DEVELOPMENTAL DYNAMICS 220:155–162 (2001)

BRIEF COMMUNICATION

Embryonic Expression of Type XIX Collagen Is Transient and Confined to Muscle Cells

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ABSTRACT **Type XIX collagen is a poorly characterized extracellular matrix component thought to be involved in the formation of specialized basement membrane zones. Here we examined the developmental expression of the mouse gene (*Col19a1*) by in situ hybridization. *Col19a1* expression during embryogenesis commences at ~E9.5 in the myotome and with a pattern that closely follows the myogenic regulatory factor *myf-5*. Like *myf-5*, *Col19a1* transcription gradually decreases in differentiating skeletal muscle progenitors and concomitantly to increased *myogenin* gene expression. Transient expression of *Col19a1* in muscular tissues is confined to a few sites of the developing embryo, such as limbs, tongue, and the smooth muscle layers of the stomach and esophagus. Additional non-muscular sites of *Col19a1* activity include the skin of the E16.5 embryos and the cerebral cortex and hippocampus of the new born brain. Unlike all other tissues, expression of *Col19a1* in the central nervous system gradually increases after birth.**

Key words: basement membranes; collagen; extracellular matrix; myogenesis; esophageal transdifferentiation

INTRODUCTION

Type XIX is the latest addition to the collagen superfamily of extracellular matrix proteins, whose various members are segregated into different groups according to structural and/or functional considerations (Brown and Timpl, 1995). Originally identified from cDNA cloning experiments, the existence of type XIX collagen was later confirmed by biochemical and immunohistochemical studies (Yoshioka et al., 1992; Myers et al., 1993, 1997; Sumiyoshi et al., 1997). Information regarding the nature and possible function of type XIX collagen aggregates is currently lacking. Structural considerations have placed type XIX collagen among the so-called fibril-associated collagens with interrupted triple helix, or FACIT (types IX, XII, XIV, and XVI) (Yoshioka et al., 1992; Myers et al., 1994; Inoguchi et al., 1995; Khaleduzzaman et al., 1997). Immunohistochemical work, on the other hand, has located type XIX collagen preferentially in basement membrane zones of several organ systems, thus suggesting that it may belong to the multiplexin group (collagens with multiple triple-helix domains and interruptions) (Myers et al., 1997).

Type XIX collagen transcripts have been detected at very low levels in mouse embryonic tissues by the RT-PCR technique, and in human rhabdomyosarcoma cell lines by Northern analysis (Myers et al., 1994; Sumiyoshi et al., 1997). A recent report has raised the intriguing possibility that type XIX collagen may also be involved in the initial stages of skeletal muscle cell differentiation. Human rhadomyosarcoma cells cultured under conditions that stimulate myoblast differentiation were in fact shown to selectively up-regulate the production of skeletal muscle myosin heavy chain and α-actinin, and of type XIX collagen as well (Myers et al., 1999). Unfortunately, the significance of this in vitro observation could not be placed into a physiologic context due to the lack of information about the developmental expression of the type XIX collagen gene. The present study was designed to fill this gap in knowledge, in addition to providing useful information for the future characterization of type XIX collagen (*Col19a1*) null mice.

The results of our in situ hybridizations demonstrate that type XIX collagen is preferentially and transiently expressed in differentiating muscle cells. Embryonic accumulation of *Col19a1* transcripts in myotomes and myotome derivatives closely resembles that of *myf-5*, in that they both decline concomitantly to the increase in *myogenin* gene activity. *Col19a1* is also expressed in some smooth muscle cells, such as those of the devel-

Grant sponsor: NIH; Grant number: AR 38468.
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Received 26 October 2000; Accepted 3 November, 2000

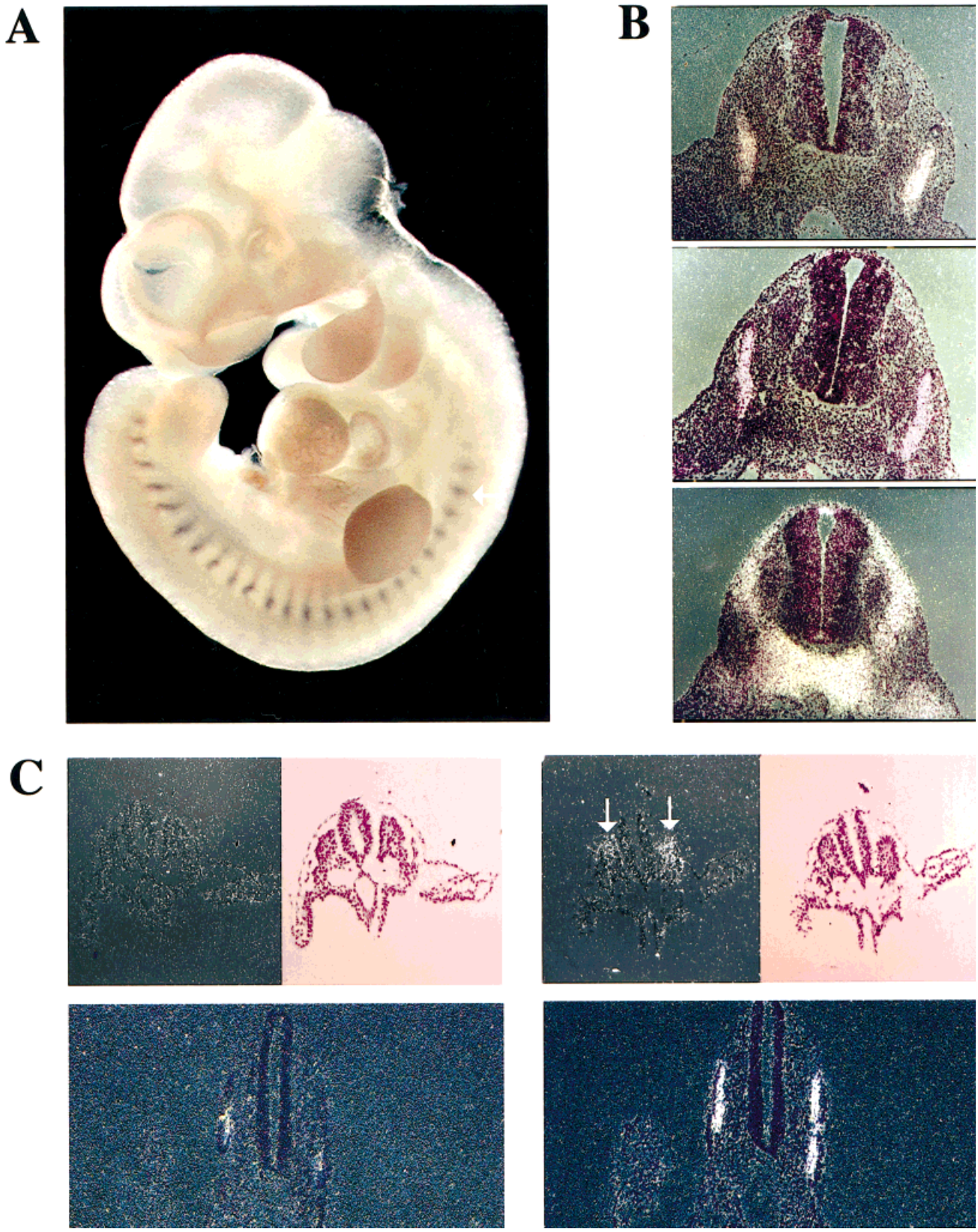

Fig. 1. **A:** Whole-mount in situ hybridization of E10.5 embryo to the *Col19a1* probe. **B:** (Top to bottom) in situ hybridization of cross-sections from a E11.5 embryo to *Col19a1*, *myf-5*, and *Col2a1* probes. **C:** In situ hybridization of cross-sections from E8.5 (top) and E9.5 (bottom) embryos to *Col19a1* (left) or *myf-5* (right) probes. Arrows point to *Col19a1* (A) and *myf-5* expression (C) in the myotome.

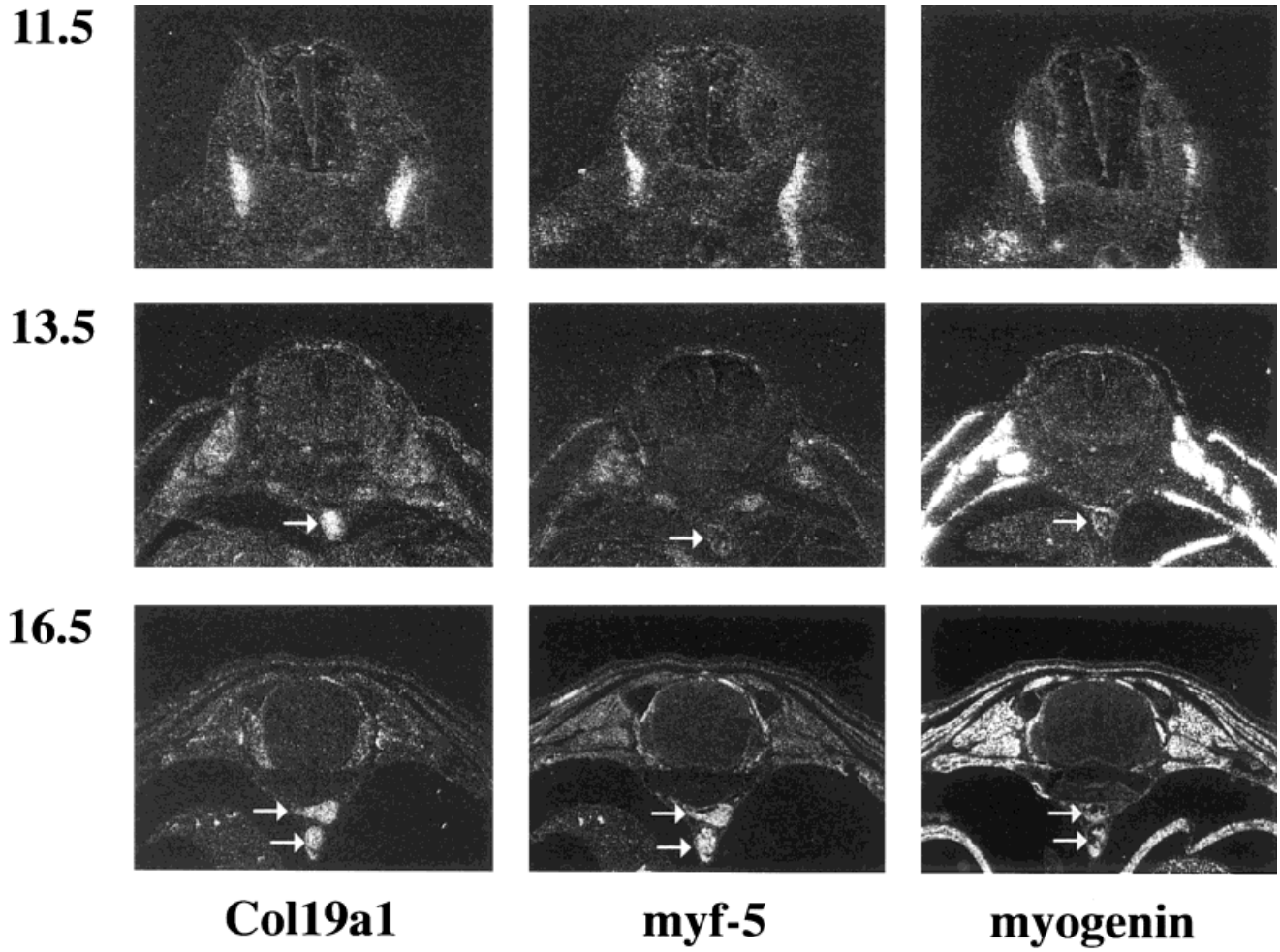

Fig. 2. Serial cross-sections from E11.5, E13.5, and E16.5 mouse embryos hybridized to the *Col19a1*, *myf-5* and *myogenin* probes. Arrows indicate unspecific signal produced by blood cells.

oping stomach and esophagus, as well as in embryonic skin and adult brain.

RESULTS AND DISCUSSION

Whole mount in situ hybridization of mid-gestation (E10.5) embryos identified the strongest *Col19a1* signal in the somites (Fig. 1A). Hybridizations of cross-sections of E11.5 embryos confirmed the restricted distribution of *Col19a1* transcripts in the myotomes, within the broader area of expression of myogenic regulatory factor (MRF) *myf-5* (Smith et al., 1994) and distinct from type II collagen (Fig. 1B). Similarly, hybridizations of E8.5 and E95 embryos documented the later appearance of *Col19a1* transcripts compared to *myf-5* (Fig. 1C). Comparison of cross-sections of E11.5 to E16.5 embryos hybridized to *Col19a1*, *myf-5*, and *myogenin* probes documented the gradual decrease in the intensity of the first two signals, and the steady increase of *myogenin* accumulation in differentiating muscles (Fig. 2). Comparison of sagittal sections of E16.5 embryos confirmed the overall decline of *Col19a1* and *myf-5* gene activity compared to *myogenin* (Fig. 3). The difference in intensity and distribution between the *Col19a1* and *myogenin* signals was most evident in the muscles of the E16.5 limbs and tongue (Fig. 4A,B). *Col19a1* transcripts in the tongue display a characteristic pattern that mirrors the histological organization of this muscle (Fig. 4C).

Col19a1 is also actively transcribed in some smooth muscle cells. Illustrative examples include the outer muscular layer of the developing stomach and the forming aductory muscles around the hair follicles of the jaw of E13.5 and E16.5 embryos, respectively (Fig. 5A,B). Expression in the stomach remains detectable only for a few days after birth (data not shown). *Col19a1* expression was also observed in the post-natal esophagus. Consistent with the transdifferentiation of smooth muscle to skeletal muscle (Patapoutian et al., 1995), we found that rostro-to-caudal progression of *myogenin* expression in the esophagus is completed

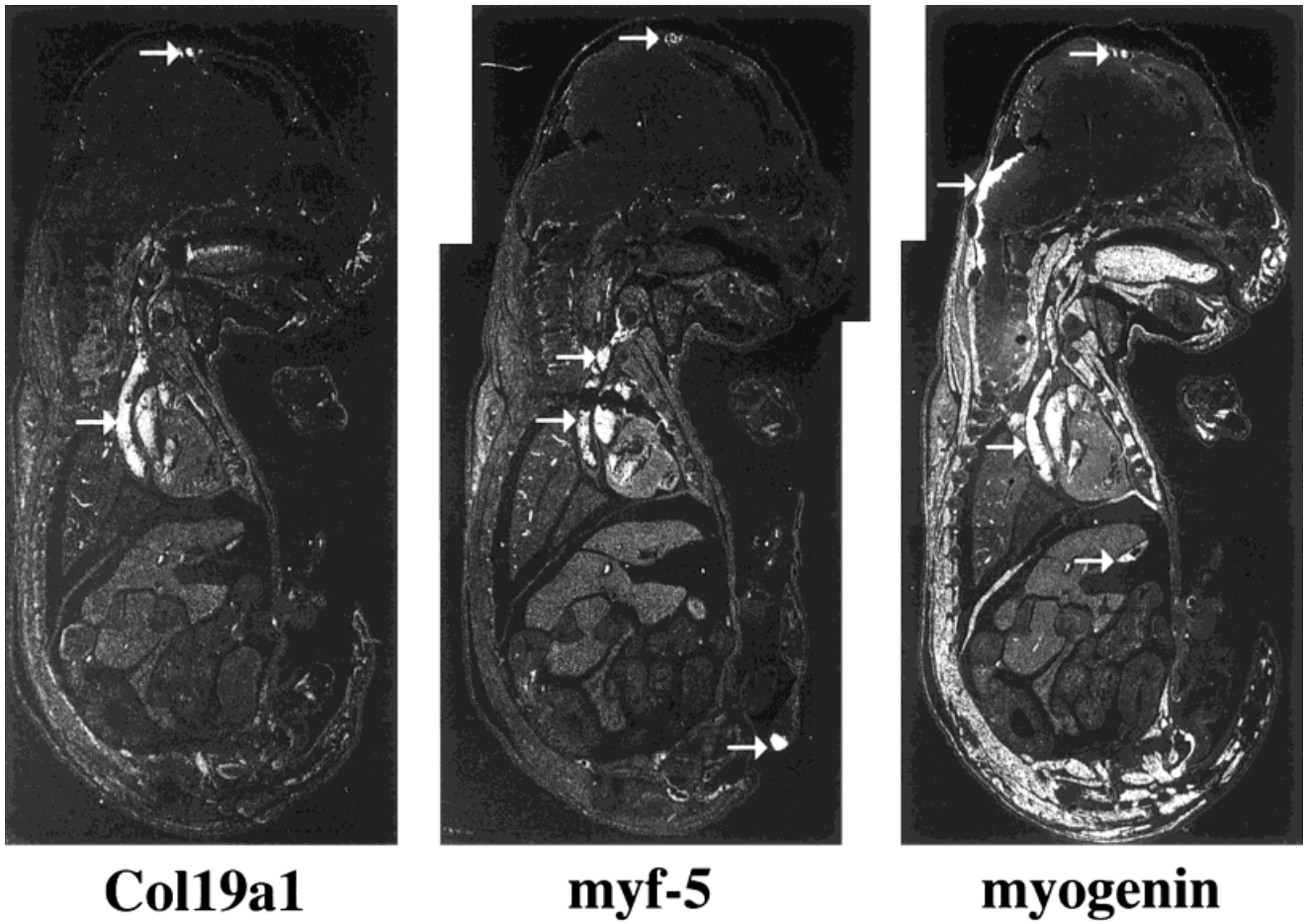

Fig. 3. Serial sagittal sections from an E16.5 mouse embryo hybridized to the *Col19a1*, *myf-5*, and *myogenin* probes. Arrows indicate unspecific signal.

within the first week of post-natal life (Fig. 5C,D). By contrast, *Col19a1* transcripts were only detected in the caudal portion of the esophagus and before onset of *myogenin* expression (Fig. 5E). The region of overlap between the *Col19a1* and *myogenin* hybridizations could be appreciated by comparing the patterns in the abdominal esophagus of P6 and P12 mice, respectively (Fig. 5F). Finally, the analysis failed to detect substantial *Col19a1* accumulation in the smooth muscle cells of the developing intestine, uterus, and blood vessels (data not shown).

The above data demonstrate that type XIX collagen is transiently expressed in muscles, irrespective of the embryonic origin of the cells. There are, however, a few discrepancies between the results of our in situ hybridizations and the immunohistolocalization in human adult tissues (Myers et al., 1997). The most evident of them includes the reported presence of type XIX molecules around all vessels and the stromal smooth muscle cells of the intestine (Myers et al., 1997). The discrepancy may simply reflect differences between the two experimental approaches; alternatively, it may indicate that type XIX collagen deposition in these particular matrices is a post-natal event.

Robust expression of the *Col19a1* gene during the early stages of skeletal muscle cell differentiation contrasts with the reported absence of type XIX proteins in mature muscles (Myers et al., 1997), unless one argues for a transient role of this collagen matrix during myogenesis. For example, activation of type XIX collagen synthesis in proliferating myoblasts soon after induction by MRFs may provide the cells with a competent extracellular environment in support of the differentiation process. Consistent with this hypothesis, onset of *Col19a1* expression was detected after *myf-5*, thus suggesting that this gene may be a downstream target of MRFs. Along these lines, we have identified two potential MRF-binding sites (E-box; Firulli and Olson, 1997) within the 0.7-kb upstream sequence of the *Col19a1* gene, and a cluster of six E-boxes 200 bp downstream of exon 1 (data not shown).

In contrast to the esophageal musculature of the embryo, which is entirely composed of differentiated

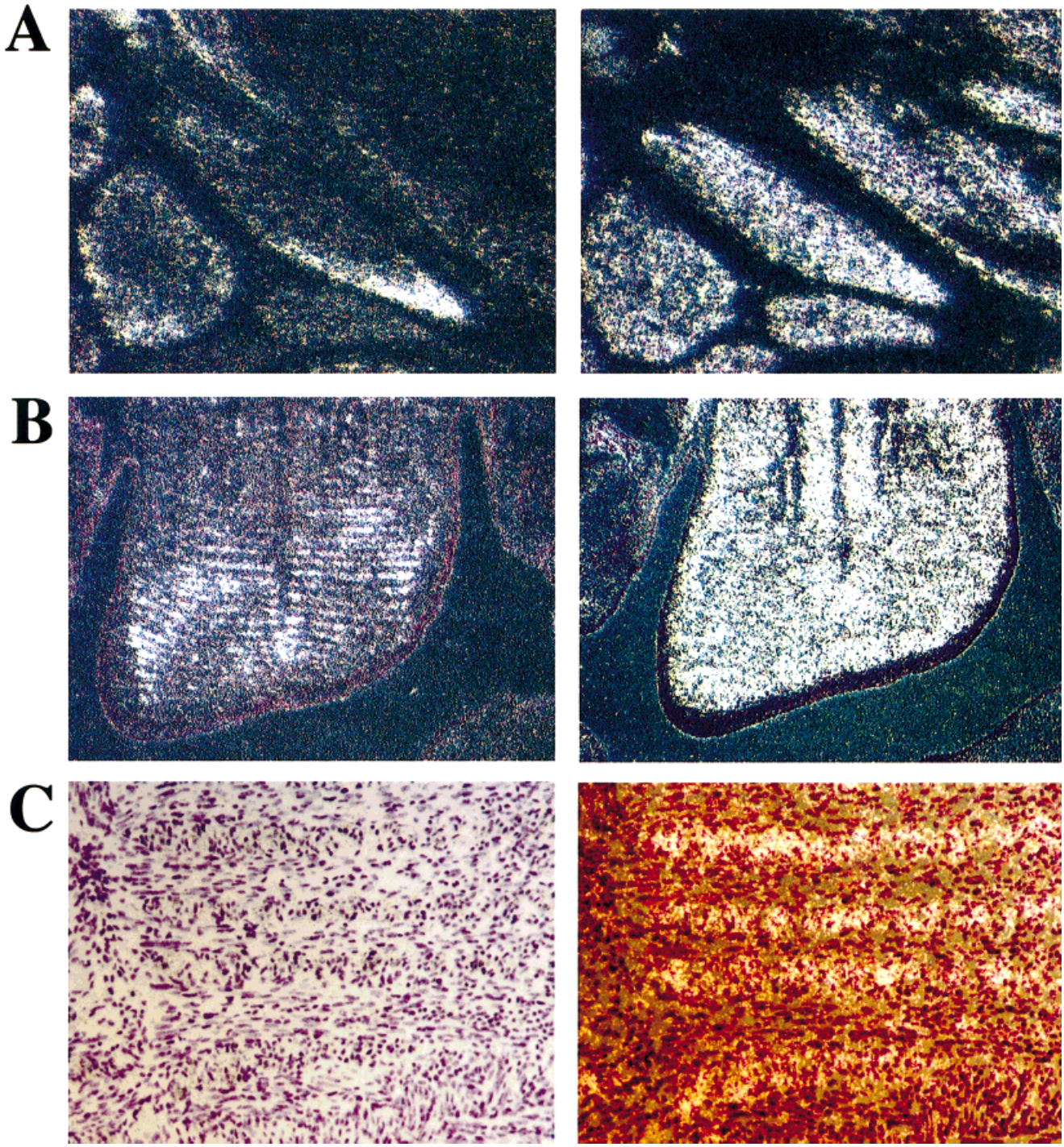

Fig. 4. In situ hybridizations of *Col19a1* (**left**) and *myogenin* (**right**) probes to tissue sections from E16.5 mouse limbs (**A**) and tongue (**B**). Note the distribution of the *Col19a1* signal at the periphery of the skeletal muscles in the limb, and in alternate rows of positive cells in the tongue. The bright (left) and dark (right) field views of the tongue in **C** correlate the *Col19a1* signal to a specific arrangement of muscle cells.

smooth muscle, the adult layer is made of striated muscle as a result of the conversion of smooth muscle to skeletal muscle (Patapoutian et al., 1995). *Col19a1* expression is restricted to the muscular layer of diaphragm-level esophagus and appears prior to *myogenin* expression and, thus, before overt muscle transdifferentiation. It is tempting to speculate that an extracellular matrix rich in type XIX collagen may endow spe-

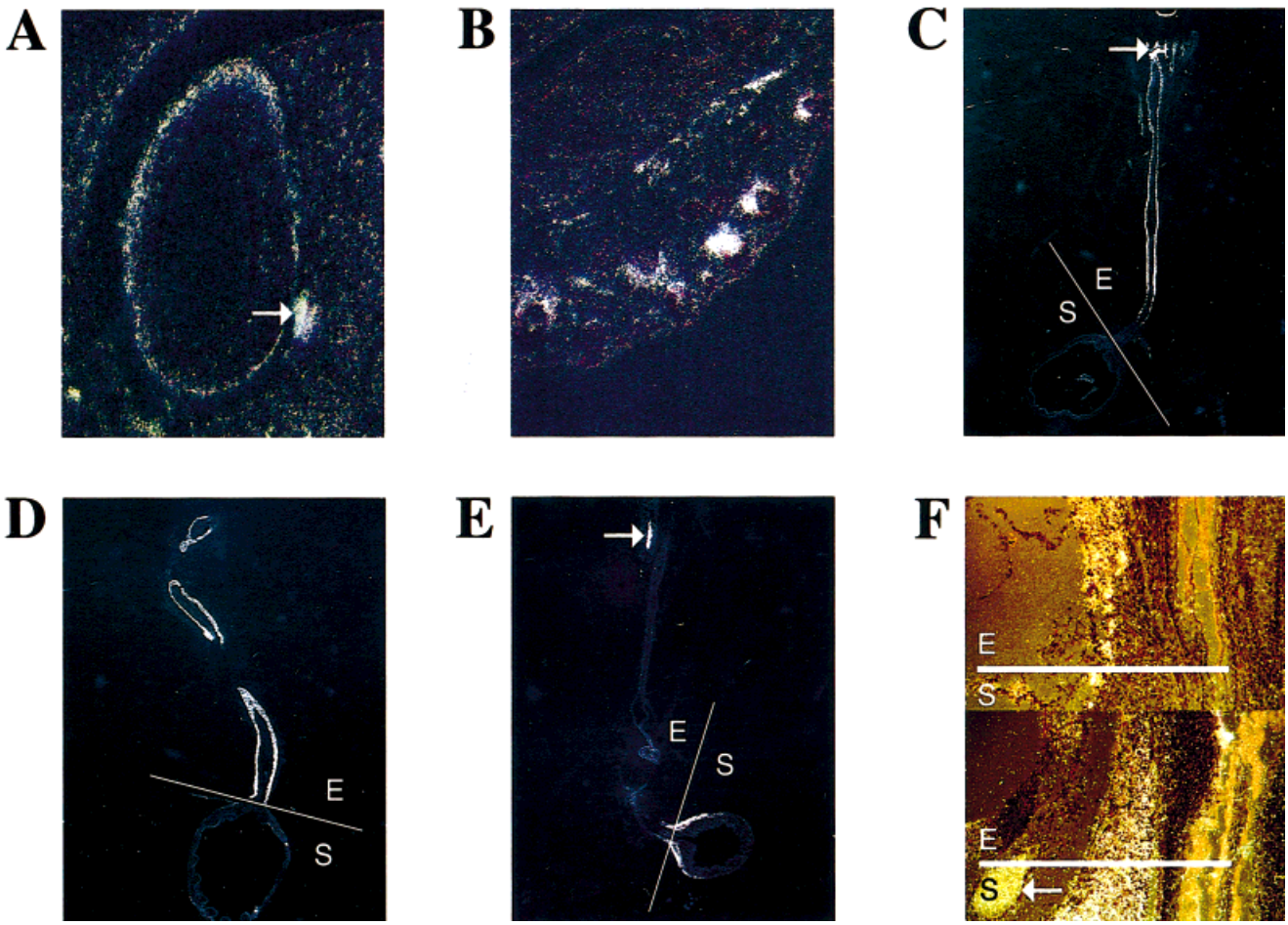

Fig. 5. *Col19a1* hybridization in the forming stomach (**A**) of the E13.5 embryo and in the jaw (**B**) of the E16.5 embryo. **C–E:** Hybridization of *myogenin* (C and D) and *Col19a1* (E) probes in longitudinal sections of the esophagus and stomach of 2- (C and E) and 6-day-old (D) mice.Letters indicate esophagus (E) and stomach (S). **F:** Closer view of the overlapping zone of hybridization of *myogenin* (top) and *Col19a1* (bottom) in the abdominal sections of the esophagus from P16 and P12 mice, respectively. Arrows indicate unspecific signal.

cialized information to the transdifferentiating cells of the abdominal esophagus.

Our analysis also documented the presence of low amounts of *Col19a1* transcripts in a few non-muscular tissues, such as skin and brain (Fig 6). *Col19a1* mRNA accumulation in the skin is maximal between E11.5 and E13.5 (Fig. 6A) and later decreases to undetectable levels (data not shown). Expression in the nervous system is clearly seen at the base of the forebrain of the P12 mouse (Fig. 6B). *Col19a1* signals are still high in the 5-week-old mouse and mostly confined in the gyrus dentatus of the hippocampus (Fig. 6C). Northern analysis of *Col19a1* mRNA accumulation in the brain during the first 2 weeks of post-natal life documented the gradual increase in mRNA accumulation from P0 to P12 (Fig. 6D). *Col19a1* expression in the skin is consistent with the immunolocalization of the protein at the dermal-epidermal junction of adult human skin (Myers et al., 1997).

In conclusion, the results of the present study are in overall agreement with the previous notion that type XIX collagen is a minorly expressed embryonic gene product (Sumiyoshi et al., 1997; Myers et al., 1997). The major new finding reported here is the discovery that expression of *Col19a1* during embryonic development is transient and confined to muscle cells. Unfortunately, there are no available antibodies against mouse type XIX collagen that could correlate the mRNA data at the protein level. With this limitation in mind, we note that *Col19a1* mRNA accumulation in muscles is at least consistent with the hypothesis that type XIX collagen-rich matrices may play specialized roles during morphogenesis. The postulate is based on the finding that transient *Col19a1* expression includes myoblasts that are undergoing differentiation; a specific organization of cells in the tongue; and the abdominal portion of the esophagus prior to muscle transdifferentiation. Ongoing generation of *Col19a1* null mice will ultimately test the validity of our hypothesis, and firmly establish the contribution of type XIX collagen to the assembly and function of the vertebrate connective tissue.

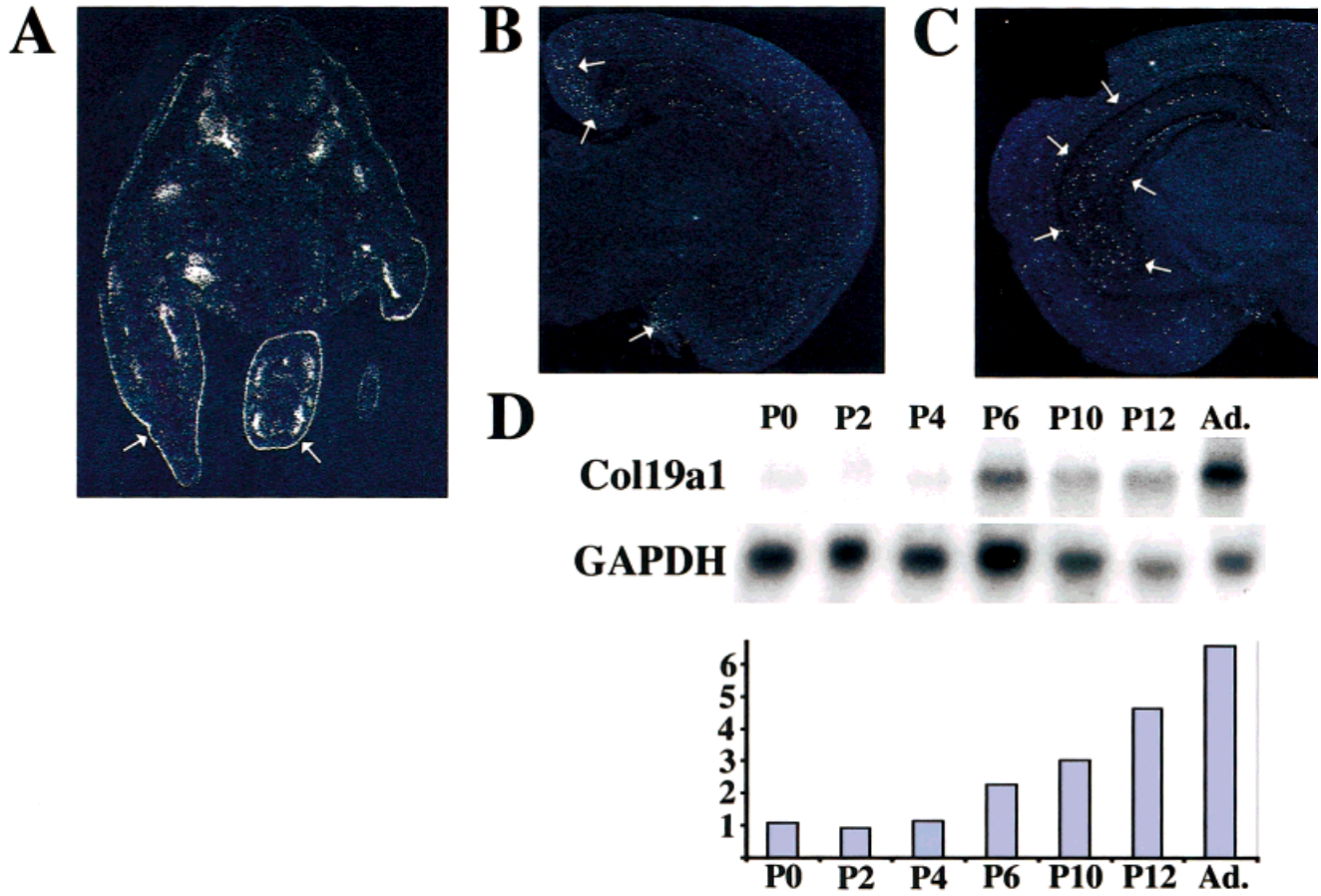

Fig. 6. **A:** *Col19a1* hybridization of a cross-section at the hind limb level of a E13.5 embryo showing an intense positive signal in the skin (arrows). **B,C:** *Col19a1* hybridization in the P12 (B) and 5-week-old (C) forebrain showing a diffuse signal in the former (arrows), which becomes gradually restricted to the hippocampal region of the latter (arrows). **D:** Northern blot hybridizations to *Col19a1* (**top**) and GAPDH (**bottom**) probes documenting the gradual increase of the *Col19a1* mRNA during the first 2 weeks of post-natal (PO-P12) life and in the 2-month-old mouse (Ad). **Bottom:** Graphic representation of *Col19a1* expression in the PO to P12 brain relative to the GAPDH signal.

EXPERIMENTAL PROCEDURES

In Situ and Northern Hybridizations

Radiolabeled ~500 bp long cDNAs for the 5′ untranslated regions *Col19a1* and *Col2a1* were used for in situ hybridization along with similar probes for mouse *myf-5* and *myogenin* (Wright et al., 1989; Ott et al., 1991; Andrikopoulos et al., 1992; Sumiyoshi et al., 1997). The MRF cDNAs were generously provided Dr. D. Sassoon, Mount Sinai School of Medicine, New York. After linearization at appropriate restriction sites, sense and antisense probes were generated by in vitro transcription with T3 and T7 polymerases in the presence of [^{35}S]-UTP. In situ hybridizations were performed essentially as previously described (Wright et al., 1989; Andrikopoulos et al., 1992) and slides were exposed at 4°C for 5–8 days. Total RNA purification from staged mouse embryos and Northern blot hybridizations were performed as previously described (Sumiyoshi et al., 1997). The relative intensity of the hybridizing bands was quantified by the public domain NIH Image program (http://rsb.info.nih.gov//nih.image).

ACKNOWLEDGMENTS

The authors thank Dr. David Sassoon for suggestions and reagents and Ms. Karen Johnson for typing the manuscript.

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