Rel protein. Beyond this, demonstrating the presence of a compound protein in insects as well as mammals fills a conceptual gap and reinforces the suggestion that these proteins may serve important regulatory roles that cannot be accomplished by separate Rel and IκB proteins. It may now be possible to test for distinct signaling pathways mediated by compound proteins in *Drosophila* using *Relish*.

We thank Gunnel Björklund and Ylva Engström for plasmids, advice, and help with the transfection experiments; Daiwu Kang for valuable suggestions for the differential display technique; and Per Kylsten for help with the prints. Ylva Engström and Pär Nordlund made valuable comments on the manuscript. This work was supported by grants from the Swedish Natural Science Research Council and the Swedish Medical Research Council. M.S.D. was supported by fellowships from the Swedish Natural Science Research Council, Wenner-Grenska Samfundet, and the Wenner–Gren Center Foundation.

1. Baeuerle, P. A. & Henkel, T. (1994) *Annu. Rev. Immunol.* **12,** 141–179.
2. Siebenlist, U., Franzoso, G. & Brown, K. (1994) *Annu. Rev. Cell. Biol.* **10,** 405–455.
3. Hultmark, D. (1994) *Nature (London)* **367,** 116–117.
4. Bours, V., Villalobos, J., Burd, P. R., Kelly, K. & Siebenlist, U. (1990) *Nature (London)* **348,** 76–80.
5. Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., Le Bail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. A. & Israël, A. (1990) *Cell* **62,** 1007–1018.
6. Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P. & Baltimore, D. (1990) *Cell* **62,** 1019–1029.
7. Meyer, R., Hatada, E. N., Hohmann, H. P., Haiker, M., Bartsch, C., Rothlisberger, U., Lahm, H. W., Schlaeger, E. J., Vanloon, A. P. G. M. & Scheidereit, C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 966–970.
8. Schmid, R. M., Perkins, N. D., Duckett, C. S., Andrews, P. C. & Nabel, G. J. (1991) *Nature (London)* **352,** 733–736.
9. Neri, A., Chang, C. C., Lombardi, L., Salina, M., Corradini, P., Maiolo, A. T., Chaganti, R. S. K. & Dalla-Favera, R. (1991) *Cell* **67,** 1075–1087.
10. Mercurio, F., Didonato, J., Rosette, C. & Karin, M. (1992) *DNA Cell Biol.* **11,** 523–537.
11. Bours, V., Burd, P. R., Brown, K., Villalobos, J., Park, S., Ryseck, R. P., Bravo, R., Kelly, K. & Siebenlist, U. (1992) *Mol. Cell. Biol.* **12,** 685–695.
12. Ip, Y. T., Reach, M., Engstrom, Y., Kadalayil, L., Cai, H., González-Crespo, S., Tatei, K. & Levine, M. (1993) *Cell* **75,** 753–763.
13. Steward, R. (1987) *Science* **238,** 692–694.
14. Reichhart, J.-M., Georgel, P., Meister, M., Lemaitre, B., Kappler, C. & Hoffmann, J. A. (1993) *C. R. Acad. Sci. Paris Life Sci.* **316,** 1218–1224.
15. Hultmark, D. (1993) *Trends Genet.* **9,** 178–183.
16. Cociancich, S., Bulet, P., Hetru, C. & Hoffmann, J. A. (1994) *Parasitol. Today* **10,** 132–139.
17. Liang, P. & Pardee, A. P. (1992) *Science* **257,** 967–971.
18. Åsling, B., Dushay, M. S. & Hultmark, D. (1995) *Insect Biochem. Mol. Biol.* **25,** 511–518.
19. Gateff, E., Gissmann, L., Shrestha, R., Plus, N., Pfister, H., Schröder, J. & zur Hausen, H. (1980) in *Invertebrate Systems In Vitro*, eds. Kurstak, E., Maramorosch, K. & Dübendorfer, A. (Elsevier/North–Holland, Amsterdam), pp. 517–533.
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1994) *Current Protocols in Molecular Biology* (Wiley, New York), Vol. 1.
21. Short, J. M., Fernandez, J. M., Sorge, J. A. & Huse, W. D. (1988) *Nucleic Acids Res.* **16,** 7583–7600.
22. Kylsten, P., Samakovlis, C. & Hultmark, D. (1990) *EMBO J.* **9,** 217–224.
23. Liang, P., Averboukh, L. & Pardee, A. (1993) *Nucleic Acids Res.* **21,** 3269–3275.
24. Samakovlis, C., Kimbrell, D. A., Kylsten, P., Engström, Å. & Hultmark, D. (1990) *EMBO J.* **9,** 2969–2976.
25. Klemenz, R., Hultmark, D. & Gehring, W. J. (1985) *EMBO J.* **4,** 2053–2060.
26. Davis, L. G., Dibner, M. D. & Battey, J. F. (1986) *Basic Methods in Molecular Biology* (Elsevier, New York), pp. 143–146.
27. O'Connell, P. O. & Rosbash, M. (1984) *Nucleic Acids Res.* **12,** 5495–5513.
28. Krasnow, M. A., Saffman, E. E., Kornfeld, K. & Hogness, D. S. (1989) *Cell* **57,** 1031–1043.
29. Swofford, D. L. (1991) PAUP: Phylogenetic Analysis Using Parsimony (Illinois Natl. Hist. Survey, Champaign).
30. Engström, Y., Kadalayil, L., Sun, S.-C., Samakovlis, C., Hultmark, D. & Faye, I. (1993) *J. Mol. Biol.* **232,** 327–333.
31. Petersen, U.-M., Björklund, G., Ip, Y. T. & Engström, Y. (1995) *EMBO J.* **14,** 3146–3158.
32. Pardue, M. L. (1986) in *Drosophila: A Practical Approach*, ed. Roberts, D. B. (IRL, Oxford), pp. 111–137.
33. Lindsley, D. L. & Zimm, G. G. (1992) *The Genome of Drosophila melanogaster* (Academic, San Diego).
34. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234,** 364–368.
35. Lin, L. & Ghosh, S. (1996) *Mol. Cell. Biol.* **16,** 2248–2254.
36. Barroga, C. F., Stevenson, J. K., Schwarz, E. M. & Verma, I. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7637–7641.
37. Brown, K., Gerstberger, S., Carlson, L., Franzoso, G. & Siebenlist, U. (1995) *Science* **267,** 1485–1488.
38. Traenckner, E. B.-M., Pahl, H. L., Henkel, T., Schmidt, K. N., Wilk, S. & Baeuerle, P. A. (1995) *EMBO J.* **14,** 2876–2883.
39. Chen, Z. J., Parent, L. & Maniatis, T. (1996) *Cell* **84,** 853–862.
40. Govind, S. (1991) *Trends Genet.* **7,** 119–125.
41. Fan, C. M. & Maniatis, T. (1991) *Nature (London)* **354,** 395–398.
42. Abastado, J. P., Miller, P. F. & Hinnebusch, A. G. (1991) *New Biol.* **3,** 511–524.
43. Roth, S., Hiromi, Y., Godt, D. & Nüsslein-Volhard, C. (1991) *Development (Cambridge, U.K.)* **112,** 371–388.
44. Inoue, J., Kerr, L. D., Kakizuka, A. & Verma, I. M. (1992) *Cell* **68,** 1109–1120.