The Drosophila fusilli (fus) gene was identified in a genetic screen for dominant maternal enhancers of an unusual dorsalizing mutation in the cactus gene, cactE10. While females that are heterozygous for the cactE10 allele produce embryos with wild-type dorsal-ventral patterning, more than 90% of the embryos produced by females that are heterozygous for both cactE10 and the fus1 mutation are weakly dorsalized. Loss of fusilli activity causes lethality during embryogenesis but not dorsal-ventral patterning defects, indicating that fusilli is important in more than one developmental process. The fusilli gene encodes a protein with RNA binding motifs related to those in mammalian hnRNP F and H, which play roles in regulated RNA splicing. The fusilli RNA is not present in the oocyte or early embryo, and germ-line clones of fusilli mutations have no maternal effect on dorsal-ventral patterning, indicating that the fusilli maternal effect does not depend on germ-line expression of the gene. Because the fusilli RNA is present in ovarian follicle cells, we propose that fusilli acts downstream of the Drosophila EGF receptor to control the biogenesis of follicle cell transcripts that control the initial dorsal-ventral asymmetry of the embryo.
Therefore predicted to modify a proteoglycan substrate (Sen et al., 1998), but that substrate is apparently not encoded by any of the known genes. Biochemical evidence suggests that the extracellular serine protein proteases required for activation of the ligand for Toll are regulated by protease inhibitors not encoded by any known gene (Misra et al., 1998). Further, the cytoplasmic portion of the signaling pathway that leads to the activation of the Rel-family protein Dorsal is homologous to the mammalian IL-1R/Toll pathways (Anderson, 2000), and counterparts to the IκB kinases and TrafS that are important in these mammalian pathways were not identified in the Drosophila maternal effect screens.

Screens for dominant enhancer mutations provide one method to identify genes that are essential for viability and play a maternal role in embryonic patterning. This approach has been useful in delineating a number of Drosophila pathways, including receptor tyrosine kinase and dpp signaling pathways (Simon et al., 1991; Raftery et al., 1995). Here we identify an essential gene, fusilli, based on its maternal effect enhancement of a weak dorsalizing muta-

**MATERIALS AND METHODS**

**Genetics and Drosophila stocks.** To identify dominant enhancers of cact190, b pr cn sp If males were mutagenized with 25 mM EMS as described by Lewis and Bacher (1968) and then mated with mid137 cn bw/CyO females. Single F1 b pr cn If +/CyO males and females were crossed to cact190 bw sp/CyO flies. The mixed F2 progeny b pr cn If +/+ b pr cn If +/cact190 bw sp, and cact190 bw sp/CyO) from each line were allowed to lay eggs and embryos were scored for dorsalization. Screening was carried out at 25°C because the interaction between cact190 and known mutations in dorsal group loci was strongest at high temperature (data not shown). Four thousand five hundred EMS-mutagenized lines were tested. In lines in which some dorsalized embryos were observed, females of the three genotypes were separated and allowed to lay eggs. Those lines in which the b pr cn If +/cact190 bw sp females produced a large fraction of dorsalized embryos were considered to carry putative enhancer mutations. Putative mutants were crossed to cact190 bw sp/CyO again to confirm the presence of a dorsalizing enhancer. Twenty-three lines that reproducibly enhanced the cact190 phenotype were identified.

Mutations on the second chromosome were mapped using standard meiotic recombination relative to the visible markers al dp b pr cn c. Further mapping was carried out relative to P[w+] transposable elements in the appropriate region (Spradling et al., 1995). One of the strongest enhancers, fusilli1 (fus1) was homozygous lethal and both the enhancer and the lethality mapped to 2R, between P[w+] (12)K03308 (51F11–13) and P[w+] (12)K0220 (52D1–2). In the two types of mapping experiments, 258 opportunities for recombination were scored and the enhancer mutation was never separated from the recessive lethality. Df(2R)WMG (52A4–B1; 52D7–E1) and Df(2R)p4 (51F13; 52F8–9) failed to complement the fusilli lethality, while Df(2R)XT11E(S13E–4; 52A6–10) and Df(2R)p5 (52A13–B3; 52F10–11) complemented fus1. Therefore fusilli mapped between 52A6–10 and 52A13–B3.

Local P-element hopping was used to generate a P[w+] insertion line of fusilli (Tower et al., 1993). Flies carrying the P[w+] insertion mutation I(2)k07207 (52A 9–11) (Iörik et al., 1993) were mated to the Sp/CyO; α-2-3 Sb/TM6, Ubx and 100 male progeny of the P[w+]/Sp; α-2-3 Sb and P[w+]/CyO; α-2-3 Sb classes were selected and crossed to w; Adv/CyO females. In the next generation 500 individual P[w+]/CyO or P[w+]/Sp males were crossed to fus1 females to test for ability to complement fus1. One line that failed to complement the lethality of fus1 was identified; this mutation was designated fus2. The fus1 mutation was caused by a P-element insertion, since it was unstable in the presence of transposase: three apparently precise and three imprecise excisions (including fus1 and fus2) were identified from 110 progeny of flies carrying both fus1 and P-element transposase. The alleles fus1, fus2, and fus1 were obtained as white derivatives from approximately 35,000 progeny of fus1 after X-irradiation (4000R).

Df (2R)WMG/Gla, Df (2R)p4/CyO, Df (2R)p5/CyO, and w; FRT42B ovo12/CyO were obtained from the Bloomington Stock Center. Df (2R)XT11E/CyO was a gift from Dr. Eileen Underwood (Bowling Green State University, Bowling Green, OH). P[w+] insertion lines were from the Berkeley Drosophila Genome Project via Dr. Istvan Kiss and Ms. Amy Beaton (University of California, Berkeley). The y w hsF122; Adv/CyO line was from Dr. Naoto Ito (University of California, Berkeley).

**Germ-line clones.** Germ-line clones homozygous for fus1 were induced using the FLP-FRT system in combination with ovo12 insertions on chromosome arm 2R (Chou et al., 1993). To make the fus1 mutant germ-line clones, males of genotype y w; P[w+]; FRT42B ovo12/CyO were crossed to females of genotype y w hsF122; P[w+]; FRT42B ovo12/CyO. First-instar larvae were heat shocked (38°C for 1 h) to induce germ-line clones. Non-CyO females from this cross were crossed to fus1/CyO, ftz-lacZ lines. To distinguish whether embryos inherited the paternal fus1 or fus1, embryos were stained with anti-β-gal antibody or anti-lacZ RNA probe.

**Isolation of genomic and cDNA clones.** DNA surrounding the fus1 P-element insertion was isolated by plasmid rescue (Moldzik et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990). A rescued 80-bp fragment of genomic DNA was used to screen a cosmid library (Tamkun et al., 1990).
native start lies within the first intron of the cDNA class A transcription unit.

**Whole-mount in situ mRNA hybridization.** In situ hybridizations to whole-mount embryos and ovaries were carried out according to established protocols (Hemavathy et al., 1997; Konsolaki and Schüpbach, 1998). Digoxigenin-labeled anti-fusilli and anti-lacZ probes were made using the DIG RNA labeling kit (Boehringer Mannheim). The snail clone was provided by Dr. Tony Ip (University of Massachusetts Medical Center, Worcester, MA).

**Antibody staining.** Embryos were stained with antibodies by blocking in PBS, 0.1% Triton (PBS-TX) and 0.5% BSA, incubating in the primary antibody in PBS-TX at 4°C overnight, washing in PBS-TX three times for 15 min at room temperature, incubating with the secondary antibody (goat anti-rabbit IgG; Vector Laboratories) at 1:200 dilution in PBS-TX for 30 min at room temperature, washing as above, and developing with a metal-stabilized DAB solution (Pierce). Rabbit anti-Twist antibody was provided by Dr. Mary Baylies (Memorial Sloan-Kettering Cancer Center, New York, NY) and was used at a dilution of 1:5000.

**RESULTS**

**Dominant Enhancers of cactus**

An unusual allele of cactus, cactE10, provided a useful tool to look for additional genes required for dorsal-ventral embryonic patterning. The cactus gene encodes an IkB-family protein with a C-terminal ankyrin repeat region that binds to Dorsal and holds Dorsal in an inactive cytoplasmic complex (Geisler et al., 1992; Kidd, 1992). In response to signaling from the Toll receptor, Cactus is targeted for degradation (Belvin and Anderson, 1995; Bergmann et al., 1996; Reach et al., 1996). Once Cactus is degraded, Dorsal enters nuclei where it promotes ventral-specific gene expression. Loss of cactus activity causes constitutive nuclear localization of Dorsal (Roth et al., 1989). The protein encoded by the cactE10 allele lacks the first 144 amino acids of the N-terminal domain and can bind Dorsal, but is not degraded in response to signal (Belvin and Anderson, 1995; Bergmann et al., 1996; Reach et al., 1996). In contrast to the recessive ventralizing phenotype of loss-of-function alleles, cactE10 causes a recessive dorsalizing maternal effect phenotype: females heterozygous for cactE10 and a loss-of-function cactus allele produce strongly dorsalized embryos (Roth et al., 1991). Despite its gain-of-function character, cactE10 does not cause a dominant phenotype: embryos laid by cactE10/+ females are wild type.

We found, however, that a dominant dorsalizing character of cactE10 was revealed in females that were also heterozygous for a mutation in any of the Dorsal group genes.
so that doubly heterozygous females produced a high fraction of weakly dorsalized embryos (Fig. 1B and data not shown). For example, all of the embryos produced by females heterozygous for both cactE10 and dorsal1 at 29°C were weakly dorsalized, 90% of the embryos produced by cactE10/++; spätzlemo7/+ females at 29°C were weakly dorsalized, and 50% of the embryos produced by cactE10/+; easter1/+ females were weakly dorsalized. The sensitivity of cactE10 to the dosage of other Dorsal group genes provided the basis of a screen for previously unidentified genes that play a role in this pathway (see Materials and Methods).

From 4500 EMS-mutagenized lines, we identified 23 lines that caused a reproducible maternal-effect dorsalization in combination with cactE10. Mapping and complementation tests indicated that 11 dominant enhancers were caused by mutations in previously identified Dorsal group genes, including 2 alleles of spätzle, 8 alleles of dorsal, and 1 allele of cactus. The remaining 12 lines were candidates to identify new components of the pathway.

One of the novel enhancers, fusilli, showed a particularly strong interaction with cactE10, more than 80% of the embryos produced by doubly heterozygous females were clearly dorsalized (Fig. 1C); this interaction was stronger than that observed with many alleles of Dorsal group genes. In addition, the fusilli chromosome was homozygous lethal. fusilli mapped near the middle of the right arm of the second chromosome, between P[w1] insertions at 51F11–13 and 52D1-2. The enhancer mutation and the recessive lethality appeared to be caused by a single mutation: the two phenotypes were not separated in 258 opportunities for recombination. We used deficiency mapping to localize the lethal mutation in this line to region 52A–B (see Materials and Methods). The deficiencies that uncovered the lethality did not show a maternal interaction with cactE10. We therefore concluded that the enhancer mutation is an unusual allele of a gene that is required for viability, which we named fusilli1 (fus1).

We isolated a P-element insertion mutation that failed to complement the lethality of fus1 using local hopping methods (Tower et al., 1993), starting from a line carrying a P[w1] insertion at 52A9–11, l(2)k07207 (Török et al., 1993; see Materials and Methods). One line was identified that failed to complement the lethality of fus1 and was designated fus2. The fus2 chromosome also acted as a dominant enhancer of the cactE10 maternal-effect phenotype (Fig. 1D); however, as described below, this interaction was the result of both the fus2 mutation and an additional mutation on the same chromosome. The additional alleles fus3, fus4, and fus5 were obtained as X-ray derivatives of fus2 that had lost the w+ gene, and fus6 and fus7 were obtained by transposase-induced imprecise excision of the fus2 P element (see Materials and Methods). All of the fus alleles caused recessive lethality at the end of embryogenesis without clear defects in the larval cuticle pattern.

The Partially Dorsalized Phenotype of Embryos Laid by cactE10/fus1 Females

The embryos laid by doubly heterozygous cactE10 fus1/ cact1 fus1 mothers (which we refer to as cactE10/fus1 fe-
fusilli Encodes a Novel Protein That Has Similarity to Human hnRNP F and H

The two fusilli transcripts had different 5′ noncoding exons, but shared the same open reading frame of 967 amino acids and a long (2 kb) 3′ untranslated region (Fig. 3A). The biochemically characterized proteins with the greatest similarity to the predicted Fusilli protein were hnRNP F and hnRNP H. Both human hnRNP F and H have three RNA binding domains (RBD) of 80 amino acids, which are believed to be important for binding specific RNA substrates (Matunis et al., 1994; Honore et al., 1995). The predicted Fusilli protein shares the same organization as the mammalian hnRNP s, with three RBDs (Fig. 3B). The sequence identity between Fusilli and hnRNP F was 26% in the homologous regions, with 43% sequence similarity. Like a number of hnRNP s, the predicted Fusilli protein had a C-terminal region of simple amino acid composition. In Fusilli, this domain was 25% alanine, 14% glutamine, 12% serine, and 6% glycine, which contrasts with the more glycine-rich C-terminal regions of other Drosophila hnRNP s (Matunis et al., 1992). A sequenced human cDNA of unknown function (Accession No. AK001778) showed the greatest similarity to Fusilli: in the homologous region, including the three RNA binding domains, the two proteins were 49% identical and 60% similar (Fig. 3A).

The EMS-induced fus1 allele contained a single base change compared to the parental chromosome, resulting in the generation of the stop codon (TAG) from glutamine (CAG) at amino acid 468. This mutant allele would encode a truncated protein that retains the first two RBDs, but lacks the third RBD and the C-terminal domain (Fig. 3). Because the fus1 allele encoded a truncated protein and fus2 enhanced the cactE10 phenotype, while deficiencies did not, we suggest that the fus1 allele produces an antimorphic product.

fusilli Is Expressed in a Dynamic Pattern during Development

Because we identified fusilli based on its maternal role in embryonic development, we examined expression of the gene in early embryos and in ovaries. In early embryos, there was no detectable fusilli RNA (Fig. 4A), suggesting that fusilli might act in the ovary prior to fertilization. Consistent with this hypothesis, fusilli transcripts were detected in both nurse cells and follicle cells during oogenesis (Figs. 5A and 5B), suggesting that one of these sites of expression is important for embryonic dorsal–ventral patterning. The RNA was enriched in follicle cells adjacent to the anterior end of the oocyte (Fig 5B); these cells appear to be the centripetally migrating follicle cells. Consistent with the zygotic requirement for fusilli during embryogenesis, fusilli was expressed in a number of embryonic tissues at later stages. fusilli RNA was first detected at stage 9 of embryogenesis, in the epithelium of the stomodeum, which develops into the foregut (Fig. 4B), and in the epithelium of the proctodeal opening. Segmentally repeated
FIG. 3. The Fusilli protein. (A) The amino acid sequence of the predicted Fusilli protein, aligned with the sequences of the human cDNA AK000178 and human hnRNPF. The position of the stop codon in fusilli is indicated. The RNA binding domains are overlined. (B) Alignment of the three RNA binding domains of Fusilli with the first RNA binding domain of hnRNPF.
expression was observed in the mesodermal layer in stage 11 (data not shown). At stage 13, segmentally repeated fusilli expression was evident, as well as expression in the epithelium of the foregut. The diffuse staining corresponds to fat body expression. All embryos are oriented with anterior to the left.

fusilli May Be Required in Follicle Cells Rather Than in the Germ Line

Homozygous fusilli mutant embryos died at the end of embryogenesis, making it impossible to test directly the effect of complete loss of maternal fusilli on embryonic development. To test the role of fusilli in the maternal germ line, we made germ-line clones homozygous for fus1 using the FLP-FRT-ovoD system (Chou et al., 1993; see Materials and Methods). Embryos produced by germ-line clone females mated with wild-type males developed into normal adults. To assess whether the paternal wild-type copy of the fusilli gene might rescue the lack of maternal gene function, we examined the expression of Twist and snail in the embryos that lacked both maternal and zygotic fusilli. Germ-line clone females were mated with fus1/CyO, ftz-lacZ males. The normal expression patterns of Twist (Fig. 3E), snail (Fig. 3F), and zen (data not shown) were seen in both wild-type (lacZ1) and homozygous (lacZ2) embryos. Thus we did not detect a requirement for fusilli in the maternal germ line for embryonic dorsal–ventral patterning.

Because fusilli was expressed in the ovarian follicle cells, it might exert its maternal effect on embryonic patterning by modifying the activity of the DER signaling pathway, which is required in the follicle cells for embryonic dorsal–ventral patterning. To investigate this possibility, we examined the phenotype of embryos produced by females that were heterozygous for fus1 and also heterozygous for mutations in genes of the DER pathway. No phenotype was seen in the embryos produced by females heterozygous for fus1 and mutations in DER (also known as torpedo (top)), gurken (which encodes the DER ligand), or squid (which encodes a protein required for Gurken localization). To test whether components of the DER pathway could have been recovered in our screen, we also tested for genetic interactions between the DER pathway and cactE10. More than 70% of the embryos...
produced by females heterozygous for cact\textsuperscript{E10} and for an allele of gurken (either grk\textsuperscript{HK} or grk\textsuperscript{WG}) were weakly dorsalized, as seen both in cuticle preparations and in a narrowed domain of Twist expression (Fig. 6). In contrast, none of the embryos produced by females heterozygous for cact\textsuperscript{E10} and top\textsuperscript{1} or squid showed an altered dorsal–ventral pattern. This interaction between the ventralizing gurken mutation and the dorsalizing cact\textsuperscript{E10} mutation with gurken was surprising, although gurken, which encodes the ligand for the EGF receptor during oogenesis, has been shown to be dosage sensitive (Neuman-Silberber and Schüpbach, 1994), whereas DER and squid are not. The interaction between cact\textsuperscript{E10} and gurken suggests that lowering the dosage of both fus\textsuperscript{1} and DER is sufficient to lower signaling through the pathway significantly. These results are consistent with the hypothesis that fus\textsuperscript{1} is an antimorphic allele and that fusilli is required for full activity of the DER pathway in the follicle cells.

**DISCUSSION**

**fusilli Encodes a Novel Protein with Three RNA Binding Domains**

The fusilli cDNA encodes a 967-amino-acid protein with homology to human hnRNP F and H (Fig. 3) (Matunis et al., 1994). Like hnRNP F and H, Fusilli has three RBDs, and the spacing between the RBDs is similar in the human proteins and Fusilli. Human hnRNP F and H are highly related proteins, based on both peptide sequence analysis and immunological reactivity. Both bind RNA but, unlike other hnRNPs, do not bind single-stranded DNA. Both hnRNPs...
hnRNPs have been characterized (Matunis et al., 1995; Gamberi et al., 1997; Chou et al., 1999; Chen et al., 1999).

Based on the genome sequence, fusilli is the gene most closely related to human hnRNP F and H in the Drosophila genome, suggesting that Fusilli also plays a role in alternative splicing. However, Fusilli is only 26% identical to human hnRNPs F/H in the homologous regions and Fusilli has a novel 280-amino-acid N-terminal domain not found in either hnRNPs F or H. Fusilli also has a much longer C-terminal domain than hnRNPs F/H, and that domain is particularly rich in alanine, glutamine, and serine. Fusilli is more closely related to a human cDNA of unknown function (Fig. 3). It is attractive to hypothesize that Fusilli and this human protein share similar RNA substrates.

The expression pattern of fusilli suggests that Fusilli is not a general regulator of RNA metabolism and is likely to have specific RNA targets. In the ovary, fusilli is expressed in both the nurse cells and the follicle cells in a stage-specific manner and is highly enriched in the follicle cells at the anterior end of the oocyte. The fus RNA is not detectable in early embryos and is expressed later in embryogenesis in a complex and dynamic pattern.

Several other Drosophila proteins related to mammalian hnRNPs have been characterized (Matunis et al., 1992). In particular, the squid (sqd) gene, which is also required during oogenesis for embryonic dorsal–ventral patterning, encodes a protein similar to hnRNPs A/B (Kelley, 1993). Squid is expressed in a variety of tissues, including the ovary. Specific mutations in sqd cause dorsalization of the embryo and the eggshell, by preventing localization of Gurken RNA to the dorsal-anterior corner of the oocyte (Neuman-Silberberg and Schüpbach, 1993; Norvell et al., 1999). However, the effect of squid on dorsal–ventral patterning depends on its expression in the germ line, while absence of fusilli in the female germ line does not alter embryonic dorsal–ventral pattern. Furthermore, Squid is related to hnRNPs proteins that shuttle between the cytoplasm and the nucleus and have been implicated in regulated nuclear export of specific mRNAs (e.g., Pinol-Roma and Dreyfuss, 1992; Hoek et al., 1998). In contrast, the hnRNPs F/H proteins that are the closest relatives of fusilli have been implicated in the very different process of regulated RNA splicing. It is therefore likely that Fusilli and Squid act in two distinct processes during oogenesis.

The Role of fusilli in Dorsal–Ventral Patterning

In embryos laid by cactE10/fus1 females, the number of cells giving rise to mesoderm, the most ventral cell type, is reduced. This is seen most clearly as a reduction in the number of cells expressing Twist and snail. The simplest interpretation of this phenotype is that the fusilli mutations act in concert with cactE10 to reduce the level of nuclear Dorsal below the threshold needed to activate Twist and snail transcription.

Based on the genetic interactions, the fus1 mutation could act at any step of the signaling pathway. For example, mutations in either dorsal or spätzle, which encode the downstream transcription factor and the putative extracellular ligand for Toll, act as dominant enhancers of cactE10 (Fig. 1 and data not shown). We also found that mutations in gurken, which acts during oogenesis to set up the asymmetry of the embryo and eggshell, act as dominant enhancers of cactE10. Because fusilli was not required in the maternal germ line for embryonic patterning and because it was expressed in the follicle cells, we think it likely that fusilli acts in the follicle cells during oogenesis in an early step required for dorsal–ventral patterning in the embryo.

It was surprising that mutations in gurken, which ventralize the embryo when homozygous, act as dominant enhancers of the dorsalization caused by cactE10. Previous studies, which showed that two sequential signaling pathways control the spatial patterning of the embryo, provide a context to suggest an explanation for the genetic interactions between gurken, cactus, and fusilli (Roth and Schüpbach, 1994; Sen et al., 1998; Anderson, 1998; Nilson and Schüpbach, 1999). The first step of embryonic dorsal–ventral patterning occurs during oogenesis, when dorsal–ventral asymmetry in the somatic follicle cells that surround the developing oocyte is established. Gurken protein localized in the dorsal anterior of the oocyte acts as a ligand for the Drosophila EGF receptor in the follicle cells, conferring a dorsal identity on those cells. The ventral follicle cells that do not experience high levels of DER signaling express the pipe gene. The amount of Gurken defines the size of the pipe expression domain in the follicle cells. Less Gurken signaling from the oocyte leads to a smaller dorsal domain in the follicle where DER activity is high and therefore a larger ventral region where pipe is expressed. After fertilization, the domain where pipe was expressed defines the region in which Spätzle protein is processed to become an active ligand for the receptor Toll. Diffusion of active Spätzle away from the site where it is processed determines the distribution of lateral and dorsal cell types in the embryo. If the amount of Gurken is decreased, the size of the domain where Spätzle is processed would expand. In embryos produced by heterozygous gurken mothers, lowering the dosage of gurken by a factor of 2 should slightly expand the domain where Spz is processed, spätzle is also dosage sensitive (Mori-sato and Anderson, 1994), indicating that a limited amount of spätzle precursor is available for processing. To account for the dominant enhancement of the cactE10 phenotype by gurken mutations, we propose that the same total amount of Spätzle is processed over a slightly wider area in embryos from gkr/ + females, leading to a slightly lower concentration of active Spätzle at any given position. In the presence of the normal downstream machinery for responding to the signal, that small decrease in the local concentration of active Spätzle does not disrupt patterning. However, when the downstream machinery is compromised by the cactE10 mutation, that decrease in the local concentration of active Spätzle is enough to decrease the amount of nuclear Dorsal protein significantly.
The data are consistent with fusilli acting either as a component of the DER pathway or at a step in the follicle cells downstream of DER. The nudel, pipe, and windbeutel genes are all required in the follicle cells at a step after the activity of the DER pathway (Stein et al., 1991; Schüpbach et al., 1991) and fusilli could help regulate the expression of nudel, windbeutel, or pipe RNAs. The nudel gene encodes a large modular protein resembling an extracellular matrix protein that contains a serine protease domain (Hong and Hashimoto, 1995). The windbeutel gene encodes a protein that appears to reside in the endoplasmic reticulum (Konsonali and Schüpbach, 1998) and is thought to be responsible for the folding and/or modification of a specific factor that is secreted from the follicle cells and participates in the activation of the ventralizing signal. The pipe gene encodes a protein related to heparan sulfotransferases, and localization of the pipe RNA to ventral follicle cells defines the polarity of the embryo (Sen et al., 1998). It is interesting to note that while only single RNA species have been detected for nudel and windbeutel, there are at least two isoforms of pipe that appear to differ by alternative splicing (Sen et al., 1998). Future experiments will be able to test whether Fusilli regulates the splicing of pipe or controls the metabolism of other target RNAs that act in the pathways of dorsal–ventral embryonic patterning.

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