The GATA factor Serpent is required for the onset of the humoral immune response in *Drosophila* embryos

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Innate immunity in *Drosophila* is characterized by the inducible expression of antimicrobial peptides. We have investigated the development and regulation of immune responsiveness in *Drosophila* embryos after infection. Immune competence, as monitored by the induction of *Cecropin A1-lacZ* constructs, was observed first in the embryonic yolk. This observation suggests that the yolk plays an important role in the humoral immune response of the developing embryo by synthesizing antimicrobial peptides. Around midembryogenesis, the response in the yolk was diminished. Simultaneously, *Cecropin* expression became inducible in a large number of cells in the epidermis, demonstrating that late-stage embryos can synthesize their own antibiotics in the epidermis. This production likely serves to provide the hatching larva with an active antimicrobial barrier and protection against systemic infections. *Cecropin* expression in the yolk required the presence of a GATA site in the promoter as well as the involvement of the GATA-binding transcription factor Serpent (dGATAb). In contrast, neither the GATA site nor Serpent were necessary for *Cecropin* expression in the epidermis. Thus, the inducible immune responses in the yolk and in the epidermis can be uncoupled and call for distinct sets of transcription factors. Our data suggest that Serpent is involved in the distinction between a systemic response in the yolk/fat body and a local immune response in epithelial cells. In addition, the present study shows that signal transduction pathways controlling innate and epithelial defense reactions can be dissected genetically in *Drosophila* embryos.

Innate immunity and the expression of gene-encoded antimicrobial peptides have been shown to play an important role as a first line of defense in higher animals including mice and men (1, 2). Inducible synthesis of antimicrobial peptides was identified first in *Drosophila* (3). Insects have been continuously proven to be very useful models for the study of innate immunity, because they are remarkably resistant to microorganisms and subject to biochemical and genetic analyses (4, 5). Recently, the molecular basis of pathogen recognition, signal transduction, and effector mechanisms have been found to share considerable similarities between insects and mammals, suggesting the existence of an evolutionary relationship in the immune defense (2, 6, 7). Insects respond to microbial infection by activating proteolytic cascades resulting in blood clotting, melanin formation, and production of an array of antimicrobial peptides with broad and overlapping specificity (reviewed in refs. 8–10). The *Drosophila Cecropin* (*Cec*) genes encode a family of peptides not only active against Gram-negative and Gram-positive bacteria (11) but also possessing anti-fungal activity (12), making them powerful weapons against most classes of microorganisms.

Expression of gene-encoded antimicrobial peptides does not require somatic gene rearrangement, and the active tissues do not have to go through processes of maturation and selection, both hallmarks of acquired immunity. Therefore, the innate immune response is rapid and, as we hypothesized, should be functional already at birth/hatching or even earlier. Phagocytosis of bacteria, a cellular component of the innate immune response, has been observed by hemocytes of late-stage *Drosophila* embryos (13). However, there are no reports on synthesis of antimicrobial peptides in earlier stages than 2nd larval instar. We decided to investigate the possibility that antimicrobial peptide synthesis can be activated during embryogenesis and to follow the development of immune responsiveness of different tissues.

The embryonic yolk in *Drosophila* embryos contain polyploid yolk nuclei called vitellophages, and the inner yolk mass. The vitellophages do not take part in the formation of the embryo proper and remain in the middle of the embryo during the processes of cellularization. It has been suggested that the large multinucleate polyploid yolk cell provides the growing embryonic cells with nutrients (14), but in fact the role of the yolk nuclei is not clear. Relatively few genes have been found to be expressed in the yolk nuclei; one such expression is the GATA factor Serpent or dGATAb (15). The analysis of *Cecropin A1* (*CecA1*) expression in embryos revealed that the embryonic yolk is a major site of antimicrobial peptide synthesis.

Surfaces of higher eukaryotes normally are covered with microorganisms but are not infected by them usually. In mammals, recent discoveries have highlighted the importance of antimicrobial peptides produced by barrier epithelia (16). When the *Drosophila* embryo hatches and becomes a crawling larva, it enters an environment crowded with microorganisms. The analysis of immune competence in embryos revealed that a large number of epidermal cells of late-stage embryos express the *CecA1* gene. The expression in the epidermis was activated by the presence of microbial cell wall products. It therefore seems that the epidermis of a hatching larva can synthesize antibiotics to provide an active barrier against microorganisms present in the surroundings.

Materials and Methods

Fly Stocks. Transgenic fly strains with *CecA1-lacZ* reporter constructs A10 (A10-1, A10-3, and A10-4), A12 (A12-1, A12-2, and A124), and A15 (A15-1, A15-2 and A15-3) are described in ref. 17, A16 is described in ref. 18, and the *diptericin 2.2-lacZ* is described in ref. 19. The *st srp*^{6G} *e* stock (kindly provided by R. Reuter, University of Tübingen, Tübingen, Germany) carries an amorphic allele of *srp* (20), in which no Srp protein is detectable (21, 22). The use of a blue balancer strain, carrying P[ftz-lacZ.ry^{+}]TM3 (Bloomington Stock Center no. BL-3218; refs. 23 and 24) enabled identification of homozygous *srp* mutant embryos by the absence of *ftz*-promoted reporter staining.

Collection, Injection, and β-Galactosidase (β-Gal) Staining of Embryos. Embryos were collected at 25°C on apple-juice agar plates for 3 h and aged to the desired stage at 18°C. Before injection, embryos

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Abbreviations: *CecA1*, *Cecropin A1* gene; Srp, Serpent; β-gal, β-galactosidase; LPS, lipopolysaccharide; RT, reverse transcriptase; AEL, after egg laying.

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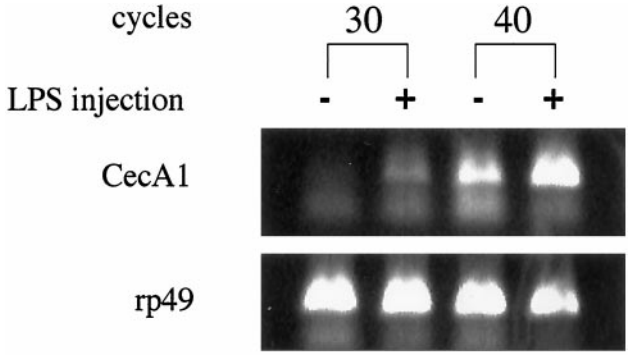

Fig. 1. LPS injection induces endogenous *CecA1* expression in embryos. Semiquantitative RT-PCR analysis was performed in embryo extracts. PCR cycles (30 and 40 rounds) were run with *CecA1*-specific primers and with *Drosophila rp49* as an internal control. The presence of a *CecA1*-specific PCR product in extracts of both control (−) and LPS-injected (+) embryos after 40 PCR cycles indicate that *CecA1* mRNA is present both before and after LPS injection. The concentration of *CecA1* mRNA was clearly higher in extracts of LPS-injected embryos than in control embryos after both 30 and 40 PCR cycles, demonstrating that the endogenous *CecA1* gene is induced in embryos by LPS injection.

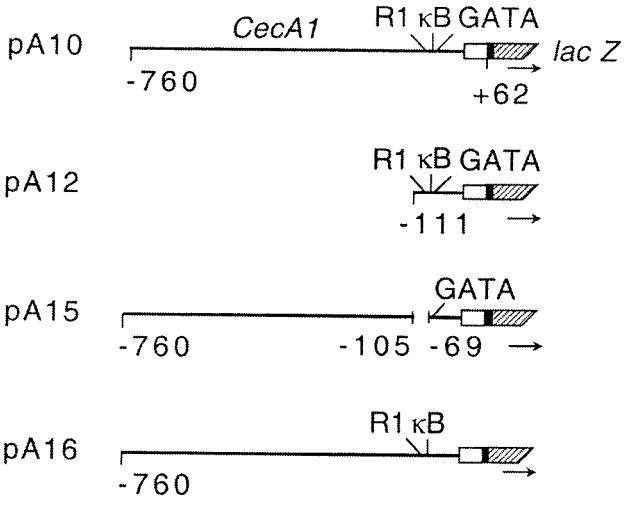

Fig. 2. Schematic representation of the *CecA1-lacZ* constructs carried by the transgenic fly strains used in this study. The constructs contain upstream fragments from the *CecA1* gene (36). Numbers refer to positions relative to the cap site. The positions of the regulatory elements R1, κB, and GATA are indicated (37).

were dechorionated in diluted sodium hypochlorite for 1 min, mounted on a coverslip with double-stick tape, and covered with 10 S voltalef oil (Elf Autochem, Norden A/S, Hartlev, Denmark). Injections were done with 10 μg/ml lipopolysaccharide (LPS) or with a 1:10 dilution of log phase *Enterobacter cloacae*, *β12*, or sterile PBS. After LPS injection, the embryos were kept at 25°C for 3–4 h to allow expression of the reporter gene. Embryos were fixed in glutaraldehyde-saturated heptane, devitellinized by hand, and stained by using 5-bromo-4-chloro-3-indolyl β-D-galactoside for 15–18 h or as indicated (25).

RNA Preparation and Reverse Transcriptase (RT)–PCR. Embryos [12–15 h after egg laying (AEL)] were injected with LPS, kept at 25°C for 2 h, and then frozen in liquid nitrogen and stored at −80°C. RNA from control embryos was treated equally apart from the LPS injections. RNA was prepared by using the RNeasy kit (Qiagen, Chatsworth, CA), and the concentration was determined by measuring *A* at 260/280 nm. A 266-bp region of the *CecA1* mRNA was amplified by RT-PCR with the rTth RNA PCR kit (GeneAmp, Perkin–Elmer) according to the manufacturer's protocol. Primers were 5′-GTC GCT CAG ACC TCA CTG CAA TAT-3′ (5′) and 5′-CGA GGT CAA CCT CGG GCA GTT GC-3′ (3′). As a positive control, rp-49 mRNA was amplified with the primers 5′-GAC CAT CCG CCC AGC ATA CAG GC-3′ (5′) and 5′-GAG AAC GCA GGC GAC CGT TGG-3′ (3′) yielding a 390-bp PCR product. The RT-PCR products were analyzed on 1% agarose gels stained with ethidium bromide.

Results

The *CecA1* Gene Is Inducible in Embryos After Microbial Challenge. To investigate whether antimicrobial peptide genes are inducible during embryogenesis, *Drosophila* embryos were injected with LPS, and the concentration of *CecA1* mRNA was measured with semiquantitative RT-PCR (Fig. 1). The *CecA1* gene was expressed constitutively at a low level in embryos, as shown by the presence of a PCR product of the correct size after 40 PCR cycles in extracts of untreated embryos. Importantly, the RT-PCR analysis demonstrated that the *CecA1* gene was induced in embryos after LPS injections (Fig. 1). In fact after 30 PCR cycles, the *CecA1*-specific PCR product could be detected only in extracts of injected embryos, and there was clearly more *CecA1* fragment produced in extracts of LPS-injected embryos than in those of uninjected ones after 40 PCR cycles.

We next investigated the maturation of immune responsiveness during embryogenesis by analyzing the expression of *CecA1-lacZ* reporter constructs in embryos of transgenic fly strains. These transformants were demonstrated previously to mimic the induction of the *CecA1* gene in terms of tissue specificity and inducibility by infectious agents in larvae and adults (16, 17). Staged embryos carrying the A10 construct (Fig. 2) were injected with LPS or bacteria at specific age intervals, and induction of the *CecA1* promoter was monitored as β-gal activity. Surprisingly, we did not observe any induction of the *CecA1-lacZ* construct in the embryonic fat body or in hemocytes, although these are tissues with high levels of *CecA1* expression during larval stages (11, 16, 25). Instead, two other tissues revealed strong expression after microbial challenge, namely the embryonic yolk and epidermis. Injections into the middle, anterior, or posterior side of the embryo or into the perivitelline space mounted a similar response, suggesting the involvement of diffusible molecule(s). The earliest stage of expression was observed in embryos injected with LPS at about 6–7 h AEL and stained for β-gal in stage 14. Staining was strong throughout the yolk sac (Fig. 3*A*). Injections into successively older embryos produced staining in the yolk also of older embryos (Fig. 3*B*). The endodermally derived epithelial cell layer of the gut was not stained (Fig. 3*B Inset*), indicating that *CecA1* expression is restricted to the yolk nuclei. As seen in Table 1, approximately 23% of embryos injected into the embryo hemocoel with LPS at 6–9 h and 9–12 h of development possessed staining in the yolk. Injections into the perivitelline space resulted in higher frequency of embryos stained in the yolk (50–60%) but also greater variation in staining intensity. We also incubated dechorionated embryos with live *E. cloacae* without damaging the vitelline membrane. Only very few of these embryos displayed staining in the yolk (about 1%), suggesting that the bacteria must enter the perivitelline space to mount a response. Injection of sterile PBS resulted in very weak yolk staining in 4% of the *CecA1-lacZ* embryos, and no staining was observed in uninjected embryos, indicating that the presence of microbial products is crucial for a robust response (Fig. 3*C*). In addition, we found that the inducible induction of a humoral response in the yolk is not restricted to *CecA1* expression, because *diptericin 2.2-lacZ* transgenic embryos also conferred LPS-inducible β-gal staining in the

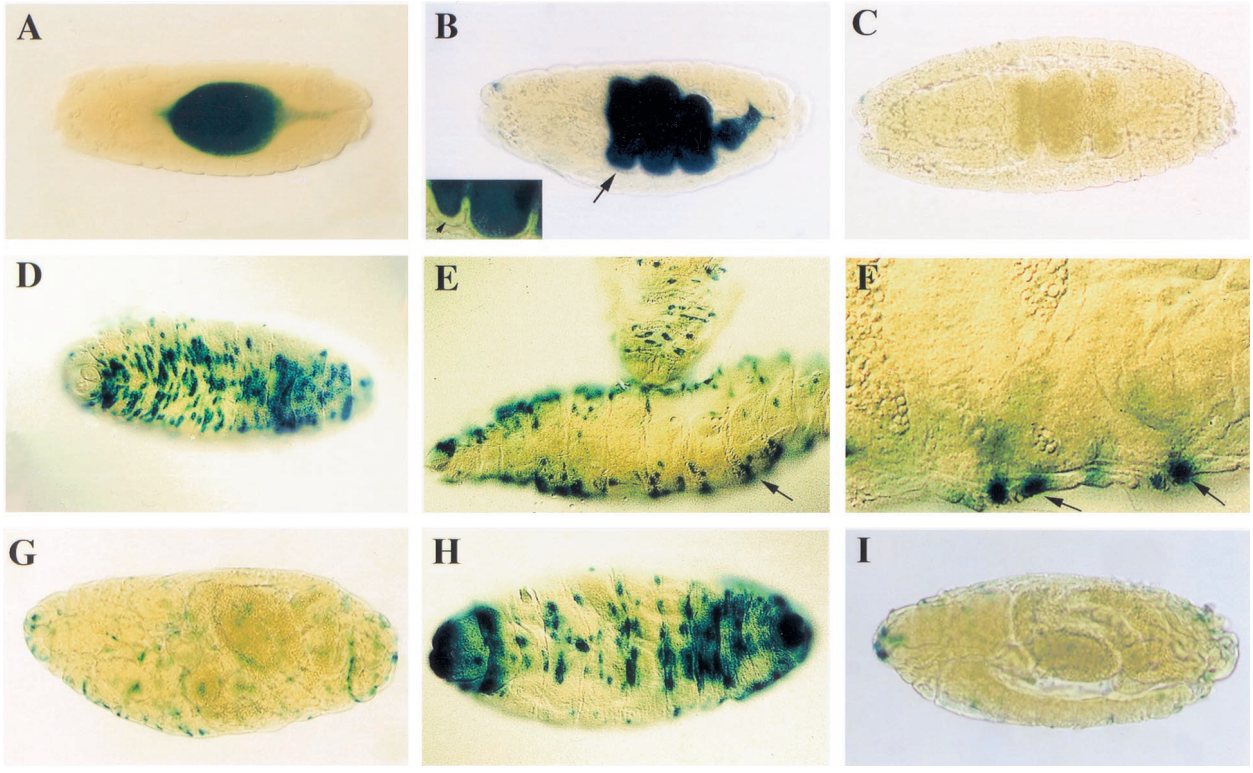

Fig. 3. Bacterial infection induces *CecA1-lacZ* expression in embryos. Anterior is to the left, and dorsal is up in lateral views. (*A–G*) Induction of β-gal expression in the yolk and epidermis of A10 transgenic embryos after injection of LPS, bacteria, or PBS 3–4 h before fixation and staining. (*A*) Dorsal view of a stage-14 embryo injected with *E. cloacae*. (*B*) Ventrolateral view of a stage-16 embryo injected with LPS. The staining is strong throughout the yolk present within the developing midgut and hindgut. The expression is within the yolk sac but excluded from midgut epithelium (arrows and *Inset*). (*C*) Lateral view of a stage-16 embryo injected with PBS. (*D*) Dorsal view of a stage-17 embryo injected with LPS around 12 h AEL. Expression no longer can be induced in the yolk region, but staining is established in epidermal cells throughout the embryo. The staining appears as an irregular pattern of transversal rows including both groups of cells as well as individual cells. (*E*) Lateral view of a stage-17 embryo injected with LPS demonstrating the uneven distribution of stained cells in the embryos with a concentration of positive cells on the dorsal and ventral sides. (*F*) Magnification of the same embryo as in *E* with a slightly different focus shows that the stained cells lie just in the embryo surface (arrows). (*G*) β-gal staining in a stage-17 A10 *CecA1-lacZ* embryo after injection of sterile PBS. (*H–I*) Induction of β-gal expression in the epidermis of embryos at stage 17 3–4 h after LPS injection carrying different *CecA1-lacZ* constructs. (*H*) Dorsal view of an A12 embryo showing the same pattern of β-gal staining as in A10 embryos. (*I*) Lateral view of an A15 embryo displaying no inducible expression. The stages of embryonic development are according to ref. 38.

yolk in equivalent numbers of embryos. The *diptericin 2.2-lacZ* expression was much weaker than the *CecA1-lacZ* expression, probably because of the lack of certain enhancer elements in the *diptericin 2.2-lacZ* (data not shown; ref. 19).

In *CecA1-lacZ* embryos injected at 9–12 h AEL, the staining in the yolk persisted in parallel with the onset of expression in the epidermis (Table 1). After 12 h of development, LPS injections promoted strong β-gal expression in a large number of scattered cells in the epidermis throughout the embryo in 85–90% of the LPS-injected embryos (Fig. 3*D*; Table 1). As shown in Fig. 3*E*, there was an uneven distribution of the responding cells, with more stained cells on the ventral and dorsal sides, than on the lateral sides of the embryo, and the number of stained cells also varied somewhat between different embryos. The stained cells were located in the epidermis in the very surface of the embryo (Fig. 3*F*) and exhibited an elongated shape along the D/V axis of the embryo (Fig. 3*D*), indicating that they are normal epidermal cells. Injections of sterile PBS in pA10 embryos mounted a response in about 18% of the embryos (Fig. 3*G*; Table 1). The staining in these embryos was much weaker than in LPS-injected embryos (Fig. 3*D*) but slightly stronger than in uninjected control embryos (data not shown). β-gal staining from the endogenous *Gal 1* gene was evident in a few tissues as previously described (25) but did not interfere with the present study. Analysis of the *CecA1-lacZ* expression in parallel with immunostaining of hemocytes with antibodies against the Croquemort protein (13) indicated that the embryonic hemocytes are not a site of *CecA1* expression (data not shown).

Promoter analysis of the *CecA1* gene has shown that a 40-bp

Table 1. Time study of *CecA1*-inducibility during embryogenesis

Time of LPS injection, hours AEL	Fixation and staining, hours AEL	Percentage stained only in yolk	Percentage stained only in epidermis	Percentage stained in yolk and epidermis	Percentage not stained	Total no. of embryos (*n*)
6–9	10–13	23 (56)	0	0	77 (44)	70 (73)
9–12	13–16	23	9	6	62	47
12–15	16–19	0	88 (97)	0	12 (3)	40 (57)

Embryos were injected in the hemocoel or in the perivitelline space (numbers in parentheses).

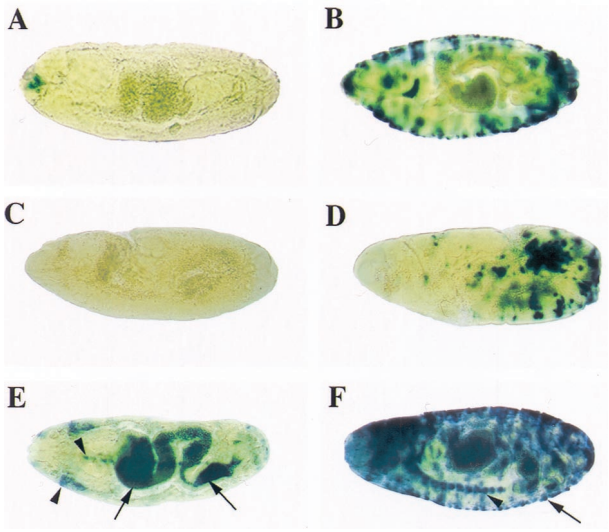

Fig. 4. Analysis of the requirement of GATA-binding factors for inducible *CecA1* expression in the yolk and epidermis, respectively. Anterior is to the left, and all embryos are in lateral view with dorsal up, except for *E* which is in ventral view. Embryos were injected with LPS before (*A*, *C*, and *E*) or 12 h after (*B*, *D*, and *F*) egg laying to induce *CecA1* expression in the yolk or in the epidermis, respectively. (*A*–*B*) β-gal staining in A16 *CecA1-lacZ* embryos with a mutated GATA site. No expression is observed in the yolk (*A*), whereas expression in the epidermis is strong (*B*). (*C*–*F*) β-gal expression in A10 *CecA1-lacZ* embryos in srp^{6G} mutant background. No *CecA1* expression could be detected in the yolk of homozygous srp^{6G} embryos (*C*) but is evident clearly in individual cells and in patches of cells in the epidermis (*D*). The development of the srp^{6G} embryos (*C* and *D*) is arrested, and the embryos do not complete germ-band retraction and dorsal closure as their heterozygous siblings (*E* and *F*). In embryos heterozygous for srp^{6G} and for the *ftz-lacZ* blue balancer, the β-gal staining is evident both from the *CecA1* promoter (arrows) in the yolk (*E* and *F*), in the epidermis (*F*), and from the *ftz* promoter (arrowheads) in a weak stripe in the anterior part of the embryo (*E*) and in the ventral nerve chord (*E* and *F*; refs. 23 and 24).

region containing a κB-like motif and an uncharacterized motif called Region 1 (R1) is required for inducible expression of the *CecA1* gene in larvae and flies. Transgenic embryos carrying the A12 and A15 reporter constructs (Fig. 2) were injected with LPS to analyze whether this region is important for *CecA1* expression also in embryos. A12 conferred the same pattern of expression as the full-length A10 construct (Fig. 3*H*; Table 1). We therefore conclude that 111 bp of upstream region is sufficient for inducible *CecA1* expression in the yolk and epidermis in embryos. Removal of the R1 and κB sites from the full-length construct (A15) abolished inducible β-gal expression in both the yolk (data not shown) and epidermis (Fig. 3*I*; Table 1), indicating that one or both of these elements are required for inducible *CecA1* expression in embryos. Future experiments will be designed to analyze the relative importance of each of these elements.

The GATA Site and the Serpent Protein Are Crucial for *Cec* Gene Expression in the Yolk but Not in the Epidermis. The GATA site, which has been shown to regulate tissue specificity of antimicrobial peptide gene expression in postembryonic stages (18), was found to be discriminative for tissue-specific expression also in the embryo. Mutagenesis of the GATA site in an otherwise wild-type *CecA1* promoter (A16; Fig. 2) had a deleterious effect on *CecA1* induction in the yolk (Fig. 4*A*), whereas the expression in epidermal cells was not affected by the lack of a functional GATA sequence (Fig. 4*B*; Table 1). Thus, expression of *CecA1* in the yolk required the GATA site, whereas in the epidermis, it was dispensable.

The *serpent* (*srp*) gene, which encodes the Srp/dGATAb transcription factor, is required for the differentiation of the midgut, hemocytes, and fat body during *Drosophila* embryogenesis (15, 21, 22, 26). Embryonic development is arrested in srp^{6G} mutant embryos, the midgut fails to develop, germ-band retraction is interrupted, and there is no dorsal closure (21). Srp protein was shown recently to be required for the activation of the *CecA1* gene in larval fat body but nonessential for the expression in adult fat body (18). We analyzed the expression of the A10 *CecA1-lacZ* transgene in homozygous srp^{6G} embryos. Homozygous srp^{6G} embryos were distinguished from heterozygous and wild-type siblings by the use of a blue balancer chromosome marked with *ftz-lacZ* (23). LPS injections did not promote activation of the A10 *CecA1-lacZ* construct in the yolk of homozygous srp^{6G} embryos (Fig. 4*C*), whereas 54% of the heterozygous srp^{6G}/*ftz-lacZ* embryos showed β-gal expression in the yolk (Fig. 4*E*). In the epidermis, β-gal staining was conspicuous in 64% of the homozygous srp^{6G} embryos, although these embryos possessed severe developmental defects (Fig. 4*D*). This staining demonstrated that Srp is not a crucial transcription factor for expression of *CecA1* in the epidermis. Therefore, different tissues utilize distinct combinations of transcriptional regulators to activate the immune response. Consequently, Srp defines two alternative modes of *CecA1* activation; one involves Srp and another bypasses Srp function.

Discussion

In this study we have demonstrated that immune responsiveness as measured by induction of the *CecA1* promoter is initiated during embryogenesis within approximately 6 h of development. Such a developmentally early inducible response against microorganisms has not been described previously to our knowledge and sheds new light on the ontogeny and function of innate immunity in higher eukaryotes.

The function of the embryonic yolk nuclei (vitellophages) in *Drosophila* has remained elusive despite the in-depth investigations of embryonic development during the last two decades. Studies of the ultrastructure of the vitellophages' cytoplasm reveal large amounts of endoplasmic reticulum and free ribosomes, among other organelles, indicating a high level of differentiation and intense protein synthesis (Rafael Cantera, personal communication). Another protein linked to immune function in insects, the hemolin protein of *Hyalophora cecropia*, was shown recently to be expressed constitutively in the yolk during oogenesis and embryogenesis, suggesting that a number of immune-related proteins are expressed in the embryonic yolk (27). Our data suggest that the yolk cell serves a very important function in defending the embryo against microbial infections by the rapid production of antimicrobial peptides.

The synthesized antimicrobial peptides, which contain a signal peptide, probably can be exported from the yolk cell to the embryo hemocoel and possibly through the amnioserosa into the perivitelline fluid also, in which they can attack invading organisms. Our data also indicate that the activation of an immune response requires contact between microbial substances and components present in the perivitelline fluid or in the embryo, because the presence of bacteria on the surface of embryos with an intact vitelline membrane did not mount a response. Therefore, the chorion and the vitelline membrane may provide a physical barrier to infection.

What then may be the normal route of infection in nature? During fertilization, the seminal fluid may be contaminated with bacteria that can enter the egg together with the sperm. The seminal receptacle and spermathecae of the female as well as the reproductive organs of the male are sites of constitutive synthesis of antimicrobial factors, indicating that it is crucial for high reproductive efficiency to minimize bacterial contamination of the seminal fluid (reviewed in ref. 28). Another threat for the embryo is infection with maternally transmitted endocellular bacteria such as *Wolbachia* of the Rickettsial family. This mi-

croorganism is transmitted vertically from the reproductive organs of the insect female to the egg, from which it migrates to the germ cells laid down in the embryo (reviewed in refs. 29 and 30). Eggs laid by infected *Drosophila* females contain bacteria scattered in the yolk region (31) and in later stages of development, bacteria are distributed throughout the somatic and germ-line tissues (32). Our study shows that the immune response is active in the yolk, in which *Wolbachia* first appear in the developing embryo.

Expression of *CecA1* in the embryonic yolk was inducible only during a relatively narrow time window. The onset of expression probably relies on the *de novo* synthesis of crucial factors in the zygotic embryo. A probable explanation for the sharp decline in expression about 12 h AEL is that microbial substances or transmitted signals cannot reach the yolk and its nuclei after the time point of dorsal and midgut closure. Our data suggest that the signal could be transmitted over the amnioserosa, which is a thin extraembryonic membrane that covers the dorsal side of the embryo during gastrulation. This route of signal transmission most likely is blocked after dorsal closure when the amnioserosa is no longer in direct contact with the perivitelline fluid.

To our surprise, *CecA1* expression was not evident in the embryonic fat body or hemocytes, although these tissues are sites of high-level expression in postembryonic stages, suggesting that important factors are limiting. Instead, the *CecA1* gene was inducible in the epidermis after 12 AEL. The results indicate that microbial substances present in the perivitelline fluid can activate the epidermal cells directly during embryogenesis and that in the absence of a hard cuticle, the signal reached numerous epidermal cells. This result suggests that the epidermal cells express transmembrane receptors that respond to the presence of microbial products in the perivitelline fluid and transduce the signal to the nucleus. Interestingly, the *Drosophila* transmembrane receptor Toll is expressed in all cells throughout the embryonic epidermis (33). Therefore, Toll together with other Toll-like receptors are possible candidates for being mediators of the immune response in embryonic epidermis. The development of an epidermal defense probably serves to protect the embryo against infection during the late stages of embryogenesis and to provide the hatching larva with an inducible immune system in the epidermis underlying the larval cuticle.

We have provided evidence for the differential requirement of the Srp protein for *CecA1* expression in different tissues of the embryo. An important outcome of these results is that it shows that the regulatory mechanisms of an inducible innate immune response can be dissected genetically in embryo-lethal mutant background. The present data show that both the GATA site in the *CecA1* promoter and the Srp protein was required for the induction of the *CecA1* gene in the yolk. We therefore conclude that Srp is a crucial factor for the activation of the *CecA1* gene in the embryonic yolk. In contrast to this result, neither the GATA site, nor the Srp protein were necessary for *CecA1* expression in the embryonic epidermis or in the larval epidermis (34). This finding shows that there is a distinction in the requirement of specific transcription factors to induce the *CecA1* gene in different tissues. The Srp protein is required for *CecA1* expression in the embryonic yolk, the larval fat body, and larval hemocytes (18). In contrast, the *CecA1* gene is expressed without the involvement of Srp or other GATA-binding factors in epidermal cells and in adult fat body (18). These tissues instead may provide other tissue-specific regulators. The presence of GATA motifs in the proximal promoter region in a large number of antimicrobial peptide genes in insects suggests that these genes may be regulated by GATA-binding factors in a similar manner as the *CecA1* gene (35). Our work indicates that the systemic immune response in the yolk, larval fat body, and hemocytes can be uncoupled from the response in the epidermis of embryos and larvae. Srp is the first regulatory protein that has been shown to distinguish between these two modes of activation. We propose that the involvement of Srp may be used as a marker of a systemic humoral immune response in *Drosophila* embryos and larvae, in contrast to local immune reactions in the epidermis, which seem to circumvent Srp function.

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