Scatter Factor/Hepatocyte Growth Factor and Its Receptor, the c-met Tyrosine Kinase, Can Mediate a Signal Exchange between Mesenchyme and Epithelia during Mouse Development

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Abstract. Scatter factor/hepatocyte growth factor (SF/HGF) has potent motogenic, mitogenic, and morphogenetic activities on epithelial cells in vitro. The cell surface receptor for this factor was recently identified: it is the product of the c-*met* protooncogene, a receptor-type tyrosine kinase. We report here the novel and distinct expression patterns of SF/HGF and its receptor during mouse development, which was determined by a combination of in situ hybridization and RNase protection experiments. Predominantly, we detect transcripts of c-*met* in epithelial cells of various developing organs, whereas the ligand is expressed in distinct mesenchymal cells in close vicinity. In addition, transient SF/HGF and c-*met* expression is found at certain sites of muscle formation; transient expression of the c-*met* gene is also detected in developing motoneurons. SF/HGF and the c-met receptor might thus play multiple developmental roles, most notably, mediate a signal given by mesenchyme and received by epithelial. Mesenchymal signals are known to govern differentiation and morphogenesis of many epithelia, but the molecular nature of the signals has remained poorly understood. Therefore, the known biological activities of SF/HGF in vitro and the embryonal expression pattern reported here indicate that this mesenchymal factor can transmit morphogenetic signals in epithelial development and suggest a molecular mechanism for mesenchymal epithelial interactions.

RECEPTOR-type tyrosine kinases and their specific ligands are essential components of intercellular signaling pathways used for the control of growth and differentiation as well as the maintenance of normal homeostasis (Ullrich and Schlessinger, 1990). In addition, evidence has accumulated that receptor tyrosine kinases can regulate morphogenic and differentiation events during development (Basler and Hafen, 1988; Chabot et al., 1988; Geisler et al., 1988; Sprenger et al., 1989; Sonnenberg et al., 1991). The important role of receptor tyrosine kinases is also emphasized by the fact that many genes encoding such proteins were identified by their oncogenic potential when mutated (Yamamoto et al., 1983; Hampe et al., 1984; Neckameyer and Wang, 1985; Bargmann et al., 1986; Birchmeier et all., 1986; Martin-Zanca et al., 1986), among them c-*met* (Cooper et al., 1984). The *met* oncogene was originally isolated by the use of the NIH3T3 transfection assay (Blair et al., 1982; Cooper et al., 1984). It is the product of a chromosomal rearrangement which fused c-*met* coding sequences of human chromosome 7 to the translocated promoter region (*tpr*)[1] locus of chromosome 1 (Park et al., 1986; Dean et al., 1987). Similar translocations between c-*met* and tpr are frequently present in human gastric carcinomas (Soman et al., 1991). The c-*met* protooncogene encodes a transmembrane glycoprotein of 190 kD, which is cleaved posttranslationally into two chains of 145 (β) and 50 kD (α). The COOH-terminal part of the β chain contains the tyrosine kinase domain and is located cytoplasmatically, whereas the NH_2-terminal part of the β and the entire α chain are exposed on the cell surface (Gonzatti-Haces et al., 1988; Giordano et al., 1989).

Recently, the ligand of the c-met receptor has been identified (Bottaro et al., 1991; Naldini et al., 1991*a*,*b*): it is scatter factor/hepatocyte growth factor (SF/HGF), a protein with structural similarities to plasminogen. SF/HGF contains four characteristic kringle domains at the NH_2 terminus and a COOH-terminal, serine protease-like domain. However, amino acids in the active site which are essential for the catalytic activities of serine proteases are altered in SF/HGF (Miyazawa et al., 1989; Nakamura et al., 1989; Weidner et al., 1991). Early studies on SF/HGF indicate that it acts in a paracrine fashion, since many fibroblast cells derived from the mesenchyme secrete SF/HGF, whereas cells of epithelial origin respond to it (Stoker and Perryman, 1985; Stoker et al., 1987; Gherardi et al., 1989). Two differ-

1. *Abbreviations used in this paper*: coxl, cytochrome oxidase; SF/HGF, scatter factor/hepatocyte growth factor; tpr, translocated promoter region.

ent biological activities, the ability to induce either cellular growth or movement, have been used to independently isolate and molecularly characterize the factor (Gherardi et al., 1989; Miyazawa et al., 1989; Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989; Weidner et al., 1990, 1991; Rosen et al., 1990; Rubin et al., 1991). Besides mitogenic and motogenic activities, SF/HGF also increases invasiveness of epithelial cells and can act as a cytostatic factor on various carcinoma cells (Weidner et al., 1990; Higashio et al., 1990; Shiota et al., 1992). In addition, a morphogenetic activity was recently discovered: MDCK epithelial cells are induced to form tubular structures when cultured in a three-dimensional collagen matrix in the presence of SF/HGF (Montesano et al., 1991*a*,*b*; Weidner et al., 1993*a*). Experiments with hybrid receptors demonstrate that the various biological responses to SF/HGF are all transduced by the c-met receptor tyrosine kinase (Weidner et al., 1993*b*).

To investigate the potential role of SF/HGF and its receptor in developmental processes, we have studied the expression of the genes encoding these two proteins during mouse embryogenesis. By in situ hybridization analysis, we find a recurring pattern of expression during development of various epithelial organs: c-*met* transcripts in specific epithelial cells and expression of the SF/HGF gene in mesenchymal cells in close vicinity. This points towards a paracrine mode of SF/HGF action and indicates a function in mesenchymal epithelial interactions. Such interactions are known to govern morphogenesis and differentiation of embryonal epithelia (Kratochwil, 1983). In addition to this distinct pattern, we find transcripts of both genes also in neuronal cells and a transient expression in developing muscles. SF/HGF and the c-met receptor could thus mediate several effects during development, in particular a morphogenetic signal exchange between mesenchyme and epithelia.

Materials and Methods

cDNA Cloning and Analysis

Mouse c-*met* and SF/HGF cDNA clones were isolated from a cDNA library synthesized from adult liver RNA (ML 1035b, obtained from Clonetech, Heidelberg, Germany) by standard techniques (Maniatis et al., 1982). For the screening, the entire coding sequence of the human c-*met* (Park et al., 1987) and SF/HGF cDNA (Weidner et al., 1991) were used as probes. The isolated murine cDNA clones were characterized by sequence analysis. Comparison of the deduced amino acid sequences revealed 100% identity between our c-*met* isolate and the previously reported sequence for mouse c-*met* (Iyer et al., 1990; Chan et al., 1988) and 90% identity between our murine isolate and the previously reported human SF/HGF cDNA (Weidner et al., 1991).

RNA Isolation and RNase Protection Assays

Various tissues from embryonal or adult mice (strain NMRI, obtained from the Zentralinstitut für Versuchstierzucht, Hannover, FRG) were dissected and shock frozen in liquid nitrogen. For determination of the age of the embryos, the morning after vaginal plug formation was considered as day 0 of embryonic development. Total RNA of the different tissues was prepared as described (Auffray and Rougeon, 1980).

RNase protection experiments were performed essentially as described (Birchmeier et al., 1987). Probes were synthesized as run-off transcripts from subclones of cDNAs in pBluescript with T7 RNA polymerase. [^{32}P]UTP with a specific activity of 400 Ci/mmol for c-*met* and SF/HGF and of 0.8–40 Ci/mmol for coxI transcripts were used (0.8 Ci/mmol for the experiments shown in Fig. 2). Probes were as follows: a 449-nucleotide transcript containing 126 nucleotides of murine SF/HGF cDNA (mSF4, see Fig. 1) in antisense orientation (corresponding to position 467–593 in the human cDNA sequence) and additional 323 nucleotides of plasmid derived sequences; a 471-nucleotide transcript containing 137 nucleotides of c-*met* cDNA (mMet2, see Fig. 1) in antisense orientation (position 3867–4004 in the murine cDNA) and 334 nucleotides of plasmid derived sequence; a 426 nucleotide transcript containing 94 nucleotides of cytochrome oxidase I (coxI) sequence in antisense orientation (position 6624–6717 in mitochondrial DNA; Bibb et al., 1981) and 332 nucleotides of plasmid derived sequence. To control absolute amounts of RNA used for the RNase protection experiment, a mixture of c-*met* or SF/HGF probe (50,000 cpm) and coxI probe (4,000–50,000 cpm; 4,000 cpm for the experiment shown in Fig. 2) were hybridized overnight to 100 μg of RNA from various tissues, digested with 30 U of RNase T2 and analyzed on denaturing acrylamide gels.

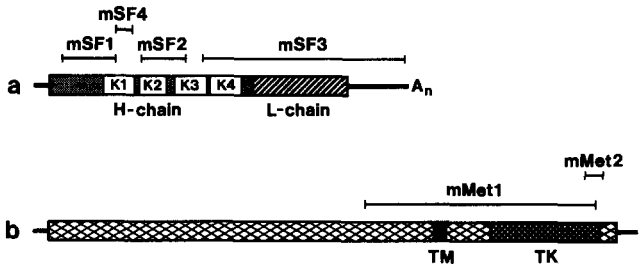

Figure 1. Schematic representation of SF/HGF and c-met cDNAs. The structure of SF/HGF (*a*) and c-*met* (*b*) cDNAs is shown. The translated sequences are indicated by boxes: the sequences encoding subdomains of SF/HGF, heavy (*H*) and light (*L*) chains and the four kringle domains (K_1-K_4) as well as subdomains in the c-*met* receptor, transmembrane domain (*TM*) and tyrosine specific protein kinase domain (*TK*), are marked. The murine cDNA subclones used for in situ hybridization (*mSF1*, *mSF2*, *mSF3*, *mMet1*) or RNase protection experiments (*mSF4*, *mMet2*) are indicated above.

Table I. Expression of the SF/HGF and c-met Genes in Various Murine Tissues

	Tissues	c-*met*	SF/HGF
Kidney	E14	+	+
	E16	+	+
	E18	+	+
	P3	+	+
	adult	+	+
Intestine	E14	+	+
	E16	+	+
	E18	+	+
Lung	E14	+	+
	E16	+	+
	E18	+	+
Liver	adult	+	+
Pancreas	E18	+	+
Stomach	E18	+	+
Muscle	E18	+	+
	adult	±	−
Total embryo	E10	+	+
ES-cells		±	−

RNA from the indicated sources was tested for SF/HGF and c-*met* expression by RNase protection with specific antisense probes. Expression of the *coxI* gene was used as internal standard (see Materials and Methods). Expression levels were determined by comparing relative signal intensities obtained with RNA from various organs and adult liver RNA; (+) denotes a strong and (±) a very weak signal; (−) denotes the absence of a specifically protected band.

In Situ Hybridization Analysis

In situ hybridization was performed as described previously (Sonnenberg et al., 1991). Probes were as follows: murine c-*met* cDNA (mMet1, see Fig. 1, corresponding to position 2298–3974 in the murine cDNA; Iyer et al., 1990; Chan et al., 1988); murine SF/HGF cDNA (mSF1, mSF2, mSF3, see Fig. 1, corresponding to positions 75–465, 676–975, and 1104–2634 in the human cDNA; Weidner et al., 1991); rat myogenin cDNA (position 791–1486; Sassoon et al., 1989). Antisense and sense RNA probes were synthesized as run-off transcripts from subclones in pBluescript with either T7 or T3 RNA polymerase and ^{35}S-labeled UTP and CTP with a specific activity of ≥ 1,000 Ci/mmol. The concentration of probes used for hybridization was 100,000 cpm/μl for c-*met* and SF/HGF and 40,000 cpm/μl for myogenin. No specific signals were observed when transcripts in sense orientation were used for hybridization.

Results

To obtain homologous probes for the analysis of SF/HGF and c-*met* expression, murine cDNAs were isolated from a cDNA library made from adult liver RNA (see Materials and Methods); this organ is known to express both, c-*met* and SF/HGF (Noji et al., 1990; Di Renzo et al., 1991; Schirmacher et al., 1992). The structure of the cDNA clones selected for in situ hybridization and RNase protection analysis are schematically shown in Fig. 1. Transcripts of the SF/HGF gene are detected in a wide variety of embryonal tissues by RNase protection experiments (Table I and Fig. 2 *A*). For example, embryonal lung, pancreas, kidney, and intestine express the SF/HGF gene; the transcript levels are low in most of the tested organs (Fig. 2 *A*). Transcripts of the c-*met* gene can be identified by RNase protection in a wide variety of embryonal tissues (Table I), as observed previously also by Northern hybridization (Iyer et al., 1990). For example, embryonal lung, pancreas, salivary gland, kidney, intestine, and muscle express the c-*met* gene (Fig. 2 *B*). Transcripts of c-*met* are also found at various developmental stages, for example during the entire development of the kidney (Table I).

To further analyze the expression patterns of the c-*met* and SF/HGF genes, in situ hybridization analysis was performed. This revealed that the genes for SF/HGF and the c-met receptor are expressed in mesenchymal and epithelial cells in a variety of parenchymal organs during development, which is shown in more detail below.

Expression of c-met in Tubular Epithelia

Lung, Pancreas, and Salivary Gland. Two major cell types, mesenchyme and epithelia, contribute to the formation of the lung, pancreas, and submandibular salivary gland. Outgrowth of epithelial cells from the endoderm into mesenchyme form the anlagen of these organs. All epithelia develop by further growth, branching, and differentiation from the first endodermal buds, a process which is dependent on mesenchymal signals (Fell and Grobstein, 1968; Lawson, 1972; Masters, 1976). We detect c-*met* transcripts in all of the developing epithelia of these organs (Fig. 3, *A*, *C*, *E*, and *G*, and data not shown). The presence of c-*met* specific transcripts was verified by RNase protection experiments (Fig. 2). The presence of transcripts for SF/HGF was demonstrated in the surrounding mesenchyme and again could be confirmed by RNase protection experiments (Fig. 3, *B*, *D*, F, and *H* and Fig. 2).

Meta- and Mesonephric Kidney. Development of the metanephric kidney starts on day 11 of embryogenesis (E11), when the ureter, a derivative of the Wolffian duct, reaches the nephrogenic mesenchyme. The ureter grows, branches, and differentiates in response to signals given by the mesenchyme, and inductive signals given by the ureter lead to conversion of mesenchyme into new epithelia, which differentiate into proximal and distal tubules as well as glomeruli (Saxen, 1987; Ekblom, 1991). During the entire develop-

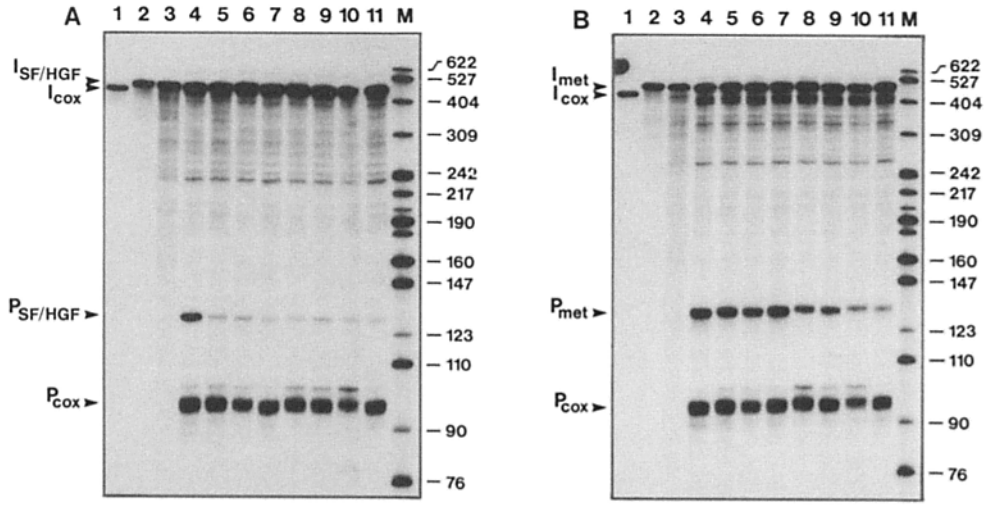

Figure 2. RNase protection analysis of SF/HGF and c-*met* expression in embryonal mouse tissues. Total RNA (100 μg) was analyzed by hybridization to ^{32}P-labeled run-off transcripts from murine SF/HGF (*A*) and c-*met* (*B*) cDNA as described in Materials and Methods. As an internal control, transcripts from *coxI* DNA in antisense orientation were mixed into the hybridization reaction. End-labeled *MspI* cut pBR322 DNA (*M*) and aliquots of the undigested probes (*A*, lane *1*, *coxI* probe; lane *2*, SF/HGF probe; *B*, lane *1*, *coxI* probe; lane *2*, c-*met* probe) were run in parallel. Tissue sources of RNA used for hybridization in *A* and *B*: lanes *3*, t-RNA from yeast; lanes *4*, adult liver; lanes *5*, embryonal (E18) intestine; lanes *6*, embryonal (E18) lung; lanes *7*, embryonal (E18) kidney; lanes *8*, embryonal (E18) submandibular salivary gland; lanes *9*, embryonal (E18) stomach; lanes *10*, embryonal (E18) pancreas; lanes *11*, embryonal (E18) muscle. The size of the marker fragments are indicated in basepairs. The arrows indicate the undigested RNA probes ($I_{SF/HGF}$: SF/HGF probe; I_{cox}: *coxI* probe; I_{met}: c-*met* probe) and the specifically protected fragments ($P_{SF/HGF}$: protected fragment of SF/HGF probe; P_{cox}: protected fragment of *coxI* probe; P_{met}: protected fragment of c-*met* probe).

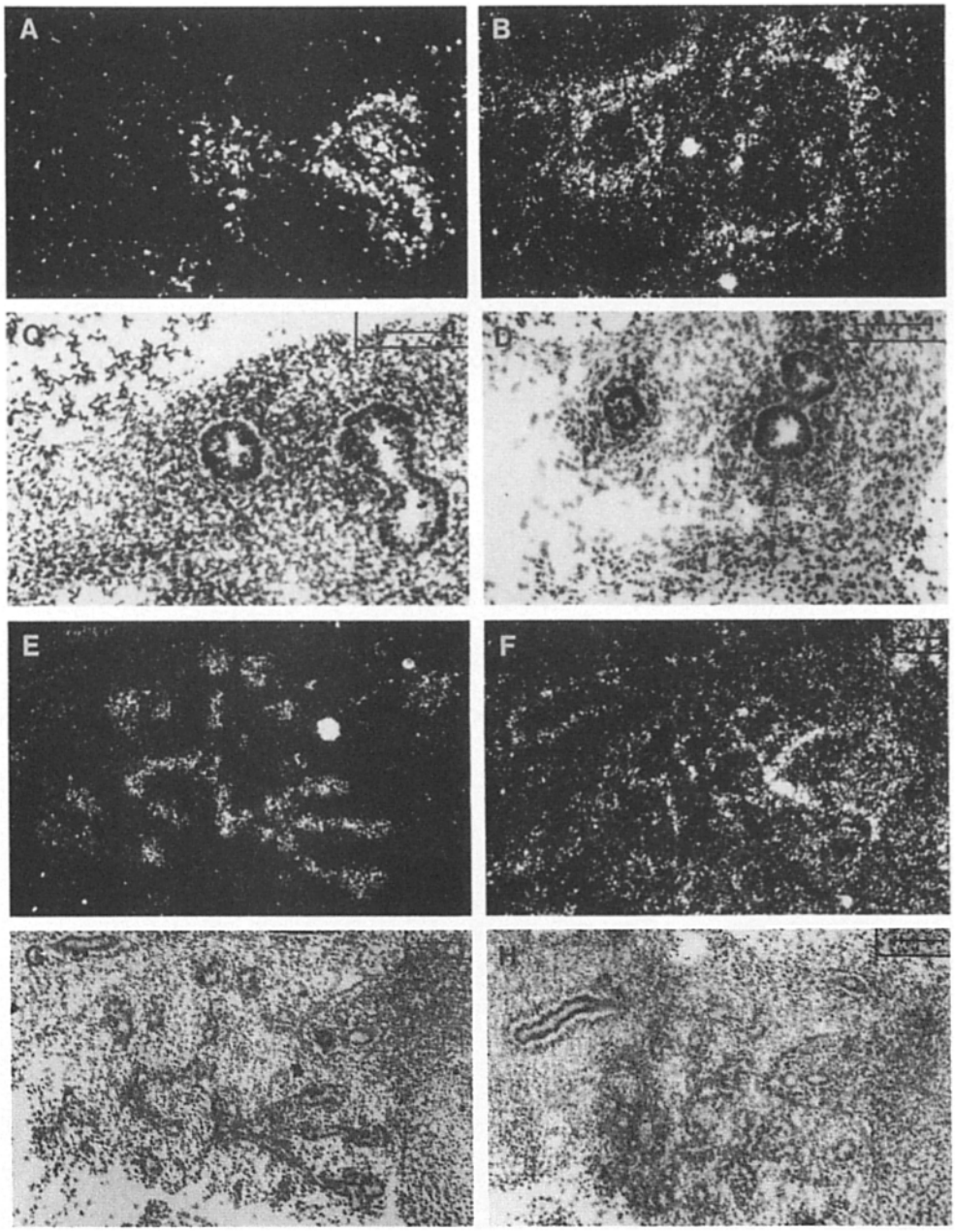

Figure 3. In situ hybridization analysis of SF/HGF and c-*met* expression in the developing lung and pancreas. Sections of embryos on day 13 of development were hybridized to c-*met* (*A* and *E*) or SF/HGF (*B* and *F*) probes. The developing lung is shown in darkfield (*A* and *B*) or brightfield (*C* and *D*) and the developing pancreas in darkfield (*E* and *F*) and brightfield (*G* and *H*). Bars, 100 μm.

ment of the kidney, we observe c-*met* transcripts in all tubular epithelia, i.e., the ureter as well as proximal and distal tubules (Fig. 4, *A*, *E*, and *G*). In contrast, no c-*met* specific transcripts are not found in the glomerular epithelium (Fig. 4 *G*). Concomitantly, SF/HGF expression is detected in the nephrogenic mesenchyme (Fig. 4 *C*). In early stages of kidney development, SF/HGF transcripts are present in the entire mesenchyme (Fig. 4 *C*); at later stages, transcripts are confined to the cortex of the kidney (data not shown). Since cells of all lineages located in the medulla correspond to later differentiation stages than cells in the cortex (Saxon, 1987), this finding indicates that mainly undifferentiated mesenchyme expresses SF/HGF. RNase protection also demonstrated the presence of specific SF/HGF and c-*met* transcripts in the developing kidney (Fig. 2). During development and regression of the mesonephros, we observe c-*met* transcripts also in tubular epithelia and the Wolffian duct, whereas SF/HGF transcripts are present in the cortical region of the nephrogenic mesenchyme (data not shown).

Expression of c-met in Non-tubular Epithelia

Digestive Tract. The intestinal anlagen are formed early in development, and consist then of a stratified, poorly differentiated epithelium which is surrounded by mesenchymal cells. Terminal differentiation is initiated on E15, when the tight cellular contacts in the epithelial layers start to dissociate and mesenchymal cells are observed to invade the stratified epithelium. This process culminates in the formation of the single layered columnar epithelium of the villi

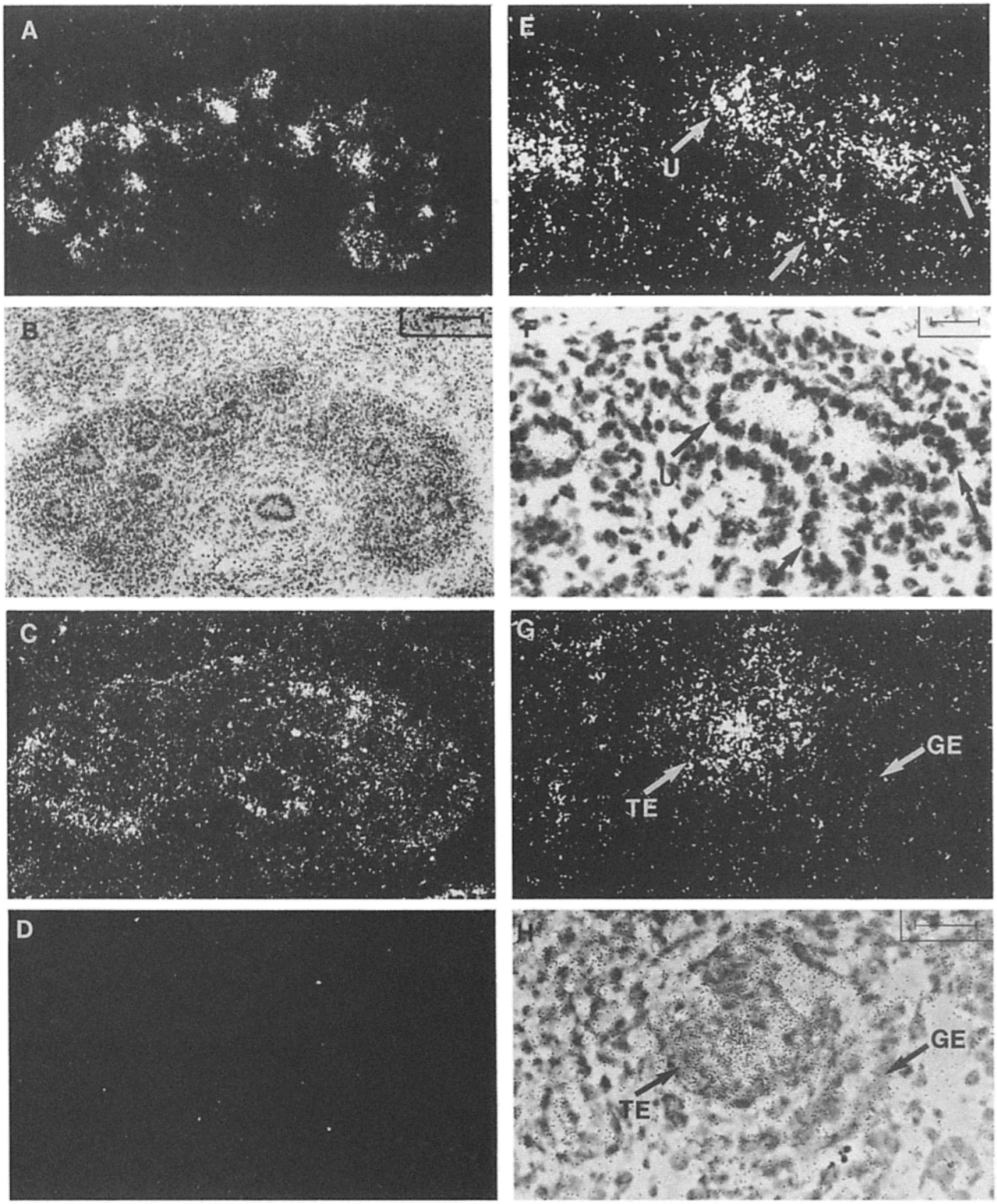

Figure 4. In situ hybridization analysis of SF/HGF and c-*met* expression in the embryonal kidney. Sections through an embryo on day 14 of development were hybridized to c-*met* (*A*, *E*, and *G*), SF/HGF (*C*) and control (*D*) probes. Consecutive sections of the developing kidney are shown in darkfield (*A*, *C*, and *D*) and brightfield (*B*). Higher magnification (*E*, *F*, *G*, and *H*) of the developing kidney. A c-*met* specific hybridization signal over a branching ureter bud (*U*) is indicated by arrows (*E* and *F*). A S-shaped body showing a strong c-*met* hybridization signal in the developing tubular (*TE*) but not the glomerular epithelia (*GE*) is indicated by arrows (*G* and *H*). Bars: (*B*) 100 μm; (*F* and *H*) 25 μm.

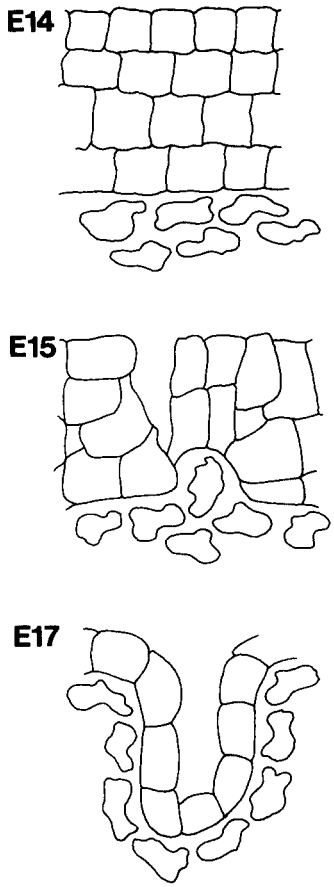

Figure 5. Schematic representation of the intestine at different developmental stages. The appearance of the murine intestine at the indicated times in development is depicted schematically. The cuboidal cells and the irregularly shaped cells represent epithelia and mesenchyme, respectively (modified after Mathan et al., 1976).

(Fig. 5 and Mathan et al., 1976). Transcripts for the c-met receptor are found in the epithelia of the intestinal anlagen and the villi, i.e., during the entire development of the mouse (Fig. 6, *A*, *B* and *E*, *F*). However, the distribution of SF/HGF transcripts shows dynamic changes during development: we detect a faint positive hybridization signal on E14 which is located in a narrow layer of mesenchymal cells around the stratified epithelium (data not shown). When the cell contacts of the epithelia have loosened and the mesenchyme has started to invade the epithelial cell layers on E15, a strong hybridization signal is found in patches of mesenchymal cells in close vicinity to the epithelia (Fig. 6, *B–D* and *H*). These correspond to mesenchymal cells which are growing into the epithelial cell layers. On E17, when the one layered villi epithelium has already formed, specific transcripts are observed in patches of mesenchyme located close to the crypts (Fig. 6, *F* and *G*).

Both the epithelia of the oesophagus and the stomach express transcripts for the c-met receptor whereas the surrounding mesenchymal cells express the gene for SF/HGF (data not shown). Expression of both SF/HGF and c-*met* is also found in the liver starting on E11. However, the resolution of in situ hybridization with ^{35}S-labeled probes does, in general, not allow positive assignment of hybridization signals to single, but rather to layers or groups of cells. Therefore, we were not able to identify the subpopulations of liver cells which express the genes for c-*met* or SF/HGF. The presence of SF/HGF and c-*met* specific transcripts in the developing intestine, stomach and liver was also confirmed by RNase protection experiments (Fig. 2).

Tooth. Two cell types, ectomesenchymal and epithelial cells, contribute to the formation of the tooth (Slavkin, 1974). The enamel organ formed by the epithelia is well developed on E15 in the mouse. At this stage and also later in development, we observe transcripts for the c-met receptor in the outer enamel epithelium (Fig. 7, *A* and *B*). In the advanced bell stage, SF/HGF transcripts are found in the mesenchymal cells of the dental papillae (Fig. 7, *B* and *C*).

Nasal Cavities. The nasal cavities develop from the olfactory pits, an ectodermal invagination, and differentiate into two functionally and histologically distinct units, an olfactory and a respiratory part (Adams, 1972). We demonstrate the presence of c-*met* transcripts in the entire epithelium of the developing nasal cavities (Fig. 7, *D* and *E*). In addition, c-*met* transcripts are identified in the epithelium of the vomeronasal organ (Fig. 7, *D* and *E*). With the appearance of Bowman's glands in the olfactory unit, c-*met* transcripts are detected in the epithelia of the glands (Fig. 7, *D* and *E*).

In the developing olfactory unit, we identify c-*met* transcripts in the entire multilayered olfactory epithelium on E11. In contrast, on E15 the hybridization signal is confined to an apical position in the epithelium (data not shown). The nuclei and the cellular bodies of the ciliated support cells are located apically which indicates that at later stages of development this epithelial cell type is responsible for the hybridization signal observed. Remarkably, a layer of mesenchymal cells in close vicinity to the epithelium of the nasal cavities expresses the ligand, SF/HGF. These mesenchymal cells are not distributed continuously around the entire epithelium, but are found only below the developing olfactory unit of the nasal cavities (Fig. 7, *E* and *F*).

The expression pattern described above, epithelia expressing c-*met* and mesenchyme in close vicinity expressing the ligand, is the prominent pattern observed during development of many parenchymal organs. However, we identified additional sites of expression for c-*met* and SF/HGF in the embryo, which are shown below.

Transient Expression of SF/HGF and c-met in Myogenic Cells

Transcripts of the c-*met* gene are found in the somites starting on day 10 of embryogenesis. To identify the population of cells within the somites which express c-*met*, consecutive sections were hybridized to c-*met* and myogenin probes. Myogenin is known to be expressed specifically in cells of the myogenic lineage (Sassoon et al., 1989). Identical hybridization patterns were observed with both probes, indicating that the myotomal compartment of the somites expresses c-*met* (Fig. 8, *A–C*). During further development, we detect c-*met* specific transcripts also in intercostal regions, in the limb buds and the mandible. Hybridization of consecutive sections with c-*met* and myogenin probes demonstrates also at these sites overlapping, but not absolutely identical patterns of expression (Fig. 8, *E* and *G* and data not shown). In addition, c-*met* expression was found on E10 in the limb bud and thus before myogenin positive cells can be located at this site (Sassoon et al., 1989). Expression of c-*met* at sites of muscle development is transient when analyzed by in situ hybridization. For example, transcripts of c-*met* are detected in the in-

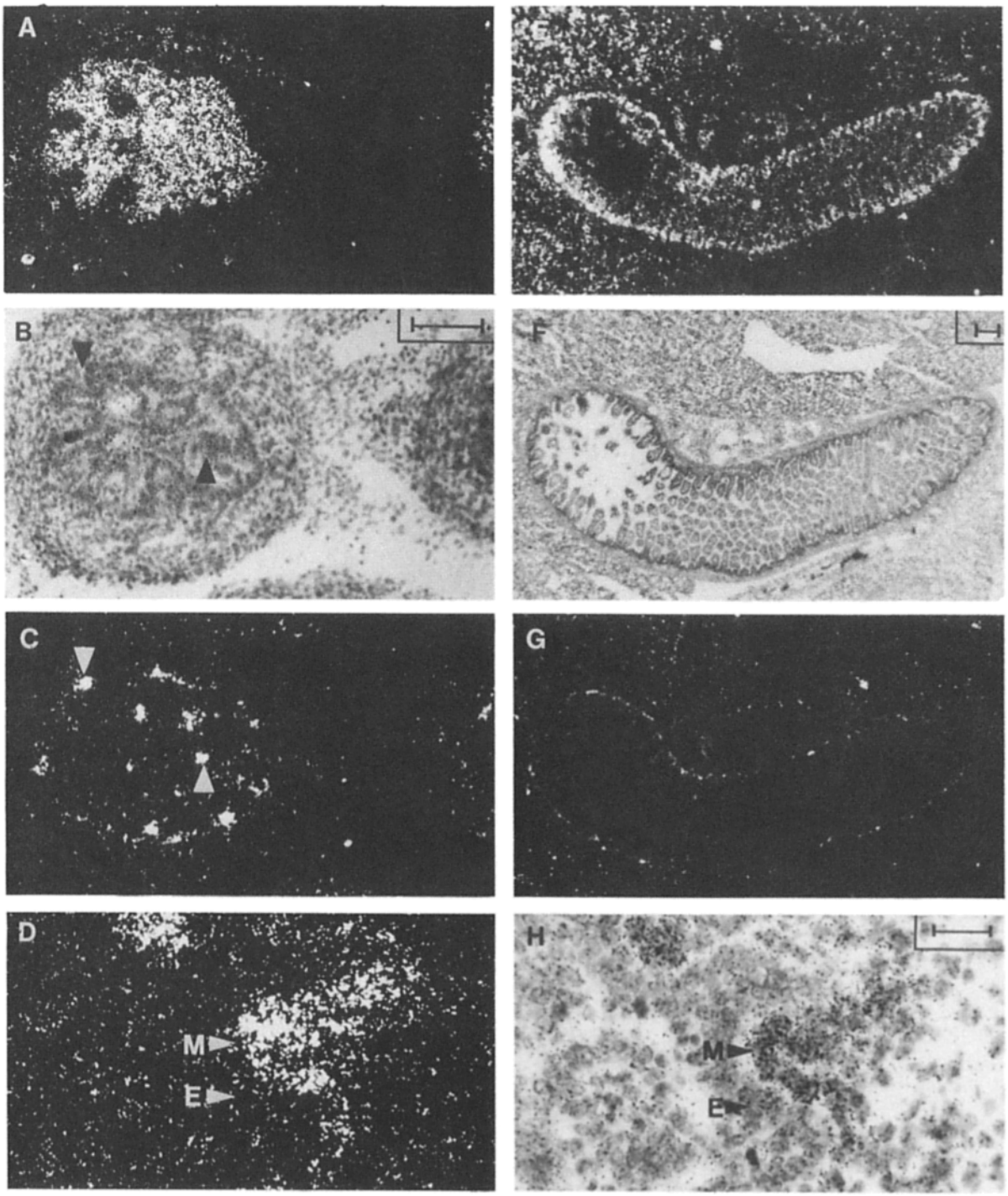

Figure 6. In situ hybridization analysis of SF/HGF and c-*met* expression in the embryonal intestine. Consecutive sections through embryos on day 15 (*A–C*) and day 17 (*E–G*) of development were hybridized to c-*met* (*A* and *E*) and SF/HGF (*C* and *G*) probes. The developing intestine is shown in darkfield (*A*, *C*, *E*, and *G*) and brightfield (*B* and *F*). The arrows in *B* and *C* point towards patches of mesenchymal cells which express the SF/HGF gene. Higher magnification of a section from a E15 embryo hybridized to a SF/HGF probe (*D* and *H*), showing positive hybridization signals over mesenchymal cells (*M*) growing into the epithelial cell layers (*E*). Bars: (*B* and *F*) 100 μm; (*H*) 25 μm. Consecutive sections in *A*, *B*, and *C* and *E*, *F*, and *G* are shown at identical magnifications.

tercostal regions between E11 and E15, and in the limbs between E10 and E15 (Table II). When analyzed by the more sensitive RNase protection method, low levels of c-*met* transcripts can be identified in RNA isolated from limb muscle of E18 embryos or adult mice (Table I).

We also detect expression of SF/HGF in the limb buds and the mandible, but not at other sites of muscle formation like the myotome. Comparison of the spatial pattern of SF/HGF and myogenin hybridization signals in consecutive sections demonstrates that myogenic cells in the limb and mandible

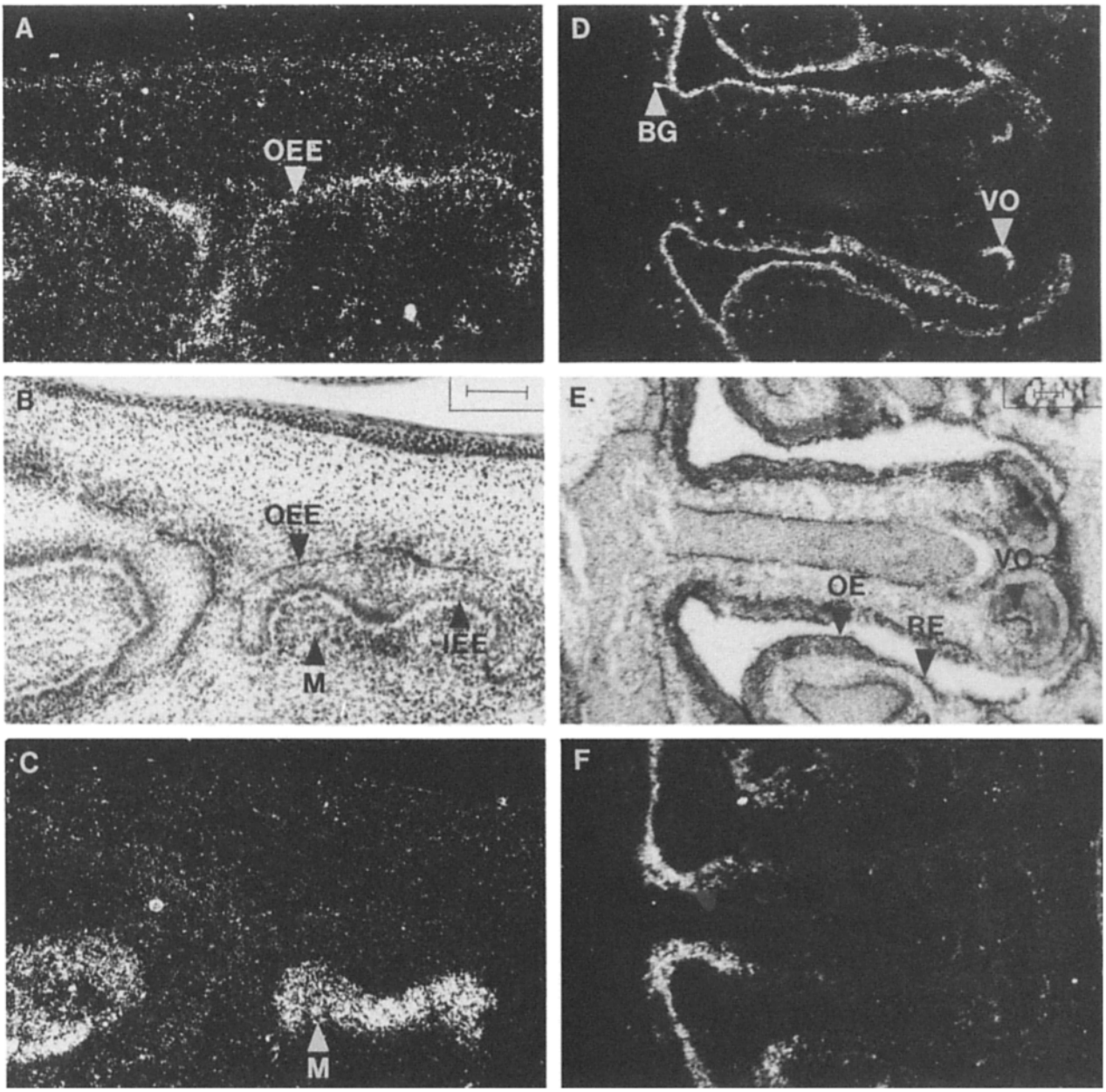

Figure 7. In situ hybridization analysis of SF/HGF and c-*met* expression in developing teeth and nasal cavities. Consecutive sections through embryos on day 18 of development were hybridized to c-*met* (*A* and *D*) and SF/HGF (*C* and *F*) probes. A developing molar is shown in darkfield (*A* and *C*) and brightfield (*B*). Arrows point toward the outer enamel epithelium (*OEE*), the inner enamel epithelium (*IEE*), and the mesenchyme of the dental papilla (*M*). The developing nasal cavity is shown in darkfield (*D* and *F*) and brightfield (*E*). Arrows point towards the vomeronasal organ (*VO*), Bowman's glands (*BG*), olfactory (*OE*), and respiratory (*RE*) epithelium. Bars, 100 μm.

express SF/HGF (Fig. 8, *G* and *H* and data not shown). However, the overlap between the hybridization signals obtained with SF/HGF and myogenin probes is not complete. SF/HGF transcripts were already observed on E10 and thus before myogenin transcripts are detected in the limb buds. In addition, not all cell groups which express SF/HGF on E11 or E13 in the limb buds express myogenin, and not all myogenin positive groups of cells express SF/HGF (Fig. 8, *G* and *H*). However, the overlap between the hybridization patterns observed with both probes appears to increase with the developmental age of the embryos. The time course of SF/HGF gene expression at sites of muscular development is again transient in nature when analyzed by in situ hybridization (Table II); for example, expression is found in the developing limb between E10 and E15. When analyzed by RNase protection, specific transcripts for SF/HGF can still be identified in RNA isolated from limb muscle of E18 embryos, but not of adult animals.

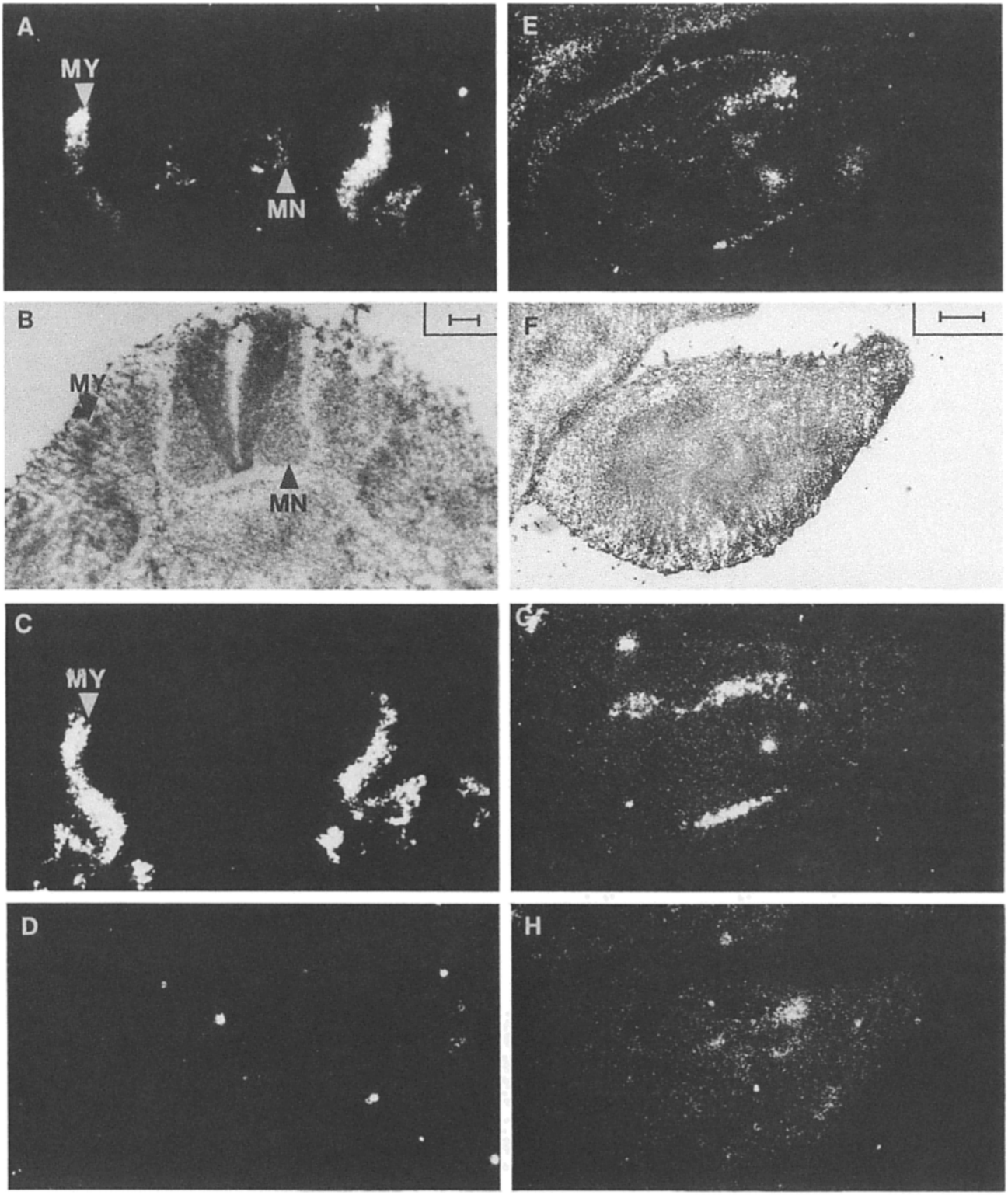

Figure 8. In situ hybridization analysis of the SF/HGF, c-*met* and myogenin expression at sites of muscular development. Consecutive cross sections of an E11 embryo shown in darkfield (*A*, *C*, and *D*) and brightfield (*B*) hybridized to c-*met* (*A*), myogenin (*C*), and control probes (*D*) are depicted at the same magnification. Arrows point to the myotome (*MY*) and motoneurons (*MN*). Consecutive sections of the limb of an E13 embryo hybridized to c-*met* (*E*), myogenin (*G*), and SF/HGF (*H*) probes are shown in darkfield (*E*, *G*, and *H*) and brightfield (*F*) at the same magnification. Bars: (*B*) 100 μm; (*F*) 200 μm.

Table II. Time Course of SF/HGF and c-met Expression at Sites of Muscle Formation and Development as Determined by In Situ Hybridization

	Somite		Axiale muscle		Limb		Mandible	
	met	SF/HGF	met	SF/HGF	met	SF/HGF	met	SF/HGF
E10	+	−	−	−	+	+	−	−
E11	+	−	+	−	+	+	+	+
E13	+	−	+	−	+	+	+	+
E15	+	−	±	−	+	±	+	±
E17	−	−	−	−	−	−	−	−

Mouse embryos at different times of gestation were analyzed for expression of SF/HGF and c-*met* at sites of muscular development; (+) denotes a strong, (±) a weak, and (−) no detectable signal by in situ hybridization.

SF/HGF and c-met Expression in Neuronal Cell Types

Transient c-met Expression in the Spinal Cord. Transcripts of the c-*met* gene are also observed in the embryonal spinal cord and confined there to the ventral horns (Fig. 9, *A* and *B*). This corresponds to the parts of the spinal cord where the nuclei and cellular bodies of the motoneurons are located (Altman and Bayer, 1984). Expression of c-*met* in the spinal cord is found only between E11 and E15. Onset of c-*met* expression correlates therefore with the "birthdate" of the motoneurons (Altman and Bayer, 1984).

Telencephalon. An additional neural tissue, the telencephalon, expresses c-*met* and the SF/HGF gene during development. Four different cellular layers located in the ventricular, subventricular, intermediate, and marginal zone form the telencephalon. Mitotic divisions take place in the ventricular zone, and give rise to cells which migrate outwards to differentiate into elongated bipolar cells. Thus the inner layer contains the immature mitotic cells, while the marginal zone is composed of postmitotic, differentiated cells (Uylings et al., 1990). Expression of c-*met* and SF/HGF can be observed in the telencephalon during prenatal development, i.e., starting around E17. The c-*met* gene is then expressed in the marginal zone, while transcripts for SF/HGF are confined to the ventricular layer (data not shown).

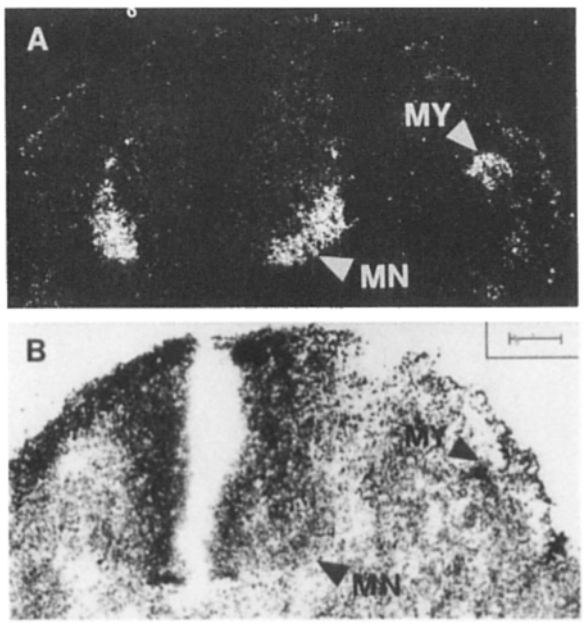

Figure 9. In situ hybridization analysis of c-*met* expression in the spinal cord. Section through an embryo on day 11 of development hybridized to c-*met* in darkfield (*A*) and brightfield (*B*). The arrows point toward the ventral horns, where the nuclei of motoneurons (*MN*) are located, and toward the myotome (*MY*). Bar, 100 μm.

Discussion

We report here the expression analysis of the genes encoding the c-met receptor tyrosine kinase and its specific ligand, SF/HGF, during mouse embryogenesis. A recurring pattern of expression is found during the development of epithelial organs: expression of the gene for c-*met* in certain epithelia, and specific transcripts for SF/HGF in mesenchymal cells in close vicinity. The observed distribution of specific transcripts indicates thus a paracrine mode of action for SF/HGF and its receptor during development of epithelial organs. Such a paracrine mode of action has been proposed originally by Stoker and colleagues (Stoker et al., 1987) and is consistent with the biological activities of SF/HGF in vitro: epithelial cells of diverse origins respond to the factor by various biological activities like mitogenesis, movement, morphogenesis or growth arrest, whereas many fibroblasts and smooth muscle cell lines derived from the mesenchyme produce SF/HGF (Stoker et al., 1987; Gherardi et al., 1989; Weidner et al., 1990; Rosen et al., 1990). In addition, novel sites of expression of the two genes were identified: we detect a transient expression of c-*met* and SF/HGF during muscle development, and transient expression of the c-met receptor in the ventral horns of the spinal cord where the developing motoneurons are located. Expression of both receptor and ligand is also detected in the telencephalon. Besides a function in mesenchymal epithelial interactions, SF/HGF and its receptor might thus also play a role in development of the brain as well as in the formation and/or innervation of muscle.

SF/HGF and c-met in Development of Epithelial Organs

During development of a variety of different organs, epithelial cells form complex branched tubular structures. In organ culture experiments, it has been demonstrated that these morphogenetic processes are dependent on mesenchymal factors (Grobstein, 1967; Saxen, 1987). It has recently also been found that epithelial MDCK cells grown in collagen gels respond to exogenous SF/HGF by the formation of branched tubuli (Montesano et al., 1991*b*). We demonstrate here that during mouse embryogenesis, various epithelia which form branching tubules, i.e. the epithelia of the lung, pancreas or salivary gland, do express c-*met*, while the surrounding mesenchyme expresses SF/HGF. In all these developing organs, mesenchymal factors are known to be essential for morphogenesis and differentiation of the epithelia (Grobstein and Cohen, 1965; Cunha et al., 1983; Saxen, 1987; Ekblom, 1991). Similarly to the observed morphogenic activity in vitro, SF/HGF might regulate such events during organogenesis in vivo.

In the kidney, two types of tubular epithelia which express

c-*met* exist, the branched ureter as well as the nonbranched proximal and distal tubuli. The ligand, SF/HGF is expressed in the nephrogenic mesenchyme. The branching epithelia of the ureter are again dependent on mesenchymal signals for proper morphogenesis (Saxen, 1987). The epithelia of the tubuli as well as the glomeruli develop from the same progenitor cells, i.e., nephrogenic mesenchyme, which is converted into epithelia by inductive signals from the ureter (Saxen, 1987; Ekblom, 1991). Expression of c-*met* in these induced epithelia is restricted to proximal and distal tubules; no expression is found in the glomeruli or the precursors of all these cell types, the uninduced mesenchyme. Therefore, the observed expression pattern is incompatible with a role of c-met and SF/HGF in the induction of new kidney epithelia; however, it is consistent with the hypothesis that the c-met receptor and SF/HGF can play a general role in the formation of tubular structures from existing epithelia.

We continuously observe transcripts for the c-met receptor in the intestinal epithelia during embryogenesis. However, expression of the ligand, SF/HGF, shows dynamic changes concomitant with morphological reorganization and differentiation of the epithelium during villi formation. The epithelium of the intestinal anlagen is multilayered; during formation of the villi, dissociation of the tight epithelial complexes is observed and secondary lumina appear. At the same time, the mesenchymal cells invade the loosened stratified epithelium (Fig. 5 and Mathan et al., 1976). Concomitant with this morphological rearrangement, we observe strong expression of SF/HGF in patches of mesenchyme associated with cells invading the epithelium. Since a prominent activity of SF/HGF on epithelial cells in vitro is the dissociation of adherent epithelial sheets (Stoker et al., 1987; Weidner et al., 1990), the observed loosening of the epithelial cell layer in the intestinal anlagen might be a direct consequence of SF/HGF production in the underlying mesenchyme.

During development of the tooth, c-*met* transcripts are found in the outer enamel epithelium. Transcripts for SF/HGF are only detected in the advanced bell stage, i.e., starting around day 17 of development, and are located in the mesenchyme of the dental papilla. Since the outer enamel epithelium subsequently disappears, the SF/HGF mediated signal might not lead to cell proliferation, but rather to growth arrest and to degeneration of these particular cells. It is noteworthy that a cytostatic and degenerative activity of SF/HGF on particular epithelial target cells has been observed before (Higashio et al., 1990; Shiota et al., 1992). In addition, a prominent hybridization signal for c-*met* is found in epithelial cells of the nasal cavities during the entire development. Again, SF/HGF expression is observed in mesenchymal cells underlying the epithelium, which consists of two functionally distinct units, an olfactory and a respiratory part. SF/HGF transcripts are restricted to a layer of mesenchymal cells in the vicinity of the developing olfactory unit starting on E11 and thus before the appearance of differentiated cell types in the olfactory epithelium (Adams, 1972). A paracrine signal mediated by SF/HGF and c-*met* during development might thus play a role in the differentiation of this complex epithelium.

The distribution of SF/HGF protein in the late rat embryo has recently been determined by immunohistochemistry (Defrances et al., 1992). Surprisingly, the protein was found to be located predominantly in the cytoplasm of various epithelia. Yet, SF/HGF mRNA is generally found in the mesenchymal but not the epithelial cell compartment. Since the high affinity receptor of SF/HGF is expressed on epithelia, the observed location of SF/HGF protein in the cytoplasm of such cells might represent internalized factor bound to the receptor.

Molecular Nature of Mesenchymal Epithelial Interactions

Mesenchymal signals are essential for differentiation and morphogenesis of epithelia, but their molecular nature is presently only poorly understood. In organ culture experiments, it has been observed that the specificities of the mesenchymal signals required by different epithelia vary considerably. The pancreas is an extreme example for the nonspecificity of mesenchymal contributions to epithelial differentiation: pancreatic mesenchyme can be replaced by virtually every heterologous mesenchyme. In contrast, no heterologous mesenchyme which can support growth or morphogenesis of the ureter epithelium has been identified (Saxen, 1987). Mesenchymal contributions to epithelial morphogenesis are therefore complex, and it is obvious that more than a single factor is involved (Grobstein, 1967). One such mesenchymal factor was recently characterized; it is epimorphin, a protein necessary for differentiation and morphogenesis of hair follicles and lung epithelia (Hirai et al., 1992).

Many genes for tyrosine kinase receptors were originally isolated as oncogenes and their protein products are therefore commonly associated with mediating growth signals (Ullrich and Schlessinger, 1990). However, evidence has accumulated recently that such receptors and their specific ligands can also induce movement, differentiation or morphogenesis of epithelia depending on the exact target cells, i.e., they regulate processes which are essential in embryogenesis. Several tyrosine kinase receptors with predominant or exclusive distribution on epithelial cells are known: (*a*) the c-met receptor, which can mediate mitogenic, motogenic and morphogenic signals (Gherardi, 1991; Weidner et al., 1993*b*); we show here that the c-met receptor is expressed in many embryonal epithelia, whereas the specific ligand is often produced in mesenchyme; (*b*) the c-ros receptor which is expressed transiently in epithelia of the kidney and intestine during development; we have suggested that this receptor receives mesenchymal signals during organogenesis (Sonnenberg et al., 1991); (*c*) the c-neu receptor; NDF/heregulin, the putative ligand of c-neu, can induce differentiation of mammary carcinoma cells (Kokai et al., 1987; Press et al., 1990; Peles et al., 1992). NDF/heregulin is expressed in embryonal mesenchyme (Dirk Meyer and C. B., in preparation). It is thus likely that some of the mesenchymal factors long known to be essential for appropriate epithelial differentiation during development, but whose molecular nature has remained elusive, will turn out to correspond to specific ligands for receptor-type tyrosine kinases (see Birchmeier et al., 1993; Birchmeier and Birchmeier, 1993).

SF/HGF and c-met Expression in the Development of Muscle

Although all skeletal muscle derives from a common precursor pool, the myotome, at least two distinct types of skeletal

muscle exist: (*a*) axial muscles (vertebral and intercostal) which derive from somite cells differentiating in situ within the somite and between the sclerotomal organization centers of the vertebrae; (*b*) the muscles of the limbs and visceral arches, which arise from cells that migrate away from somites and invade the lateral regions of the embryo, where they differentiate. Precursor cells for the two types of muscle are located at distinct subregions of the somite (Ordahl and Le Douarin, 1992). We detect transient expression of c-*met* and SF/HGF in myogenic precursor cells. However, whereas c-*met* is expressed in both types of differentiating muscle, SF/HGF transcripts are confined to myogenic cells in the limbs and mandible. In the limb buds, prominent expression of both genes is found already on E10, i.e., before myogenic markers are expressed. In addition, we observed that the spatial distribution of SF/HGF, c-*met* and myogenin expressing cells is not completely overlapping even in later stages of development. It is therefore possible that SF/HGF and c-*met* are not only expressed in myogenic but also in other mesenchymal cells in the limb buds. Alternatively, myogenic precursors might exist which express the SF/HGF and c-*met* genes but not yet markers of myogenic differentiation like myogenin.

Because of the partial overlap of c-*met* and SF/HGF expression patterns in the limb buds, it is conceivable that an autocrine signaling system operates at distinct sites and times during muscle development. However, we use for our in situ hybridization experiments ^{35}S-labeled antisense RNA probes. The resolution of this method allows assignment of positive hybridization signals to groups or layers, but not to single cells. Therefore, different subpopulations or differentiation stages among the apparently homogenous cells might exist, which express either c-*met* or SF/HGF.

Expression of SF/HGF and c-*met* at sites of muscular development is transient in nature and found between E10 and E15. Interestingly, this transient expression correlates in time with the transient expression of the c-*met* receptor gene in developing motoneurons. Therefore, the c-met receptor and its ligand might play a role also in development and/or innervation of the muscle.

Conclusion

The detailed expression analysis of c-*met* and SF/HGF reported here points towards multiple roles for the receptor and its ligand in embryogenesis. First and most notably, the receptor is expressed in epithelia, whereas transcripts for the ligand are found in distinct mesenchymal cells in close vicinity. This suggests that the receptor and its ligand mediate a signal exchange between mesenchyme and epithelia, a mechanism long known to govern morphogenesis and differentiation of parenchymal organs. The second notable site for expression are myogenic cells, where transcripts for both ligand and receptor are found at distinct times and sites of muscular development. Since motoneurons concurrently also express c-*met*, a role in muscle differentiation and/or innervation is possible. Our results provide a basis for further analysis of receptor and ligand function, for example by ablating SF/HGF or c-met activity in muscle cell or organ culture experiments by adding antagonistic antibodies or peptides. Alternatively, genetic approaches like targeted mutagenesis of the gene(s) encoding ligand or receptor, ectopic overexpression of the ligand or expression of a dominant negative allele of c-*met* during development could lead to a further understanding of the developmental role of this signaling system.

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