THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 280, No. 30, Issue of July 29, pp. 27856–27865, 2005
Printed in U.S.A.

A Causative Role of Stromelysin-3 in Extracellular Matrix Remodeling and Epithelial Apoptosis during Intestinal Metamorphosis in *Xenopus laevis**

Received for publication, November 24, 2004, and in revised form, May 26, 2005
Published, JBC Papers in Press, June 1, 2005, DOI 10.1074/jbc.M413275200

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The matrix metalloproteinases are a family of proteases capable of degrading various components of the extracellular matrix. Expression studies have implicated the involvement of the matrix metalloproteinase stromelysin-3 (ST3) in tissue remodeling and pathogenesis. However, the *in vivo* role of ST3 has been difficult to study because of a lack of good animal models. Here we used intestinal remodeling during thyroid hormone-dependent metamorphosis of *Xenopus laevis* as a model to investigate *in vivo* the role of ST3 during postembryonic organ development in vertebrates. We generated transgenic tadpoles expressing ST3 under control of a heat shock-inducible promoter. We showed for the first time *in vivo* that wild type ST3 but not a catalytically inactive mutant was sufficient to induce larval epithelial cell death and fibroblast activation, events that normally occur only in the presence of thyroid hormone. We further demonstrated that these changes in cell fate are associated with altered gene expression in the intestine and remodeling of the intestinal basal lamina. These results thus suggest that ST3 regulates cell fate and tissue morphogenesis through direct or indirect ECM remodeling.

The matrix metalloproteinases (MMPs)[1] are a superfamily of matrix-degrading proteases, including collagenases, gelatinases, stromelysins, and membrane type MMPs (1–3). They are either secreted extracellularly or bound to plasma membrane. Membrane type MMPs and stromelysin-3 (ST3) are activated intracellularly through a furin-dependent process (4, 5). The other MMPs are secreted as inactive proenzymes, which are activated upon the removal of the propeptide through various mechanisms (6–8). The mature (*i.e.* activated) MMPs are capable of cleaving, at least *in vitro*, ECM components with distinct but overlapping substrate specificity (2, 9).

The ECM is a complex structure made of numerous proteinaceous and other components (10, 11). It serves as the structural scaffold to hold cells together in various organs or tissues. It can also influence cell fate and behavior through direct interactions with cells or indirect effects on cellular function by regulating cell-cell interactions and controlling the availability of various factors such as growth factors present in the ECM (12, 13).

MMPs are expected to exert their effects on cells, at least in part, through their ability to remodel the ECM by cleaving specific ECM components. In addition, a number of studies suggest that MMPs can also cleave non-ECM proteins (2, 9, 12), providing potentially other means for MMPs to regulate cell fate and behavior.

MMPs are expressed in a number of developmental and pathological processes (14–16). Animal studies with natural and synthetic MMP inhibitors have provided supporting evidence for the involvement of MMPs in tumor invasion (16, 17), although clinical trials with synthetic MMP inhibitors in advanced human cancers have produced disappointing results (18). In addition, MMPs, including ST3, have more recently been implicated to regulate tumor initiation and growth (16, 19–21). Similarly, a number of *in vivo* studies by using MMP inhibitors and/or MMP knock-out mice support the involvement of at least some MMPs in mammalian development (22–27). For example, mice lacking membrane type 1 MMP, although apparently normal at birth, develop a number of severe defects including dwarfism, craniofacial dysmorphism, impaired endochondral ossification, and angiogenesis and die within several weeks (23, 24). On the other hand, mice lacking any one of other MMPs, including ST3, often show little or weak phenotypes (21, 25–27). This is probably due to redundancy in the activity of MMP genes in development. In addition, the importance of MMPs in development may vary in different organs for a given MMP, although it may be expressed in many organs. This would result in MMP knock-out animals that appear normal but have defects in certain organs/tissues. For example, mice lacking MMP13 (collagenase 3) have abnormal skeletal growth plate development (25), and mice lacking MMP2 (gelatinase A) or MMP3 (stromelysin-1) have altered mammary gland branching morphogenesis (26). Thus, a proper model system is highly beneficial for studying the function of MMPs in development *in vivo*.

We have been using amphibian metamorphosis as a model to study the role of MMPs in postembryonic organogenesis in vertebrates. Postembryonic development is difficult to study in mammals due to an inability to manipulate the uterus-enclosed embryos. Amphibian metamorphosis involves changes in essentially all tissues/organs of the tadpole as it changes into a frog and has been used for nearly a century as a model for studying postembryonic organ remodeling and development (28). Although different tissues/organs undergo drastically dif-

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[1] The abbreviations used are: MMP, matrix metalloproteinase; ST3, stromelysin-3; ST3m, mutant ST3; T3, thyroid hormone; ECM, extracellular matrix; GFP, green fluorescent protein; RT, reverse transcription; IFABP, intestinal fatty acid-binding protein; rpL8, ribosome protein L8; TUNEL, terminal dUTP nick-end labeling.

ferent changes, such as total resorption and *de novo* development, all changes are controlled by thyroid hormone (T3), and they can be easily manipulated by simply controlling the availability of T3 to the tadpoles or even *in vitro* cultures of tadpole organs (28). Furthermore, a number of MMPs have been shown to be activated by T3 during metamorphosis in *Xenopus laevis* and *Rana catesbeiana* (28, 29). In particular, the expression of the *Xenopus* ST3 but not several other MMPs correlates tightly with larval or tadpole cell death (apoptosis) in different organs (30–32).

Our own studies focus on the function of ST3 during intestinal metamorphosis in *X. laevis*. The tadpole intestine is a simple tubular organ consisting predominantly of a single layer of larval epithelial cells surrounded by a sparse layer of connective tissue (except in the single epithelial fold, the typhlosole) and thin layers of muscles (33). During metamorphosis, the larval epithelial cells are induced by T3 to die through apoptosis, and concurrently, the undifferentiated adult epithelial cells and cells of the connective tissue and muscles proliferate extensively (28, 33). Toward the end of metamorphosis, the adult epithelial cells differentiate to form a multiply folded adult epithelium surrounded by elaborate connective tissue and muscles. During this process, the expression of *Xenopus* ST3 is spatially and temporally correlated with not only larval epithelial cell death but also the remodeling of the basal lamina, the ECM that separates the epithelium and the connective tissue (32, 34). These results suggest that ST3 is likely to influence cell behavior during intestinal metamorphosis by directly or indirectly modifying the ECM.

To investigate whether ST3 can remodel the ECM and influence cell fate *in vivo*, we have adapted the restriction enzyme-mediated transgenesis (35) to overexpress ST3 in *X. laevis*. Our initial studies showed that overexpression of ST3 in embryos leads to embryonic lethality, thus preventing the analysis of ST3 function in postembryonic development (36, 37). Here, we generated transgenic animals expressing wild type ST3 or a catalytically inactive mutant under control of a heat shock-inducible promoter. We show that precocious expression of wild type but not mutant ST3 leads to ECM degradation and larval epithelial cell death in the intestine, consistent with a role of ST3 in ECM remodeling as a mechanism of T3-induced apoptosis during intestinal metamorphosis.

MATERIALS AND METHODS

Animals and Transgenesis—Wild type tadpoles were purchased from *Xenopus* I, Inc. (Dexter, MI), and, when indicated, stage 54 premetamorphic tadpoles at a density of 2 tadpoles/liter were treated with 5 nM T3 for 3 days. Adult wild type *X. laevis* were purchased from Nasco. Transgenesis was carried out as described (35, 36). Transgenic animals were generated by using a double promoter construct containing the γ-crystalline promoter driving the expression of GFP3, a different version of green fluorescent protein (GFP) that contains a number of amino acid substitutions (38) and heat shock-inducible promoter driving either the wild type or catalytically inactive ST3 fused to GFP (pCGHSwG or pCGHSmG, respectively) (36, 37). The latter construct, pCGHSmG, was generated by excising the coding region for the inactive mutant ST3 from the CSmG construct (36) with AgeI digestion and inserting it into AgeI-digested pCGHSwG, replacing the ST3 coding region. When indicated, the F0 transgenic animals (*i.e.* those generated directly from the transgenesis procedure) were reared to adulthood. Male F0 transgenic animals were sacrificed to obtain the sperm to generate F1 transgenic animals as described (39).

Wild type and transgenic tadpoles at the indicated stages were heat-shocked at 33 °C for 30 min twice with a 30-min space at 18 °C (37, 40). The wild type and transgenic tadpoles were reared and treated with heat shock together in a single container. When needed, the transgenic animals were identified under a UV dissecting microscope with a Chroma filter set for GFP expression in the eyes. Tadpoles were photographed by using an RT Spot digital camera (Diagnostic Instruments, Inc.) attached to the dissecting microscope.

Intestine Histology and TUNEL Assay—Wild type and transgenic tadpoles at the indicated stages were heat-shocked to induce transgene expression. Tissue fragments were isolated from the anterior part of the small intestine, rinsed in 0.6× phosphate-buffered saline to remove food contamination, and fixed in 4% paraformaldehyde, 0.6× phosphate-buffered saline for 2 h at room temperature or overnight at 4 °C. The fixed specimens were then infiltrated in 0.5 M sucrose in 0.6× phosphate-buffered saline for 2–3 h and embedded in OCT cryogenic embedding medium (Electron Microscopy Science). Cross-sections were cut at 6 μm and placed on Fisher Probe-On charged slides and dried on a slide warmer at 37 °C for 2 h or overnight prior to use. The sections were hydrated in 1× phosphate-buffered saline and stained with methyl green-pyronin Y (Muto, Tokyo, Japan) for histologic observation (41). A TUNEL assay on tissue sections was performed as previously described (32).

Electron Microscopy—Four days after the initial heat shock treatment, the wild type and transgenic tadpoles were sacrificed and dissected as described above. The anterior part of intestines were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.5) at 4 °C for 2 h, washed in 0.2 M sucrose in 0.1 M cacodylate buffer (pH 7.5), and postfixed with 1% osmium tetroxide in the same buffer at 4 °C for 2 h. They were then stained en bloc with uranyl acetate and embedded in epoxy resin. Ultrathin sections were stained with lead citrate and examined with a JEOL 200CX electron microscope.

In Situ Hybridization—A partial cDNA encoding *Xenopus* ST3 was obtained by reverse transcription (RT)-PCR with mRNA extracted from *Xenopus* intestine at stage 61 with the primers 5′-TGGGACAAGACAAACCTGACGTAC-3′ (forward) and 5′-TGGAGATAGAAGAATGCTGCAGGC-3′ (reverse). The RT-PCR product was cloned into pCR II-TOPO vector (Invitrogen) and verified by sequencing. To synthesize an antisense RNA probe, this plasmid was linearized with BamHI and transcribed with T7 RNA polymerase (Roche Applied Science). *In situ* hybridization of sections was performed as described (42).

RT-PCR and Northern Blot—Total RNA from wild type and transgenic embryos or tadpole tissues was extracted with TRIzol reagent according to the manufacturer's instructions (Invitrogen) and analyzed by Northern blot hybridization or RT-PCR. Northern blot hybridization was done as previously described with a ^{32}P-labeled ST3 probe (43). RT-PCR was performed by using Superscript One-Step RT-PCR (Invitrogen) from 0.1 μg of total RNA as described (44). RT-PCR for the transgene was done with the primers 5′-GGT ATC CTC ACC TGA TCA GTC AAG-3′ (in the ST3 coding region) and 5′-CTT CAG CAC GTG TCT TGT AGT TCC-3′ (in the GFP coding region). Other primers used were 5′-CAT CTT CTT CAA GGA CGA CGG G-3′ and 5′-AGT TGC ACG CCG CCG TCT TCG-3′ for GFP3 and 5′-CGA AGG TTA TGT ACA GGA AAG AAC T-3′ and 5′-TTC CAT CTT CAA TGT TGT GTC TAA-3′ for the GFP originating from pS65T-C1 (Clontech) and fused to the ST3 gene at its C terminus (37). All RT-PCRs included an internal control amplifying a fragment of ribosome protein L8 (rpL8) with primers 5′-CGT GGT GCT CCT CTT GCC AAG-3′ and 5′-GAC GAC CAG TAC GAC GAG CAG-3′ (45). The RNA for RT-PCR was treated with RNase-free DNase I (Ambion) to remove any DNA contamination and repurified with TRIzol reagent before RT-PCR (Invitrogen). RT-PCR products were run on 2% agarose gels, visualized with ethidium bromide staining under UV lights, and photographed with an Eastman Kodak Co. imaging system (Gel Logic 100 Imaging System). When indicated, quantitative RT-PCR was also carried out to quantify the gene expression levels. In brief, 100 ng of total RNA each was reverse transcribed in 20 μl of the reaction system according to the manufacturer's instructions for the High Capacity cDNA Archive Kit (Applied Biosystems), and 4 μl of each cDNA was subjected to quantitative PCR analysis with primers and probes (Table I) synthesized through Assay-by-Design (Applied Biosystems). Mixed total RNA isolated from tadpoles of all stages encompassing the entire metamorphosing period was reverse transcribed at a higher concentration (1 μg of total RNA in 20 μl of the reaction system) to serve as standard samples to produce a standard curve for the quantitative PCR analysis, where the cDNA was diluted at series of 1:2 or 1:3 and covered the whole range of the tested samples. All of the quantitative PCRs were carried out with a quantitative PCR machine (Model 7000 Sequence Detection System; Applied Biosystems). A set of primer/probe specific for rpL8 was used as a control for RNA input of each sample, and the expression level of the gene of interest within each sample was normalized to that of rpL8.

RESULTS

Our earlier studies showed that transgenic expression of ST3, but not a catalytically inactive mutant ST3 (ST3m), with

TABLE I
Primer/probe sets for quantitative RT-PCR

The primer/probe set for the endogenous ST3 had the forward primer located in the 5′-untranslated region; the primer/probe set for the transgenic ST3 fused to GFP (ST3-GFP) had the reverse primer in the GFP region; the primer/probe set for both the endogenous ST3 and transgenic ST3-GFP had the primers and probes all in the ST3 coding region.

Targeted gene	Forward primer	Reverse primer	Probe
ST3	5′-CACTTGTAGCCATTGTATCACACTCA-3′	5′-GCCATGATCTTTCTGAGGCTTTTC-3′	5′-ATGCATTCTCACAAGCTGT-3′
ST3-GFP	5′-GTCAAGCAAGCTCTACATTTGTTAATTCA-3′	5′-ATTGGGACAACTCCAGTGAAAAGT-3′	5′-TCGCCACCATGGGTAAA-3′
ST3/ST3-GFP	5′-GTGGTCGAAATCGTCAGAAACG-3′	5′-CGGATGATCTTGTACGTCAGGTT-3′	5′-ACGCTGGGACAAGACA-3′
RpL8	5′-AGAAGGTCATCTCATCTGCAAACAG-3′	5′-CAATACGACCACCTCCAGCAA-3′	5′-CAACCCCAACAATAGCT-3′
IFABP	5′-AAGGTTGACAGAAGTGAAAACTATGAGAA-3′	5′-CCATCTTGCTGGATTATGACCTTCA-3′	5′-CTTCCGTTTCACTATATTTAC-3′
Gel A	5′-GACGATGATGAGCTTTGGACACTA-3′	5′-GAACTCTCCATCTGCATTACCATACTT-3′	5′-ACAACTTGGCCTTCTCC-3′
TIMP2	5′-GGAGATCGCTGGAAAGAAGGAATAT-3′	5′-GCACGATGAAGTCACACAGGATTA-3′	5′-ATGCATCTTTCCATCACCATC-3′

GFP fused at the carboxyl terminus, under control of the ubiquitous cytomegalovirus promoter led to lethality of *X. laevis* embryos or early stage tadpoles (36). To overcome this embryonic lethal phenotype, we developed a double promoter construct to express the ST3 fusion proteins under control of a heat shock-inducible promoter as well as GFP3, a different version of GFP that contains a number of amino acid substitutions (38), under control of the lens-specific γ-crystalline promoter (Fig. 1*A*) (37).

To verify the independent function of the two promoters in transgenic animals, ST3-GFP transgenic tadpoles at premetamorphic stage 54 were treated with heat shock. RNA was isolated from the intestine and head, which included the eyes where γ-crystalline promoter functions, and was subjected to RT-PCR analysis by using primer sets specific for GFP (present in the ST3-GFP), ST3-GFP (*i.e.* one primer in ST3 and another in GFP coding region), or GFP3 (driven by the γ-crystalline promoter) (Fig. 1*B*). The results showed that GFP3 was expressed in the head of transgenic tadpoles (Fig. 1*B*, *lane 4*) but not in the head of wilt type animals or the intestine of any tadpoles (Fig. 1*B*, *lanes 1–3*). In contrast, ST3-GFP and the GFP moiety of ST3-GFP were expressed in both the head and intestine of heat-shocked transgenic but not wild type tadpoles. These results indicated that the two promoters were regulated independently and that the ST3-GFP transgene was expressed only upon heat shock but ubiquitously. Therefore, identification of transgenic animals could be accomplished subsequently by simply looking for GFP expression in the animal eyes. This allowed us to keep the wild type and transgenic animals together to avoid variation in treatment and rearing conditions between the two groups.

When wild type and transgenic animals were reared in the absence of heat shock treatment, they developed normally through metamorphosis and into adulthood without detectible ST3-GFP expression (data not shown). On the other hand, as shown in Fig. 1*B*, heat shock treatment of both transgenic and wild type F0 tadpoles (*i.e.* the animals generated directly from the transgenesis procedure) led to ST3-GFP expression only in the transgenic animals, with ubiquitous expression similar to that found in the cytomegalovirus promoter-driven ST3 transgenic animals based on GFP fluorescence of the fusion protein (37) (also see below). (It should be pointed out that the restriction enzyme-mediated transgenic methods produces animals that are germ line-transgenic, although individual F0 animals are probably different genetically due to variations in the insertion site and transgene copy number (35)). Northern blot analysis of the wild type and transgenic animals confirmed that the ST3 transgene mRNA of the expected size was induced within 1 day of heat shock treatment in the transgenic but not wild type animals (Fig. 2*A*).

To quantitatively compare the transgenic ST3 expression upon heat shock with endogenous ST3 expression, we used a quantitative PCR primer/probe sets specific for transgenic ST3-GFP, endogenous ST3, or both (Fig. 2*B*). Quantitative PCR was carried out on intestine RNA isolated from premetamorphic (stage 54) wild type and ST3-GFP transgenic tadpoles treated with or without heat shock for the indicated number of days (during the experimental period, the animals remained at premetamorphic stages, between stages 54 and 56, with few changes morphologically or cellularly for control animals (data not shown; also see below)). For comparison, we also used intestine RNA from tadpoles at the climax of natural metamorphosis (*i.e.* stage 60) and premetamorphic tadpoles induced to metamorphose with T3 treatment (Fig. 2*B*). Endogenous intestinal ST3 mRNA was expressed only in tadpoles undergoing natural metamorphosis (Fig. 2*B*, *left panel*, *lane 11*) or T3-induced metamorphosis (Fig. 2*B*, *left panel*, *lane 10*). Transgenic ST3-GFP mRNA was expressed after 1–7 days of heat shock treatment, with the highest levels after 4 days for unknown reasons (Fig. 2*B*, *right panel*, *lanes 2–4*). During the heat shock treatment, the tadpoles remained at the premetamorphic stage, and expression of endogenous ST3 was not detectable in the intestine of both transgenic (Fig. 2*B*, *left panel*, *lanes 1–4*) and wild type animals (Fig. 2*B*, *left panel*, *lanes 5–8*). The quantitative PCR analysis using the primer/probe set specific for both the endogenous and transgenic ST3 showed that the ST3-GFP expression levels after 1–7 days of heat shock treatment were comparable with or severalfold higher than those of endogenous ST3 during natural or T3-induced metamorphosis (Fig. 2*B*, *middle panel*, compare *lanes 2–4* with *lanes 10–11*).

In situ hybridization was then carried out to determine where ST3-GFP transgene was expressed in the intestine after heat shock treatment. As expected, a probe specific for the ST3 coding region showed that no ST3 was detected in the intestine of premetamorphic wild type tadpoles with or without heat shock and transgenic tadpoles without heat shock (Fig. 3, *A–C*). High levels of endogenous ST3 expression were detected in the connective tissue of the intestine of wild type tadpoles at the metamorphic climax (stage 61, Fig. 3*D*) or T3 treatment of premetamorphic (stage 54) wild type tadpoles (Fig. 3*E*), in agreement with earlier findings (32, 43). In transgenic animals treated with heat shock, strong expression of ST3 was present in the epithelium, whereas only much lower levels were present in the connective tissue and muscle layers (Fig. 3*F*). Since there was no endogenous ST3 expression in wild type tadpoles at this stage (Figs. 2 and 3*B*), all ST3 in the transgenic animals was derived from the transgene. It is unclear why the transgene was strongly expressed only in the epithelium given the ubiquitous nature of heat shock response in general. On the other hand, because ST3 is a secreted protein, the exact location of the cells expressing the transgene mRNA is unlikely to affect ST3 function significantly in the intestine.

Transgenic Expression of ST3 in Tadpoles Leads to Precocious Intestinal Remodeling and Epithelial Cell Death—The ability to precociously express ST3 in premetamorphic tadpoles under an inducible promoter offers an opportunity to study the

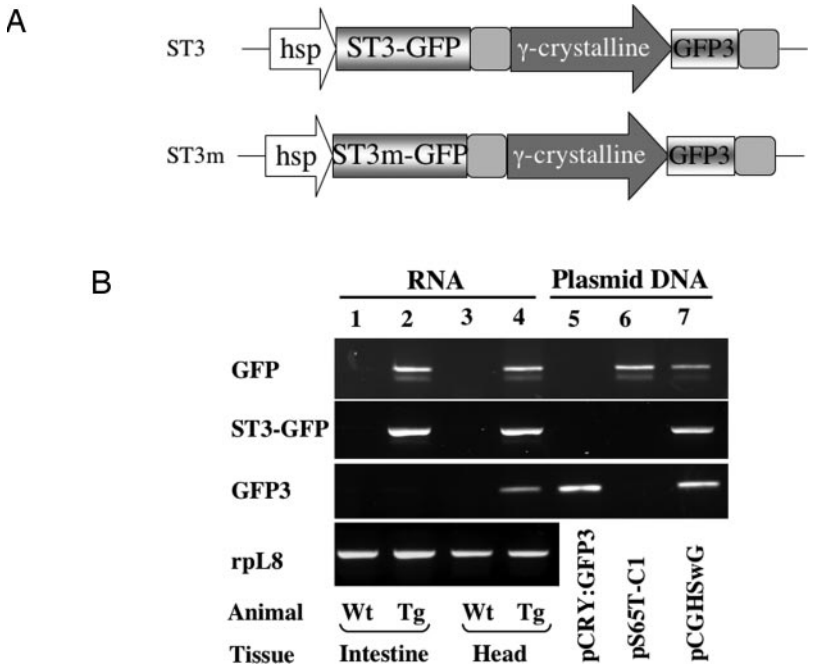

FIG. 1. **Generation of transgenic tadpoles expressing ST3.** *A*, schematic diagrams of the double promoter constructs for transgenesis. The entire coding region for the wild type (ST3) or catalytically inactive mutant (ST3m, containing an E204A substitution) (36) was cloned in frame with the coding region for GFP at the 3′ terminus into a double promoter construct under control of a heat shock promoter (*hsp*). The construct also contains the coding region of GFP3, a different version of GFP that contains a number of amino acid substitutions (38), under control of the lens-specific γ-crystalline gene promoter for the identification of transgenic animals (37). *B*, RT-PCR shows that heat shock treatment activates only the heat shock-inducible promoter driving the expression of ST3-GFP but not the lens-specific γ-crystalline gene promoter, which drives the GFP3 in the eyes. Transgenic (*lanes 2* and *4*) and wild type (*lanes 1* and *3*) animals at stage 54 were heat-shocked for 4 days. Total RNA was isolated from the intestine (*lanes 1* and *2*) or head (*lanes 3* and *4*) and subjected to RT-PCR with a pair of primers specific for the GFP fused to ST3 (*GFP*), transgenic ST3-GFP (*ST3-GFP*; *i.e.* one primer in ST3 and the other in GFP coding region), or GFP3 under control of the lens-specific γ-crystalline gene promoter (*GFP3*), respectively. A primer pair specific for rpL8 was included as a control for RNA input. Note that heat shock led to the expression of ST3-GFP (as shown when analyzed for either GFP or ST3-GFP) but not GFP3 in the intestine (*lane 2*). GFP3 is constitutively expressed in the head (which included the eyes) as expected (*lane 4*). *Lanes 5*, *6*, and *7*, 100 ng of plasmid pCRY:GFP3 (*lane 5*), pS65T-C1 (containing GFP used to generate ST3-GFP) (*lane 6*), or pCGHSwG (used for transgenesis) (*lane 7*) was used as PCR template to validate the specificity of each pair of primers. *Wt*, wild type animals; *Tg*, transgenic animals.

effects of ST3 on tissue transformation. We and others have previously shown that ST3 expression is tightly correlated with T3-dependent cell death during metamorphosis (30–32, 34). More importantly, our earlier organ culture studies indicated that ST3 is required for T3-induced apoptosis of the intestinal epithelial cells during metamorphosis (34). These findings raised the possibility that expression of ST3 alone may be sufficient to cause precocious cell death in the tadpole intestine although it had little effect on overall morphology. Thus, we heat-shocked transgenic and wild type tadpoles at premetamorphic stage 54, just prior to the onset of natural metamorphosis or the expression of endogenous ST3 (43) to induce ST3 transgene expression in transgenic animals. Morphologically, no changes were observed in the transgenic animals with or without heat shock treatment compared with the wild type animals after 4–7 days. As our earlier studies strongly supported a role of ST3 in intestinal remodeling during metamorphosis, we focused entirely on the intestine for subsequent analyses. Four days after the onset of the initial heat shock, the intestine of transgenic tadpoles appeared to be normal (Fig. 4*A*) compared with wild type animals with or without heat shock (Fig. 4*C*, data not shown). On the other hand, 7 days of heat shock treatment led to apparent degeneration of the epithelial cells in the transgenic (Fig. 4*B*) but not wild type animals (Fig. 4*D*), suggesting that continuous, precocious ST3 expression resulted in precocious intestinal transformation. This high level of expression is comparable with the duration of endogenous high levels of ST3 expression during natural metamorphosis (43), where the ECM is dramatically altered and cell death occurs naturally (33, 34).

During natural and T3-induced intestinal remodeling, the most dramatic event is the larval epithelial degeneration, where the epithelial cells undergo programmed cell death or apoptosis (33). To investigate whether the epithelial degeneration caused by ST3 expression also occurred through apoptosis, we examined whether apoptosis took place prior to the histologic degeneration as seen after 7 days of ST3 expression. Thus, intestinal sections from stage 54 tadpoles subjected to daily heat shock for 4 days were analyzed by TUNEL for detection of apoptotic cells. The results showed that precocious overexpression of ST3 (Fig. 5, *A* and *D*) but not the catalytically inactive mutant (Fig. 5, *B* and *E*) led to apoptosis specifically in the intestinal epithelium, as observed during metamorphosis. As expected, heat shock treatment of wild type animals failed to induce any cell death in the intestine (Fig. 5, *C* and *F*). These results indicate that ST3 expression leads to precocious apoptosis in the tadpole epithelium.

To further characterize the cell death induced by ST3 expression, the intestines isolated from the above animals were examined by electron microscopy. The intestinal epithelium from the transgenic tadpoles expressing ST3 was found to contain many epithelial cells with numerous lysosomes (Fig. 6*A*), a sign of cell death. In contrast, the intestinal epithelium from transgenic tadpoles expressing ST3m (Fig. 6*B*) or wild type tadpoles (Fig. 6*C*) lacked these large, dark lysosomes. Such lysosome-rich epithelial cells were also observed during natural metamorphosis as the epithelium degenerates (Fig. 6*D*, stage 60). Furthermore, at higher magnification, it was clear that the dying cells caused by ST3 overexpression were undergoing apoptosis. This is exemplified by the condensation of chromatin

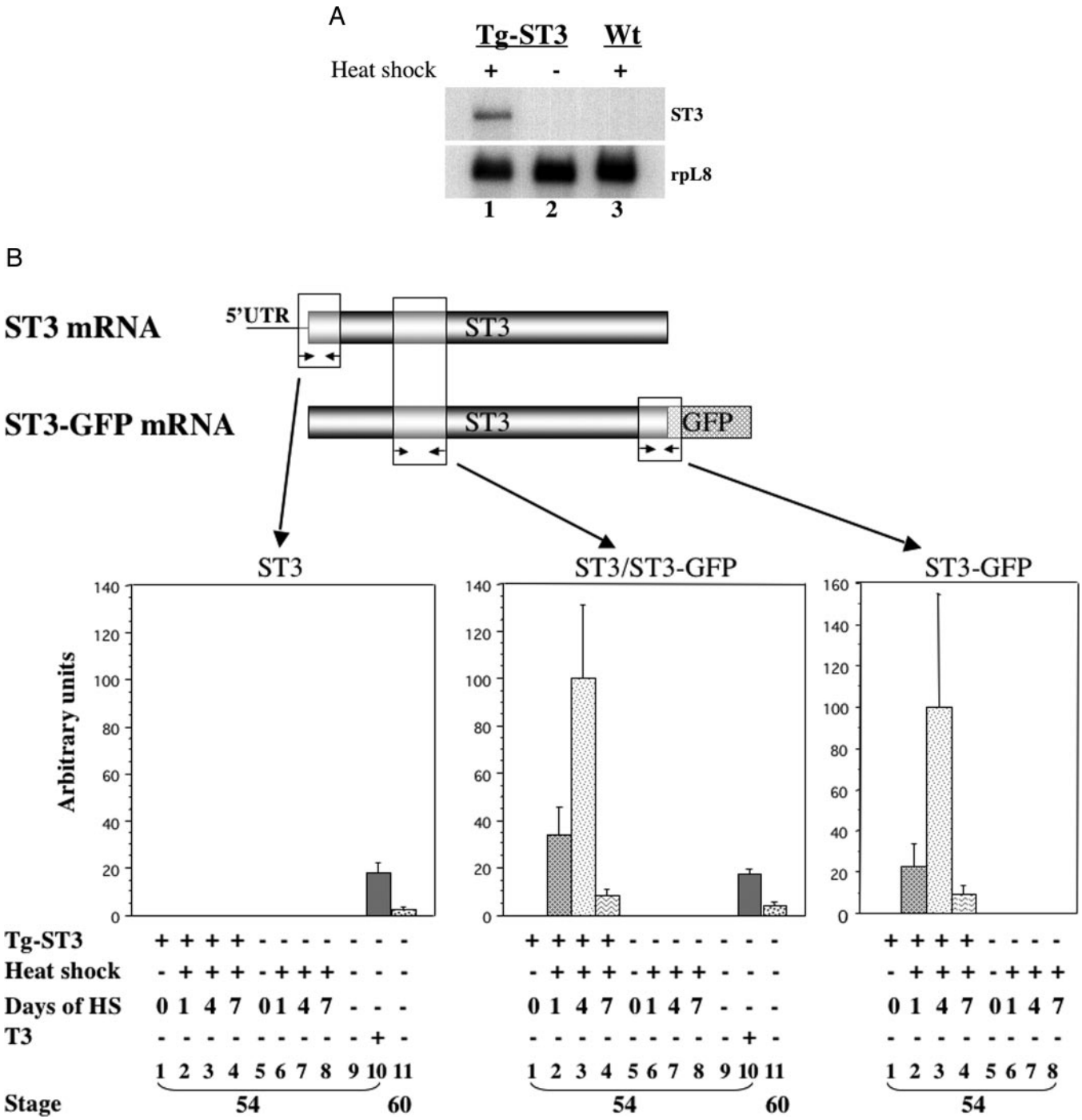

FIG. 2. **Transgenic but not endogenous ST3 is expressed in the transgenic tadpoles upon heat shock.** *A*, Northern blot analysis reveals the expression of transgenic mRNA upon heat shock. Transgenic (*Tg-ST3*) and wild type (*Wt*) tadpoles at stage 46 were treated with or without heat shock. One day later, whole body total RNA was isolated, and 5 μg of RNA was electrophoresed on a denaturing agarose gel. The RNA was transferred from the gel to a nylon membrane and probed with a ^{32}P-labeled ST3 cDNA to detect the transgenic mRNA. The same membrane, after removing the ST3 probe, was reprobed with a ^{32}P-labeled rpL8 (ribosomal protein L8) cDNA probe as an RNA loading control. *B*, quantitative RT-PCR shows that the intestine of premetamorphic transgenic animals express ST3-GFP transgene but not endogenous ST3 upon heat shock and that the transgene expression level is comparable with or higher than that of endogenous ST3 at the climax of natural metamorphosis (*i.e.* stage 60) or in premetamorphic tadpoles induced to metamorphose with T3 treatment. Transgenic and wild type animals at stage 54 (premetamorphic) were heat-shocked (*HS*) for 0, 1, 4, and 7 days. Total RNA was isolated from the intestine of these tadpoles as well as stage 60 (metamorphic climax) wild type animals or stage 54 wild tadpoles treated with or without 5 nM T3 for 3 days. The total RNA was subjected to reverse transcription and quantitative PCR with primer/probe sets specific for endogenous ST3 (*left*), the transgene ST3-GFP (*right*), or both ST3-GFP and endogenous ST3 (*middle*). A primer/probe set specific for the rpL8 gene was used as the RNA control. The ST3-GFP or ST3 signals were normalized to those of rpL8, with the highest expression level achieved after 4 days of heat shock in the transgenic tadpoles set to 100. The schematic diagram at the *top* illustrates the location of the specific primer/probe sets for ST3, ST3-GFP, or both (*boxed* with *arrows* indicating the direction of forward or reverse primers).

along the nuclear membrane (Fig. 6*E*), the formation of membrane-enclosed apoptotic bodies (Fig. 6*F*), and the removal of the apoptotic bodies through macrophage engulfment (Fig. 6*G*), all of which occur during natural metamorphosis (33). Thus, ST3 expression alone activated the epithelial apoptotic pathway that normally occurs when T3 is present.

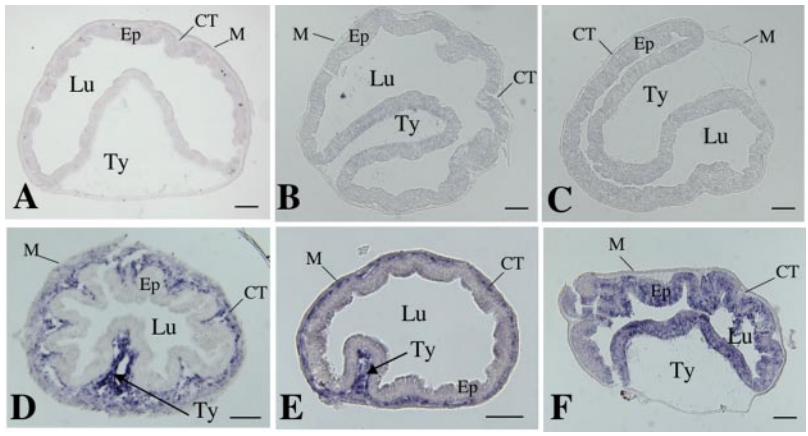

FIG. 3. **Heat shock treatment leads to the expression of ST3-GFP transgene mainly in the epithelium of the intestine of transgenic animals.** Wild type tadpoles (*A* and *B*) and transgenic tadpoles containing ST3-GFP transgene (*C* and *F*) were reared to premetamorphic stage 54 and treated with (*B* and *F*) or without (*A* and *C*) heat shocks. The intestines from these animals as well as from wild type tadpoles at the climax of metamorphosis (stage 61) (*D*) or at stage 54 but induced to undergo metamorphosis with 5 nM T3 treatment for 2 days (*E*) were subjected to *in situ* hybridization with ST3 antisense probe in the ST3 coding region (*i.e.* detecting both the transgene or endogenous ST3. However, as shown in Fig. 2 as well as here, in these animals, there was no overlap in transgene or endogenous ST3 gene expression). *CT*, connective tissue; *Ep*, epithelium; *Lu*, lumen; *M*, muscle; *Ty*, typhlosole. *Bar*, 100 μm.

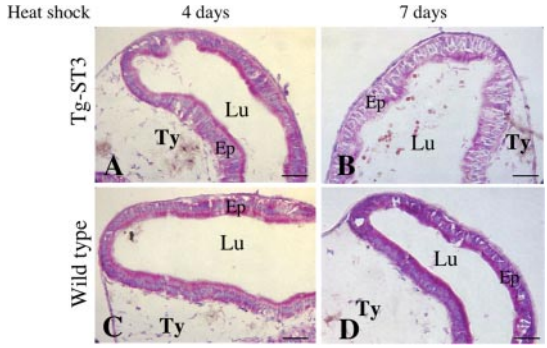

FIG. 4. **Transgenic overexpression of ST3 leads to intestinal epithelial degeneration.** Transgenic (*A* and *B*) or wild type (*C* and *D*) tadpoles were reared to stage 52–54 and then subjected to daily heat shock treatment to induce transgene expression. After 4 or 7 days, the intestine was isolated, fixed in 4% paraformaldehyde, and cryosectioned at 6 μm. The sections were stained with methyl green-pyronin Y. After 4 days of transgene expression, the intestine appeared normal (compare *A* and *C*), but after 7 days of transgene expression, degenerative changes were apparent throughout the epithelium (compare *B* and *D*). *Ty*, typhlosole (the only epithelial fold in the larval intestine, where connective tissue is abundant); *Lu*, lumen; *Ep*, epithelium. *Bar*, 50 μm.

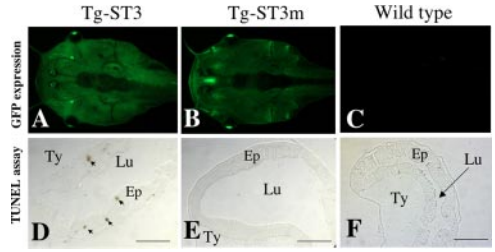

FIG. 5. **Transgenic overexpression of ST3 but not ST3m leads to intestinal epithelial cell death as revealed by histology.** Transgenic tadpoles containing ST3 (*A* and *D*) or ST3m (*B* and *E*) transgene and wild type tadpoles (*C* and *F*) were reared to stage 54 and subjected to daily heat shock to induce transgene expression. Four days after the initial heat shock treatment, the tadpoles were examined under a fluorescent dissecting microscope to verify the transgene expression (due to the C-terminally fused GFP) (*A–C*) and were then sacrificed. The intestine was isolated for the TUNEL assay to detect apoptotic cells (*D–F*, *short arrows*). *Ty*, typhlosole; *Lu*, lumen; *Ep*, epithelium. *Bar*, 100 μm.

ST3 Expression Leads to ECM Remodeling and Fibroblast Activation in the Tadpole Intestine—As a matrix metalloproteinase, ST3 is expected to cleave ECM proteins. ECM remodeling is known to affect cell fate *in vitro*. For example, inhibiting ECM-cell interactions leads to apoptosis for many types of cells such as mammary gland epithelial cells (46–48), a phenomenon referred as "anoikis" (49). The intestinal epithelial cells are in tight contact with the underlying ECM, the basement membrane or basal lamina, and alterations in the ECM may affect their behavior. On the other hand, unlike other MMPs, ST3 has only weak activities toward known ECM proteins but much stronger activities against non-ECM proteins such as α1-proteinase inhibitor and insulin-like growth factor binding protein-1 (50–52). To investigate whether ST3 induced intestinal cell death through ECM remodeling, we examined the epithelial connective tissue interface using electron microscopy. In wild type premetamorphic tadpoles, a thin but continuous basal lamina was found to be present underlying the larval epithelium, separating it from the connective tissue (Fig. 7*A*, *a*). The same was true for transgenic tadpoles carrying the ST3m transgene with or without heat shock to induce the transgene expression (Fig. 7*B*, *b*; data not shown). In contrast, the basal lamina becomes amorphous or absent in wide areas of the intestine in transgenic tadpoles expressing ST3 after heat shock but not without heat shock to induce the transgene expression (Fig. 7*C*, *c*; data not shown). In addition, these transgenic tadpoles had activated fibroblasts (*i.e.* containing well developed rough endoplasmic reticulum) just beneath the epithelium (Fig. 7*C*). Such activated fibroblasts were often found in the tadpole intestine during natural metamorphosis or T3-induced intestinal remodeling (Fig. 7*E*). Furthermore, as during natural and T3-induced metamorphosis (Fig. 7*F*), we also observed cell-cell contacts between epithelial cells and the activated fibroblasts in transgenic tadpoles expressing ST3 but not ST3m (Fig. 7*D*; data not shown).

Some differences, however, existed between transgenic animals expressing ST3 and wild type animals undergoing natural or T3-induced metamorphosis. First, during natural metamorphosis, in addition to the degeneration of the tadpole epithelium, adult epithelial cells, whose origin is yet unknown, proliferate first as islets in between the dying larval epithelium and the underlying connective tissue. They subsequently differentiate to form a multiply folded epithelium (33). In heat-shocked transgenic animals, larval epithelial degeneration took place, but proliferating islets of adult epithelial cells were not observed (data not shown). Second, the basal lamina un-

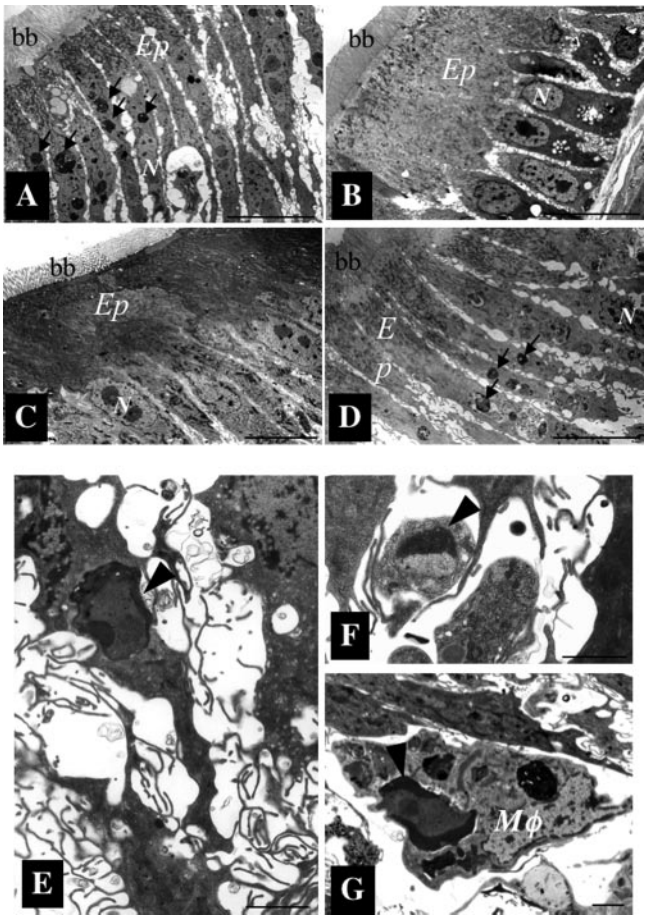

FIG. 6. **Electron microscopic examination reveals that the cell death induced by transgenic overexpression of ST3 is apoptosis.** *A–D*, wild type and transgenic tadpoles were treated as in Fig. 4. Ultra thin sections of isolated intestines were examined under an electron microscope. The intestine of the transgenic tadpoles at stage 54 expressing ST3 (*A*) but not ST3m (*B*) or wild type stage 54 animals (*C*) had numerous lysosomes (*arrows*), as also observed during natural metamorphosis (stage 60) (*D*). *E–G*, higher magnification of epithelial cells at various stages of apoptosis in the intestine of stage 54 transgenic tadpole expressing ST3 revealed chromatin condensation close to the nuclear membrane (*arrowheads*) in an epithelial cell (*E*), an apoptotic body containing a nuclear fragment with condensed chromatin close to the nuclear membrane in the intercellular space prior to engulfment by a macrophage (*F*), and an apoptotic body engulfed by a macrophage (*Mϕ*) (*G*). *bb*, brush border; *Ep*, epithelium; *N*, nucleus. *Bar*, 10 μm (*A–D*) or 1 μm (*E–G*).

derlying the epithelium becomes thicker during natural or T3-induced metamorphosis (Fig. 7*E*) (33). On the other hand, the basal lamina of the intestine in transgenic tadpoles expressing ST3 was amorphous or simply absent (Fig. 7, *C* and *D*). These differences suggest that overexpression of ST3 induces only part of the metamorphic program that normally takes place during natural development, which would not be surprising, since many other genes, in addition to ST3, are induced during metamorphosis (28).

Transgenic ST3 Alters the Expression of Some Late T3 Response Genes—Like all other changes that occur during metamorphosis, intestinal remodeling is controlled by thyroid hormone (T3), and many genes that are known to be regulated by T3 during intestinal remodeling have been isolated and characterized (28). The similarities between the changes caused by transgenic expression of ST3 and those during T3-dependent metamorphosis prompted us to examine whether transgenic expression of ST3 alters the metamorphic gene regulation program that is normally controlled by T3. Thus, we analyzed the expression of genes that are known to be regulated by T3 during intestinal remodeling by RT-PCR. We first analyzed several early or direct T3 response genes, which are regulated by thyroid hormone receptors directly at the transcription level. None of them, including the endogenous ST3 gene (data not shown), was induced by the expression of transgenic ST3. We then analyzed the expression of late or indirect T3 response genes. The genes included the T3-down-regulated intestinal fatty acid-binding protein (IFABP) (53) and T3-up-regulated gelatinase A (29), tissue inhibitor of matrix metalloproteinase 2, MMP13 (collagenase 3), and membrane type 1 MMP. Among them, tissue inhibitor of matrix metalloproteinase 2, MMP13, and membrane type 1 MMP were not up-regulated by transgenic expression of ST3, whereas IFABP and gelatinase A were down-regulated and up-regulated, respectively (data not shown). Quantitative RT-PCR was then carried out on intestinal RNA samples to determine the extent of the regulation of IFABP and gelatinase A by the transgenic ST3. The results showed that gelatinase A was up-regulated by about 2-fold and that IFABP was down-regulated by about 50% in the transgenic animals expressing ST3 (Fig. 8). These changes were similar to, although less dramatic than, those observed in wild type siblings treated with T3 (Fig. 8). In addition, as a control, quantitative PCR also showed that tissue inhibitor of matrix metalloproteinase 2 expression was induced by T3 treatment of wild type tadpoles but was not affected by the transgenic ST3 expression in the intestine of transgenic animals (Fig. 8), again consistent with the RT-PCR data. These results suggest that ST3 overexpression affects some genes downstream in the T3-dependent gene regulation cascade responsible for intestinal remodeling.

DISCUSSION

The ECM plays a critical role in organogenesis and tissue remodeling by serving as a structural support and a medium for cell-cell interactions. Numerous *in vitro* studies have implicated a role for MMPs in ECM remodeling or degradation to facilitate organogenesis and tissue remodeling during development. Few studies, however, have addressed MMP functions *in vivo*. Our study here has now for the first time provided strong *in vivo* evidence to suggest that 1) ST3 remodels the basal lamina, 2) ST3 is sufficient to induce physiological cell death, 3) ST3 causes fibroblast activation and cell-cell contact between the activated fibroblasts and epithelial cells, and 4) the catalytic activity of ST3 is required for these *in vivo* functions.

Intestinal Metamorphosis as a Model for Studying ECM Remodeling and MMP Function in Cell Fate Determination—The formation of the adult frog intestine involves two phases (33). The first is the development of the tadpole intestine during embryogenesis, leading to the formation of simple tubular organ consisting of predominantly a single layer of tadpole/larval epithelial cells surrounded by thin layers of connective tissue and muscles. After a period of premetamorphic tadpole growth, the rising concentration of endogenous T3 triggers the second phase of intestinal development. During this metamorphic transformation, the entirely larval epithelium degenerates through programmed cell death or apoptosis. Concurrently, the adult epithelial cells, whose origin is yet unknown, proliferate and eventually differentiate to form a multiply folded adult epithelium supported by elaborate connective tissue and muscles. The major transforming tissue, the epithelium, is separated from the connective tissue by a special ECM, the basement membrane or basal lamina. Based on tissue culture studies, the presence of connective tissue is not needed for larval epithelial cell death, but it is important for adult epithelial development (54). Similarly, the epithelium also plays a role in the metamorphic transformation of the connective tissue (55). Thus, alterations of the basal lamina may contribute to intestinal

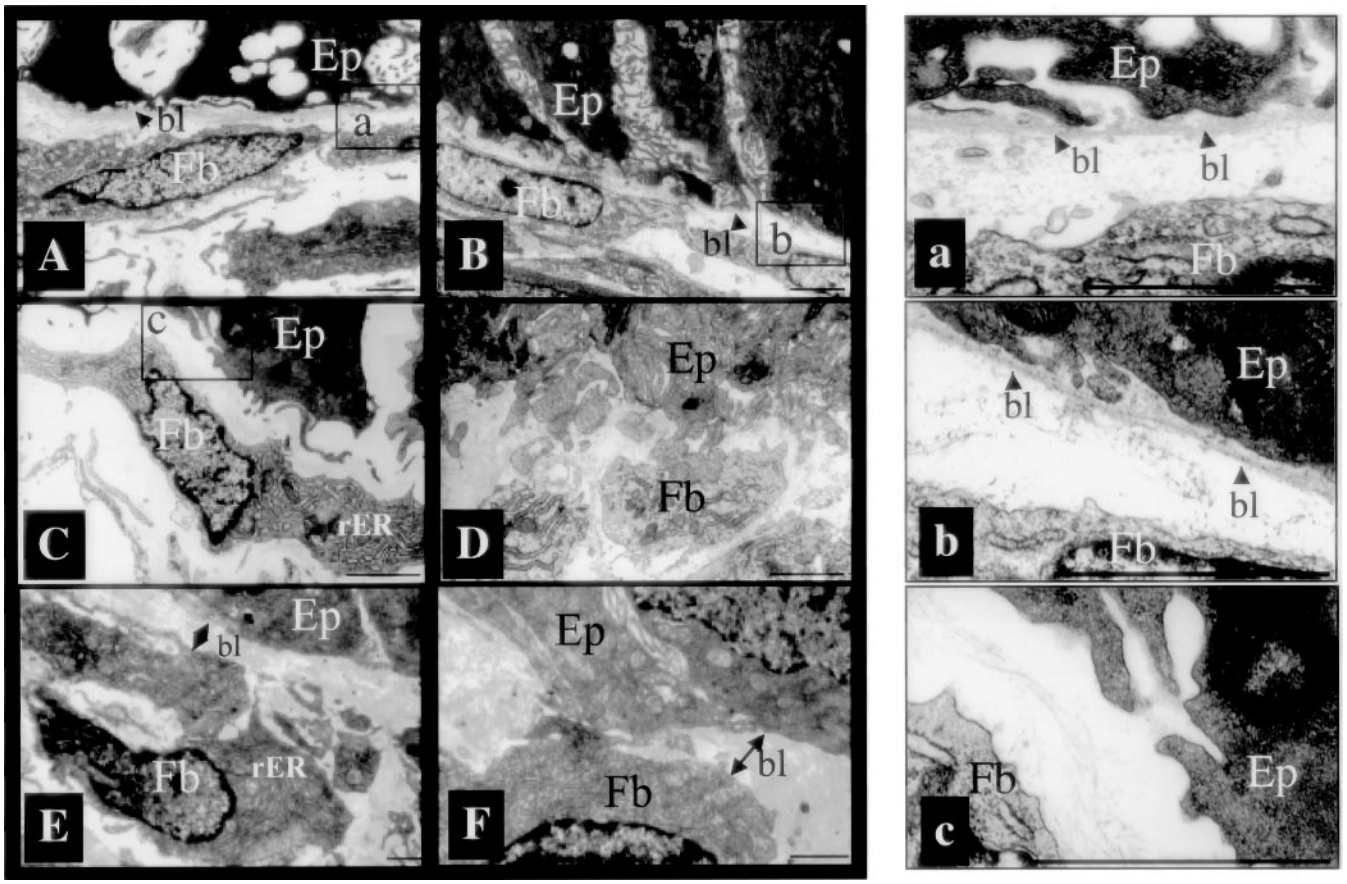

FIG. 7. **Transgenic overexpression of ST3 but not ST3m leads to the loss of the basal lamina and establishment of direct cell contacts between epithelial cells and fibroblasts.** The intestines from wild type tadpoles at metamorphic climax (stage 61) and stage 54 wild type or transgenic tadpoles with heat shock treatment as described in the legend to Fig. 4 were isolated and analyzed under an electron microscope. *A* and *B* and *a* and *b*, a thin and continuous basal lamina (*bl*, *arrowheads*) separates the epithelium from the connective tissue in the intestine of stage 54 wild type (*A*, *a*) or ST3m transgenic (*B*, *b*) tadpoles with heat shock, similar to wild type animals without heat shock (data not shown). *a* and *b* show the basal lamina in the *boxed regions* of *A* and *B*, respectively, at a higher magnification. *C*, *c*, the basal lamina becomes amorphous or absent in wide areas of the intestine in transgenic tadpoles expressing ST3. Furthermore, overexpression of ST3 led to the activation of fibroblasts just beneath the epithelium, as reflected by the presence of well developed rough endoplasmic reticulum in these cells. *c* shows the *boxed region* of *C*, revealing the lack of basal lamina in the ST3 transgenic tadpole. *D*, epithelial cell-fibroblast contacts are present in tadpoles overexpressing ST3 but not in wild type or ST3m transgenic animals at stage 54 (not shown). *E*, the basal lamina (*double-headed arrow*) becomes much thicker at the climax of metamorphosis (stage 61), when the fibroblasts are also activated as shown by the presence of well developed rough endoplasmic reticulum. *F*, epithelial cell-fibroblast contacts are also present, across the thickened basal lamina (*double-headed arrow*) in the intestine during natural metamorphosis (stage 61). *Ep*, epithelium; *Fb*, fibroblast; *bl*, basal lamina; *rER*, rough endoplasmic reticulum. *Bar*, 1 μm.

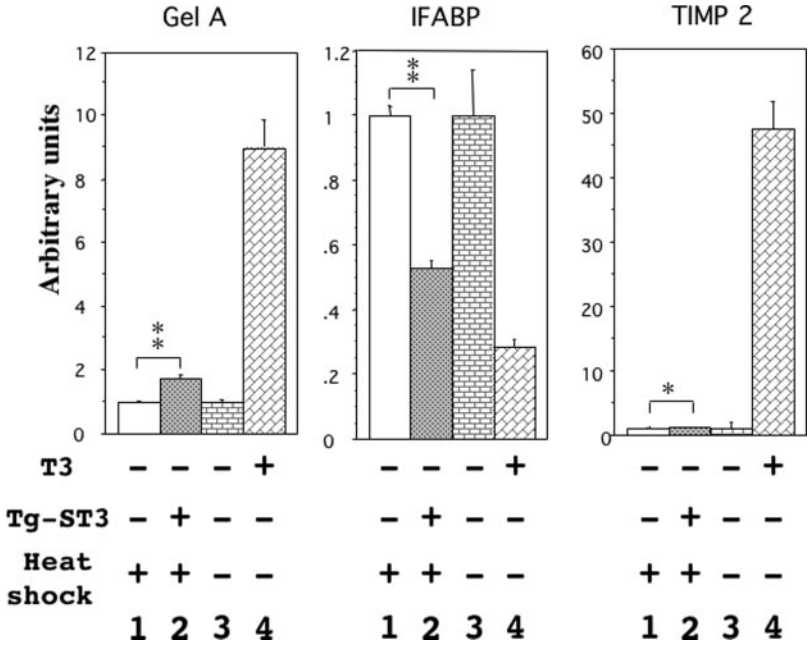

FIG. 8. **Transgenic expression of ST3 results in the regulation of some late T3 response genes in the intestine.** Wild type (*Tg-ST3−*, *columns 1*, *3*, and *4*) and transgenic (*Tg-ST3+*, *column 2*) tadpoles at stage 54 were heat-shocked for 7 days (*columns 1* and *2*) or treated with (*column 4*) or without (*column 3*) 5 nM T3 for 3 days. Total RNA was isolated from the intestine and subjected to quantitative PCR analysis for the expression of the indicated genes. All expression levels were normalized against the control gene rpL8, with the signal from wild type animals without T3 treatment set to 1. Note that GelA was up-regulated, whereas IFABP was down-regulated in transgenic animals (compare *column 2* with *column 1*), similar to that observed during T3 treatment of wild type animals (*columns 3* and *4*). *, $p = 0.94$; **, $p \leq 0.005$.

remodeling by affecting cell-cell interactions. In fact, during both natural and T3-induced metamorphosis, the basal lamina changes from a continuous but thin structure to an amorphous, discontinuous, but thick structure just when the epithelial transformation starts (33). The remodeled basal lamina appears to be more permeable, because extensive direct contacts across the basal lamina exists between the proliferating adult epithelial cells and the underlying fibroblasts, and numerous macrophages migrate across it from the connective tissue to the larval epithelium to participate in the removal of the apoptotic cells (33). Thus, it is quite possible that the remodeling of the basal lamina plays a critical role for cell fate determination and cell behavior during intestinal metamorphosis.

Based on the ability to induce intestinal remodeling in organ cultures, we have previously cultured isolated epithelial or fibroblastic cells from tadpole intestine (56, 57). We showed that T3 could induce the proliferation of both cell types, leading to apoptosis of epithelial cells but not fibroblasts, just like during intestinal metamorphosis *in vivo*. Surprisingly, adult epithelial cells from metamorphosing intestine also undergo apoptosis when treated with T3 in cell cultures (56), suggesting that removing the support from the basal lamina makes the cells vulnerable to T3-induced death. In support of this, when tadpole epithelial cells were cultured on various ECM-coated dishes, the larval epithelial cells became more resistant to T3-induced death (56). Such an effect of ECM on cell fate is similar to that observed for many tissue culture cells *in vitro*, where blocking cell-ECM interactions leads to apoptosis (46, 49, 58, 59). Thus, the remodeling of the basal lamina during intestinal metamorphosis is likely to play a critical role in removal of the larval epithelium and development of the adult epithelium, making this process an excellent model to study the developmental function of ECM remodeling.

ST3 Plays a Role in the Spatial and Temporal Regulation of Cell Death during Intestinal Metamorphosis—ST3 is regulated by T3 directly at the transcriptional level in intestinal fibroblasts and thus is believed to function at early stages of intestinal remodeling (43). Furthermore, ST3 expression is temporally and spatially correlated with apoptosis in different organs during metamorphosis and with the remodeling of the basal lamina in the intestine (30–32). These findings suggest that ST3 may function to remodel the ECM, thereby affecting cell fate and behavior during metamorphosis. In support of this, we have shown earlier in intestinal organ cultures induced to metamorphose with T3 that inhibiting ST3 activity with a function-blocking antibody led to inhibition of not only larval epithelial cell death but also the invasion of the adult epithelial primordia into the underlying connective tissue, a process that is critical for the morphogenesis of the adult epithelium (*i.e.* epithelial fold formation) (34). More importantly, the T3-dependent remodeling of the basal lamina (*i.e.* thickening and folding) was also inhibited by the ST3 antibody, consistent with the model that ST3 may function through ECM remodeling. All of these earlier studies provide strong support for a role of ST3 in intestinal remodeling. This makes intestine an ideal system for *in vivo* studies of ST3 function. In contrast, much less is known about the potential function of ST3 in other organs/tissues during development, and thus it would be difficult to ascertain whether any observed effects from transgenic expression of ST3 in those other organs would be physiologically relevant. For this reason, we focused our studies on the intestine. Our results here for the first time provide *in vivo* evidence complementing the *in vitro* studies to implicate that ST3 alone is sufficient to alter the ECM and induce epithelial cell death *in vivo*. Furthermore, we also observed the activation of the fibroblasts in the connective tissue and cell-cell contacts between epithelial cells and the fibroblasts. All of these also occur during natural and T3-induced intestinal remodeling. These results argue that during metamorphosis, T3 induces the expression of the ST3 gene. ST3, in turn, may cause either directly or indirectly the remodeling of the basal lamina and the alteration of cell-ECM interactions, leading to larval epithelial cell death and fibroblast activation. Furthermore, the observed contact between the activated fibroblasts and larval epithelial cells in transgenic animals in the absence of T3 suggests that such interactions may facilitate the dedifferentiation of larval epithelial cells to become precursors of adult epithelial cells. The origin of adult epithelial cells, which has so far remained unknown (33), is probably the larval epithelium.

It should be pointed that although the transgenic ST3 was expressed mainly in the epithelial cells instead of the fibroblasts as during metamorphosis, this should not affect our conclusion as ST3 is secreted (our earlier studies showed that ST3-GFP was also secreted as expected (36)). More importantly, the effects of the transgenic ST3 that we observed are highly specific and entirely consistent with the potential function of ST3 during metamorphosis based on our earlier expression studies and *in vitro* organ culture studies (*e.g.* inducing cell death in the larval epithelium but not connective tissue). In addition, transgenic overexpression of stromelysin-1 in mouse mammary gland epithelial cells, instead of the fibroblasts as the endogenous gene, also had effects consistent with the predicted function based on expression studies (60–62), again supporting the view that it is not critical for a secreted protease to be expressed in the same cells as the endogenous gene to properly affect target cells.

Ever since the isolation of ST3 gene as a breast cancer-associated gene in humans (63), extensive studies have been carried out to determine its physiological and pathological functions and its biochemical and molecular properties. These studies have shown that ST3 expression is correlated with cell death during development and tissue remodeling and with cancer development (14, 63–66). However, like other MMPs, it has been much more difficult to determine the role of ST3 in these processes. ST3 knock-out mice are apparently normal and fertile, with no observable behavior defects (21). This is probably due to redundancy in the activity of MMP genes in development, a result consistent with weak or absent phenotypes from mice lacking other MMPs (21, 25–27) with the exception of membrane type 1 MMP knockouts (23, 24). In addition, the difficulty of manipulating mammalian embryos also makes it hard to study the roles of MMPs in development in mammals. Our studies here, in conjunction with our earlier work showing the correlation of ST3 expression with larval epithelial cell death and the requirement of ST3 for cell death in organ culture, thus provide the first *in vivo* evidence to implicate a role of ST3 in ECM remodeling and cell fate determination.

Metamorphosis is controlled by T3, which induces the expression of many direct response genes including ST3. The fact that many events induced by T3 can be reproduced simply by expressing ST3 suggests that ST3 is a major player in T3-dependent intestinal remodeling. On the other hand, a few differences do exist when compared with natural or T3-induced intestinal metamorphosis. These include the lack of the proliferation of adult epithelial development (33). In addition, the basal lamina underlying the epithelium of the intestine in transgenic tadpoles expressing ST3 became amorphous or simply absent instead of thicker during natural or T3-induced metamorphosis (33). These differences are also consistent with our analysis of the expression of known T3 response genes in the intestine of transgenic animals,

which suggests that transgenic expression of ST3 alone is able to induce only part of the gene regulation program that is normally controlled by T3. Whereas the underlying molecular mechanisms for these different changes remain to be determined, the results are not surprising, and at least a few factors could contribute to the difference in ECM remodeling. First, *de novo* synthesis of new ECM proteins would increase the basal lamina as seen in T3-induced or natural metamorphosis. Second and perhaps more importantly, the small intestine reduces its length by as much as 10-fold during natural metamorphosis (33). Since the ECM proteins are not totally degraded due to the selective activation of MMP genes (32, 43), the reduction in the length of the intestine would lead to increased thickness of the basal lamina as the intestine contracts. In contrast, in transgenic animals, the transgenic ST3 and/or the resulting up-regulated gelatinase A may lead to direct or indirect degradation of some components of the ECM. In the absence of intestinal contraction or new ECM synthesis, the basal lamina would then become amorphous or lacking in some areas of the intestine.

How ST3 induces ECM remodeling and cell fate changes remains to be determined. As described above, ST3 may cleave some ECM components directly. In addition, ST3 expression causes the up-regulation of some genes known to be activated during natural metamorphosis. In particular, the up-regulation of GelA would contribute to ECM remodeling. Finally, we have recently isolated the 67-kDa laminin receptor as a substrate of ST3 through a yeast two-hybrid screen (67). The laminin receptor is known to facilitate cell binding to laminin, a major component of the basal lamina in the intestine. Thus, ST3 may function by cleaving the laminin receptor. This would alter intestinal epithelial cell-ECM interaction, leading to changes in cell fate and behavior. It is conceivable in the future that by making use of this T3-dependent intestinal remodeling, where the whole organ undergoes drastic remodeling within a short developmental period, we should be able to determine the molecular mechanisms by which ST3 remodels the ECM and regulates cell fate determination through not only identifying genes whose expression is affected by ST3 but also studying the effects of the cleavage of substrates such as laminin receptor by ST3 on intestinal metamorphosis.

Acknowledgment—We thank Dr. Yamaguchi from Dokkyo University School of Medicine for technical help in electron microscopic analyses.

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