The extracellular serine proteases of the trypsin family are well-known for their functions in blood in the formation and dissolution of blood clots, the regulation of blood pressure and the activation of complement. In all of these processes, it is crucial that protease activity be localized to the site where it is needed and that the protease be rapidly inactivated after it has performed its function (Hecht and Anderson, 1992). Serine proteases are initially synthesized as inactive zymogens containing an N-terminal pro-domain and a C-terminal catalytic domain, and activation requires proteolytic clipping of the zymogen at a defined site by a specific activating protease. Activation is frequently localized to the surface of specific cells by the requirement for a membrane-bound cofactor. Localized activation is often amplified through a positive feedback loop and terminated through a negative feedback loop. Further, specific protease inhibitors prevent the spatial and temporal spread of proteolytic activity away from the localized activation site.

The best-studied example of the role of serine proteases in development is the requirement for four members of the trypsin family during development of the early Drosophila embryo (Morisato and Anderson, 1995). These proteases are encoded by four of the twelve maternal-effect genes that are known to encode components of a signaling pathway that establishes the first dorsal-ventral asymmetry of the embryo. A key step in this pathway is the ventral activation of the membrane receptor encoded by Toll, apparently by a novel ligand encoded by spätzle. Spätzle is secreted as a freely soluble protein into the extracellular space between the plasma membrane of the syncytial embryo and the eggshell, and is proteolytically processed to generate an active ligand, apparently only on the ventral side of the embryo (Morisato and Anderson, 1994; Schneider et al., 1994). Proteolytic activation of Spätzle requires the sequential action of four different members of the trypsin family. The first protease in this pathway is encoded by nudel, which is expressed in the somatic follicle cells of the ovary that secrete the eggshell (Hong and Hashimoto, 1995), whereas the other three proteases are expressed by the germline cells. gastrulation defective (gd) is closely related to the trypsin family, but the protein encoded by gd lacks a number of amino acid residues crucial for protease activity, so its biochemical function is not clear (Konrad and Marsh, 1990). Downstream of gd is the protease encoded by snake (DeLotto and Spierer, 1986; Smith and DeLotto, 1994), which acts upstream of the final protease known in this pathway, Easter (Chasan and Anderson, 1989; Chasan et al., 1992). The Easter protease is likely to be the direct proteolytic activator of Spätzle.

The proteases in this pathway have been ordered genetically using constitutively active forms of the Snake and Easter proteases that consist of only the catalytic domain and should...
therefore bypass the requirement for activation of the zymogen (Chasan et al., 1992; Smith and DeLotto, 1994; Smith et al., 1994). However, it has not been demonstrated biochemically that zymogen activation of any of the proteases occurs in vivo, and the events that regulate zymogen activation and subsequent activity of the proteases have not been characterized.

Here we show that the Easter zymogen undergoes proteolytic processing in vivo to generate the cleaved form of the protein that should correspond to the active protease. The active form of Easter is present in extremely low amounts in the embryo because it is converted into a high molecular mass covalent complex. The identification of this complex as a form of Easter that has gone through the activation process has allowed us to demonstrate directly that the Nudel, Gastrulation defective and Snake proteases are required for activation of the Easter zymogen and has also revealed the existence of a feedback loop that can regulate the amount of Easter that is activated in the embryo.

**MATERIALS AND METHODS**

**Drosophila strains and transformants**

Oregon R was used as the wild-type strain. The dorsal group mutants used have been described previously: ndl[11]/ndl[11], gd[7]gd[2], snk[29]/snk[29] and snk[3]/snk[3] (Anderson and Nüsslein-Volhard, 1984); ea[7]/ea[7] (Chasan and Anderson, 1989); T{SUP}Breq{SUP} (Anderson et al., 1985); Df(3R)hkhXM[2] Df(3R) tub[56] and p[7]{SUP} (Hecht and Anderson, 1993); cac[99] (Roth et al., 1991); and dil/dil (Nüsslein-Volhard, 1979).

The intron-less eaΔN cDNA (Chasan et al., 1992) was placed downstream of the 0.4 kb fragment of genomic DNA that lies 5′ to the start site of transcription of easter, which had previously been shown to direct normal levels of expression of easter (Jin and Anderson, 1990) and then cloned into the CaSpeR3 vector for transformation. The construct was injected into w− flies, and w− trans revertant strains were selected. Similar trans revertants containing the wild-type easter cDNA were also constructed; these fully rescued the ea− phenotype, demonstrating that there is no essential regulatory information present in the intron sequences. The P[eaΔN] allele used in the experiments described here is an insertion onto the third chromosome. The construction of the ea S338A mutation was described previously (Jin and Anderson, 1990).

**RNA injections and immunohistochemical staining of embryos**

Synthetic SP6 RNA transcripts were generated by in vitro transcription and injected into the center of 0-1 hour embryos (Chasan and Anderson, 1989). Embryos were allowed to develop until the time of gastrulation and harvested by aspirating into a pipette. Expression of the Twist protein was assayed with a rabbit anti-Twist antibody (Roth et al., 1989), a gift from Siegfried Roth; injected embryos were fixed as described by Stein et al. (1991).

**Preparation and immunoblotting of embryo extracts**

Embryo extracts of dechorionated embryos were prepared as described at 4°C (Morisato and Anderson, 1994), but without PMSF and at lower protein concentration (1-5 mg/ml). Unless otherwise noted, all embryo extracts were prepared from 0-4 hour embryos (collected at 22°C). Extracts of injected embryos were prepared by homogenization in gel loading buffer. Extract proteins were boiled for 5 minutes with SDS loading buffer at a final concentration of either 100 mM dithiothreitol (DTT; reducing) or 20 mM N-ethylmaleimide (nonreducing) and spun for 1 minute at 13,000 revs/minute. Samples were separated on SDS-polyacrylamide gels and transferred to nitrocellulose (S&K) in 25 mM Tris, 190 mM glycine, 0.1% SDS, and 20% methanol (Towbin et al., 1979). Western blots of gels run under nonreducing conditions were incubated 20 minutes in 20 mM DTT in 1× TBS (30 mM Tris pH 7.5/150 mM NaCl). Membranes were incubated with 1:1000 dilution of rat anti-Easter antibody (Chasan et al., 1992) and then goat anti-rat HRP-conjugated antibody (Jackson ImmunoResearch) in 5% nonfat dry milk in TBS/0.1% Triton X-100. The immunoblots were visualized using ECL (Amersham).

**RESULTS**

A form of Easter that does not require zymogen activation promotes development of the most ventral cell types in the embryo

Embryos that lack maternally provided easter RNA and protein are completely dorsalized: they lack all ventrally and laterally derived cell types including the mesoderm and ventral nerve cord (Anderson and Nüsslein-Volhard, 1984). We previously demonstrated that an N-terminal deletion mutant easter, eaΔN, in which the pro-domain is deleted and the signal sequence is fused directly to the catalytic domain, is biologically active and bypasses the requirement for zymogen activation (Chasan et al., 1992). When eaΔN transcripts were synthesized in vitro and injected into dorsalized easter− embryos, the embryos developed laterally derived cell types all around the embryonic circumference, but the ventral-most cell type, the mesoderm, was not induced. This could either indicate that the eaΔN RNA did not produce enough active protein to fully activate the pathway or that some other component is required, in addition to the Easter catalytic domain, to promote the most ventral fates.

We constructed females homozygous for a P[eaΔN] transgene and lacking wild-type Easter activity; these females produced lateralized embryos, similar to those seen in the eaΔN RNA injection experiments (Fig. 1A). To test whether high concentrations of eaΔN would be sufficient to induce more ventral fates, we injected eaΔN RNA into embryos produced by these P[eaΔN], ea− females. Cells at all dorsal-ventral positions near the injection site developed like the most ventral cell type, the mesoderm, as assayed by the expression of the Twist protein (Fig. 1B). Thus activation of the Easter zymogen appears to be an essential step in the production of all lateral and ventral cell types in the embryo.

**Proteolytic processing of catalytically inactive Easter can be detected in vivo**

The product of the maternally expressed easter gene is required during the syncytial blastoderm stage, from about 1.5-3 hours after fertilization, as judged by the time at which it is possible to rescue the dorsalized phenotype of mutant embryos by injection of the transcript of the easter gene and by the temperature-sensitive period of temperature-sensitive alleles (Anderson and Nüsslein-Volhard, 1984, 1986). Western blot analysis of embryo extracts separated on reducing gels using antibodies to the Easter protein showed that 0-4 hour wild-type embryos contained protein the size of the 50 kDa Easter zymogen (Fig. 2). Proteolytic activation of the Easter zymogen should produce two smaller protein fragments, the catalytic
domain and the pro-domain. The catalytic domain should co-migrate with the 35 kDa product encoded by the P[eaΔN] allele present in the transfectants, but no band the size of the Easter catalytic domain or the smaller pro-domain was detected in wild-type embryo extracts (Chasan et al., 1992; Fig. 2).

Although no processed Easter was detected in wild-type embryos, we did detect processing of certain mutant forms of Easter. The ea8 null allele is a mis-sense mutation that changes the amino acid at the entrance of the binding pocket from glycine to glutamic acid (Jin and Anderson, 1990). This mutation should preclude the binding of the basic amino acid of either a substrate or protease inhibitor that would fit into the binding pocket of wild-type Easter protein. The ea8 allele produces a normal amount of a normal-sized zymogen (Jin, 1991). In contrast to wild type, we detected a form of Ea8 protein that co-migrated with the Easter catalytic domain produced by P[eaΔN] transformants (Fig. 3A), revealing that this Easter protein is processed to generate a protein the same size as the active catalytic domain, as predicted for a typical serine protease. The processed N-terminal pro-domain was not detected, apparently because the polyclonal antibody used did not recognize this region of the protein. Another ea8 mutant in which the active site serine was replaced by alanine (S338A) (Jin and Anderson, 1990) showed the same pattern of proteins; the Easter zymogen was produced and, again, a protein the size of the processed catalytic domain was present (Fig. 3B). These results present a conundrum: we could detect proteolytically activated Easter only in mutants of easter that lack catalytic activity and not in wild type.

Activated Easter forms a stable high molecular mass complex

The lack of accumulation of processed Easter in wild-type embryos could reflect rapid degradation of the processed protein (dependent on its own catalytic activity) or conversion of processed Easter to another form. In wild-type embryos, in addition to the 50 kDa Easter zymogen band, a higher molecular mass band of 80-85 kDa was recognized by the Easter antibody (Fig. 2). This high molecular mass band (which we call Ea-X) was not present in embryos that lacked easter RNA, so it appeared to be a novel form of the Easter protein. The Ea-X complex was very stable; it was not disrupted by boiling in SDS for 30 minutes (data not shown).

Neither the Ea8 nor the EaS338A protein made an Ea-X complex (Fig. 3). Thus the Ea-X complex forms only with catalytically active Easter protein. The results suggest a sequence of events that occur in vivo: Easter zymogen is activated by proteolytic cleavage and then is rapidly converted into Ea-X, but only if the Easter protein has the potential for catalytic activity (Fig. 4).

The Ea-X complex contains proteolytically processed Easter protein

It should be possible to determine whether the wild-type Easter in Ea-X has been proteolytically cleaved at the zymogen activation site based on the size of the Easter protein in Ea-X. Embryos laid by transfectants carrying the P[eaΔN] construct as their only source of easter (ea− P[eaΔN]/ea−) lacked the 50 kDa Easter zymogen, as expected, and also contained both the 35 kDa catalytic domain (EaΔN) and a 80-85 kDa EaΔN-X complex (Fig. 5A). EaAN-X comigrated with the Ea-X complex from wild-type embryos on reducing gels, as predicted if the wild-type Easter in Ea-X has been
proteolytically clipped at its activation site and X is tightly bound to the catalytic domain of Easter.

Based on the distribution of cysteines in the wild-type Easter protein, the N-terminal pro-domain is expected to remain disulfide bonded to the C-terminal catalytic domain after zymogen activation (Chasan and Anderson, 1989). If the activated Easter in Ea-X is a two-chain protease with the pro- and catalytic domains associated by a disulfide bond, then wild-type Ea-X would migrate more slowly on non-reducing gels, where the pro-domain should remain associated with the complex, than on reducing gels, which should contain only the Easter catalytic domain and X (see Fig. 4). Under non-reducing conditions, Ea-X migrated more slowly than the EaN-X, confirming that the wild-type Ea in Ea-X had been proteolytically cleaved at the zymogen activation site and demonstrating that the pro-domain of the wild-type enzyme remained disulfide-bonded to the catalytic domain after zymogen cleavage (Fig. 5B).

Upstream genes are required for activation of the Easter zymogen

We previously found that the eaΔN mutation bypasses the requirement for the activity of five other components of this signaling pathway, pipe, nudel, windbeutel, gastrulation defective (gd) and snake (Chasan et al., 1992), which suggested that those genes are normally required for the activation of the Easter zymogen. Since Ea-X contains proteolytically activated Easter, we can use Ea-X as a biochemical indicator of Easter activation. We examined extracts made from nudel, gd and snake embryos and found that, in each case, the Easter zymogen was present at the same level as in wild-type embryos, but neither activated Easter nor Ea-X was detected (Fig. 6). Because nudel, gd and snake are required for formation of Ea-X, these experiments provide biochemical evidence that three genes act by controlling the activation of the Easter zymogen.

The activation of Easter appears to be regulated by a negative feedback loop from the end of the pathway

As predicted from the genetic analysis, mutations that block this signaling pathway at steps downstream of Easter do not block the formation of Ea-X (Fig. 6). In fact, all these downstream mutants contain 3- to 5-fold more Ea-X than wild-type embryos (Figs 6, 7). Even mutant embryos that lack spätzle RNA and protein showed increased levels of Ea-X (data not shown), indicating that Ea-X is not a stable complex of Easter with its putative substrate Spätzle. In ventralized cactus embryos, we observed that the amount of Ea-X was less than half the amount present in wild-type embryos (Fig. 6). Since the amount of Ea-X reflects the amount of activated Easter, these results suggest that a feedback loop regulated by nuclear Dorsal acts back across the plasma membrane of the syncytial embryo to regulate activation of the Easter zymogen.

The accumulation of Ea-X in downstream mutants occurs at the same time that signaling through the pathway occurs. While the zymogen form of Easter was present at fertilization, Ea-X did not appear until 1 hour after fertilization, at approximately the time that the Easter protein is active in the embryo (Fig. 7), and increased during the next 2 hours of development. As soon as Ea-X was detectable, there was more Ea-X in Toll" than in wild-type embryos (Fig. 7). Thus, well before the final gradient of Dorsal is achieved at about 2.5 hours after fertilization (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989), the level of Easter processing appears to be modified by this feedback loop.

Fig. 3. Processing of the Easter zymogen is observed in catalytically inactive Easter mutants. (A) The ea4 missense mutation in the binding pocket of Easter allows the detection of the processed catalytic domain of Easter, which comigrates with the product of the P[eaΔN] transgene and the prevents formation of the Ea-X complex. The processed N-terminal pro-domain is not detected, apparently because the polyclonal antibody used does not recognize this region of the protein. Extracts were prepared, analyzed and immunoblotted with α-Ea antibodies as in Fig. 2, from embryos laid by females of the following genotypes: lane 1, wild type (Oregon R); lane 2, ea4/eα 5022 rx1; lane 3, eaα4/eaα 5022 rx1; lane 4, P[eaΔN] eaα4/ P[eaΔN] eaα* . (B) A null mutant form of easter in which the active site serine-338 is replaced with alanine (ea s338A) (Jin and Anderson, 1990) is processed to give the catalytic domain-sized fragment and fails to make Ea-X. Extracts were prepared by ea4/eaα 5022 rx1 females were injected with transcripts encoded by wild-type ea cDNA (lane 1) or ea s338A mutant cDNA (lane 2). Extracts were prepared from 15 injected embryos and immunoblotted with α-Ea antibodies as in Fig. 2.

Fig. 4. The sequence of proposed events in Easter activation.

Based on the distribution of cysteines in the wild-type Easter with its putative substrate Spätzle. In ventralized cactus embryos, we observed that the amount of Ea-X was less than half the amount present in wild-type embryos (Fig. 6). Since the amount of Ea-X reflects the amount of activated Easter, these results suggest that a feedback loop regulated by nuclear Dorsal acts back across the plasma membrane of the syncytial embryo to regulate activation of the Easter zymogen.
Fig. 5. The Ea-X complex forms after zymogen cleavage, based on migration of the complex on reducing and non-reducing gels. (A) The Ea-X complex in embryos encoded by the wild-type Easter gene and the EaAN complex encoded by a P[eaAN] transgene comigrate under reducing conditions. This suggests that the Ea protein in the Ea-X complex is cleaved between the pro and catalytic domains, and that X is expressed (no zymogen), but a band comigrating with Ea-X is present, corresponding to EaAN-X. The doublet band below Ea-X is a cross-reacting band variably recognized by the anti-Ea antibodies; compare to the ear lane in Fig. 2. Note that in the P[eaAN] transformants a substantial amount of the EaAN protein does not enter the EaAN-X complex; this is consistent with biochemical observations that the proteolytic activity of EaAN is substantially less than that of the properly activated wild-type Ea (Hecht, 1993). Embryo extracts were prepared as in Fig. 2, incubated with dithiothreitol (DTT) as described in the Materials and methods, and analyzed by immunoblotting a 7.5% SDS-polyacrylamide gel with α-Ea antibodies. Extracts were prepared from embryos laid by females of the following genotypes: lane 1, wild type (Oregon R); lane 2, ea+/ea-; lane 3, Toll5BRE/Toll5ORE; lane 4, P[eaAN] ea+/P[eaAN] ea-; lane 5, P[eaAN] ea7/ea7; lane 6, gd7/gd7; P[eaAN]+/+; lane 7, gd7/gd7. (B) The Ea-X complex migrates more slowly than the EaAN-X complex under non-reducing conditions. The pro-domain of Ea is expected to remain disulfide bonded to the catalytic domain of wild-type Ea, causing the Ea-X complex to migrate more slowly than under reducing conditions, where the pro-domain is not bound. Embryo extracts were incubated with N-ethylmaleimide instead of DTT (as described in Materials and Methods) and analyzed as in part A. Extracts were prepared from embryos laid by females of the following genotypes: lane 1, wild type (Oregon R); lane 2, ea7/ea7; lane 3, Toll5BRE/Toll5ORE; lane 4, P[eaAN] ea7/ea7; lane 5, P[eaAN] ea7/ea7; lane 6, gd7/gd7; P[eaAN]+/+; lane 7, gd7/gd7. Both Ea-X and EaAN-X can be seen in the P[eaAN] ea7/ea7 and P[eaAN] ea7/ea7 lanes. EaAN-X but not Ea-X is present in the P[eaAN] ea7 lane, which lacks wild-type zymogen, and there the Ea-X complex is present. The ratio of Ea-X protein to Easter zymogen, we estimate that less than 10% of total Easter protein in the wild-type embryo is processed. Therefore, the amount of Easter activity in the embryo is limited by the amount of zymogen activation allowed by the upstream proteases.

**DISCUSSION**

**Regulated activation of the Easter zymogen**

Previous results on Easter activation were somewhat paradoxical. Site-directed mutagenesis experiments had shown that the identities of the particular amino acids at the zymogen activation site are critical for Easter activity, arguing that proteolytic processing at that site is necessary for activity (Chasan et al., 1992). Furthermore, the pre-processed catalytic domain of Easter was active in the absence of upstream genes, indicating that those genes are required for activation of the zymogen (Chasan et al., 1992). Despite this molecular genetic evidence, no cleaved, activated protease was detected in the embryo.

We have now demonstrated biochemically that the Easter zymogen is proteolytically processed in vivo to form a catalytic domain of the predicted size and we have discovered that the proteolytically activated protein is rapidly sequenced into a high molecular mass complex, Ea-X. Using the presence of Ea-X to monitor the activation process, we find that activation of the Easter zymogen depends on the serine protease family members encoded by nudel, gastrulation defective and snake, as predicted from the genetic experiments. In contrast, Easter zymogen activation does not depend on Easter activity, since the proteases encoded by the null alleles ea8 and eaS38A are activated. Based on the molecular mass complex, Ea-X. Using the presence of Ea-X to monitor the activation process, we find that activation of the Easter zymogen depends on the serine protease family members encoded by nudel, gastrulation defective and snake, as predicted from the genetic experiments. In contrast, Easter zymogen activation does not depend on Easter activity, since the proteases encoded by the null alleles ea8 and eaS38A are activated. Based on the

**Fig. 6.** Mutants that act upstream of easter in the genetic pathway prevent the formation of the Ea-X complex, and mutants that block the pathway downstream of easter produce more Ea-X. Embryo extracts were separated and blotted as described in Fig. 2 and probed with α-Ea antibodies. (A) Extracts were prepared from 0-4 hour embryos laid by females of the following genotypes: lane 1, nudel1; nudel1; lane 2, snk277/snk277; lane 4, ea7/Ea7; lane 5, spz178/Df(3R)spz178; lane 6, Ti3BaBET/Ti3BaBET; lane 7, Df(3R)u607;Df(3R)u607; lane 8, pill20/pill20; lane 9, cact16/cact16. (B) Extracts were from embryos laid by females of the following genotypes: lane 1, wild type (Oregon R); lane 2, ea7/ea7; lane 3, dorsal/dorsal. 

A. Reducing  B. Non-reducing
Activation of the Easter zymogen is first detected at about 1 hour after fertilization. Therefore there must be some event that begins after fertilization that triggers the activity of the proteases upstream of Easter. The Toll receptor is first detected on the surface of the embryo at about 90 minutes after fertilization (Hashimoto et al., 1991). The ligand for Toll, presumably the proteolytically processed Spätzle protein, appears to be made ventrally, but can diffuse freely in the perivitelline space if it is not sequestered by binding to Toll (Stein et al., 1991). If the ligand were activated by proteolysis before the receptor were present, it could diffuse to the dorsal side of the embryo and activate Toll ectopically. It therefore seems likely that timing of Easter activation is important for patterning.

The high molecular mass Easter complex appears to contain a protease inhibitor

Ea-X is a very stable complex between the activated catalytic domain and another component. The appearance of Ea-X correlates with activation of the Easter zymogen, suggesting that Ea-X is a complex of Easter with another protein that either activates or inhibits Easter activity. The formation of the Ea-X complex depends on previous proteolytic activation of the zymogen and on residues essential for catalysis, indicating that the X protein binds specifically to the activated enzyme through its active site. The most likely molecules to bind the active site are substrates and inhibitors. Because Ea-X is extremely stable and because Ea-X does not include Spätzle, its likely substrate, we favor the hypothesis that Ea-X is an inhibited complex and that the other component of Ea-X is an inhibitor. A serine protease inhibitor could play an important role in patterning by preventing the spread of Easter activity away from its initial site of activation.

The Ea-X complex has the properties expected for a complex with a protease inhibitor of the serpin family. The serpins are a family of proteins that act as suicide substrates, with the active site serine of activated serine proteases, thereby irreversibly inactivating the protease (Potempa et al., 1994). Most serpins are 40-60 kDa in size, which would be consistent with the size difference between the predicted masses of the catalytic domains of Easter and Ea-X. As with other serpin-protease complexes, Ea-X formation requires the active site serine and the protease-binding pocket (Laskowski and Kato, 1980; Potempa et al., 1994). Also, like other serpin complexes, the Ea-X complex is extremely stable. Only a single cloned Drosophila serpin-encoding gene has been well characterized; this serpin is transferred from the male to the female during copulation and its function is not known (Coleman et al., 1995). Other serpin genes have been identified because of their proximity to cloned genes (FlyBase: http://cbridges.harvard.edu:7081/). Additional serpins have been identified in other insects (e.g. Jiang et al., 1994), making it very likely that Drosophila will have a number of different serpin-encoding genes. The reverse genetic approach of purifying the high molecular mass complex, microsequencing and gene cloning will ultimately make it possible to test whether the other component of Ea-X is a serpin or other protease inhibitor.

A feedback loop may modulate Easter zymogen activation

Recent data from a variety of signaling pathways suggests that secreted inhibitors play key roles in defining the realm of action of extracellular signals. For example, activity of the Drosophila EGF receptor induces the secretion of the Argos protein, which inhibits EGF receptor activity and thereby limits the domain of activity of the receptor, apparently by competing with the ligand for the receptor (Golembio et al., 1996). Our data suggest that a negative feedback loop may also operate in the Toll signaling pathway. Mutations in any one of the downstream genes required for pathway activity, spätzle, Toll, tube, pelle or dorsal, lead to an increase in the amount of Easter that is activated in the blastoderm embryo, as detected by the increase in the amount of Ea-X present. A similar phenomenon was previously reported with Spätzle: more proteolytically processed, activated Spätzle was detected in extracts from embryos in which the pathway was blocked downstream of Spätzle than in wild-type embryos (Morisato and Anderson, 1994). Such a feedback loop could regulate the activation of the Easter zymogen directly, or it could act at any step upstream of Easter in the pathway. Because mutations in dorsal lead to increased Easter activation, we propose that the initial component of the feedback loop is a transcriptional target of Dorsal. This hypothesis suggests that there is a more dynamic interaction between maternal and zygotic products in initial dorsal-ventral patterning than previously thought, a possibility that should be experimentally testable.

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