Expression and Functional Interaction of Hepatocyte Growth Factor–Scatter Factor and its Receptor c-*met* in Mammalian Brain

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Abstract. Hepatocyte growth factor–scatter factor (HGF-SF) is a pleiotropic cytokine with mito-, morpho-, and motogenic effects on a variety of epithelial and endothelial cells. HGF-SF activity is mediated by the c-*met* protooncogene, a membrane-bound tyrosine kinase. Here, we demonstrate that both genes are expressed in developing and adult mammalian brains. HGF-SF mRNA is localized in neurons, primarily in the hippocampus, the cortex, and the granule cell layer of the cerebellum, and it is also present at high levels in ependymal cells, the chorioid plexus, and the pineal body. c-*met* is expressed in neurons, preferentially in the CA-1 area of the hippocampus, the cortex, and the septum, as well as in the pons. In the embryonic mouse, brain HGF-SF and c-*met* are expressed as early as days 12 and 13, respectively. Neuronal expression of HGF-SF is evolutionary highly conserved and detectable beyond the mammalian class. Incubation of septal neurons in culture with HGF-SF leads to a rapid increase of c-*fos* mRNA levels.

The results demonstrate the presence of a novel growth factor–tyrosine kinase signaling system in the brain, and they suggest that HGF-SF induces a functional response in a neuronal subpopulation of developing and adult CNS.

HEPATOCYTE growth factor–scatter factor (HGF-SF) is a multifunctional, heterodimeric cytokine. It serves as a para- and endocrine mitogen for many different epithelial and endothelial cells, as well as melanocytes (Nakamura et al., 1989; Zarnegar and Michalopoulos, 1989; Matsumoto et al., 1991; Bussolino et al., 1992), is motogenic for various epithelial cell lines (Stoker et al., 1987; Weidner et al., 1990), induces kidney epithelial cells to form tubular structures (Montesano et al., 1991), and induces hepatocytes to grow in cords (Michalopoulos et al., 1993).

Recently, the c-*met* protooncogene, a membrane-bound heterodimeric tyrosine kinase (Park et al., 1987), was demonstrated to represent the high affinity HGF-SF receptor (Naldini et al., 1991; Bottaro et al., 1991) that is able to mediate all the known effects of HGF-SF (Weidner et al., 1993). The HGF-SF/c-*met* signaling system seems to be important in mesenchymal–epithelial interactions during fetal development (Stern et al., 1990; Montesano et al., 1991; Sonnenberg et al., 1993), but is also constitutively expressed in normal adult liver (Schirmacher et al., 1992) and activated during parenchymal regeneration (Zarnegar et al., 1991; Ishiki et al., 1992) in adult animals.

HGF-SF and c-*met* have been detected in the brain (Di Renzo et al., 1991; Zarnegar et al., 1990; Tashiro et al., 1990), but the cells expressing these molecules have not been clearly identified. Several growth factors, especially those interacting with receptor tyrosine kinases, are active in the brain and some of them are involved in the regulation of neurotrophic processes. Further understanding of the possible role of HGF-SF and c-*met* in the brain requires the exact localization of the expressing cell populations during different stages of mammalian brain development.

Therefore, we have characterized the expression of HGF-SF and c-*met* in the developing and adult mammalian brain in more detail. Transcripts of both genes are present in distinct neuronal cell populations and in the case of HGF-SF also in nonneuronal cells, suggesting a local, possibly paracrine role for HGF-SF in the mammalian brain. Furthermore, we demonstrate that c-*met*–expressing septal neurons exert a functional response after stimulation with recombinant HGF-SF.

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1. *Abbreviations used in this paper*: HGF-SF, hepatocyte growth factor-scatter factor; NGF, nerve growth factor; rtPCR, reverse transcription PCR.

Materials and Methods

Preservation and Preparation of Tissues and Cells

Tissues were obtained by autopsy and were immediately snap frozen in liq-

uid nitrogen and continuously stored at −70°C. Animal experiments were performed according to an officially accepted experimental animal protocol.

Adult and 4-d-old (P4) Wistar rats were killed after receiving ether anesthesia, and various brain areas were rapidly excised and frozen. Fetal mice were obtained after controlled mating for one night and their age (designated Ex in days after conception) was additionally controlled by histological examination in comparison with established standards (Theiler, 1972). Expression analysis was perfomed on sagittal, 12-μm thick cryostat sections of adult brain, and in the case of the fetal brain, on parasagittal sections of whole fetuses and horizontal sections of the brain.

Plasmids and Probes

Hybridization for HGF-SF mRNA was partly carried out with a 1.4-kb EcoRI-fragment covering the 3′ translated part of the rat HGF-SF cDNA (Tashiro et al., 1990), subcloned into pBluescript SK(+) kindly supplied by T. Nakamura (Stratagene, La Jolla, CA) and also in part with rtPCR-amplified and sequenced rat, mouse, and human HGF-SF cDNA fragments subcloned into pSK(+) (Stratagene; see below). Hybridization for c-*met* mRNA was performed with a 1.3-kb fragment, spanning the tyrosine kinase domain of the human c-*met* cDNA (Park et al., 1987), subcloned into pGEM3Z(+) (Promega Corp., Madison, WI). For Northern and Southern hybridization experiments, the purified HGF-SF and c-*met* cDNA fragments were labeled by primer extension, either with ^{32}P or digoxigenin-coupled dCTP (Feinberg and Vogelstein, 1983). Single-stranded, [^{35}S]-UTP-labeled riboprobes were prepared by linearizing the vector downstream of the inserted cDNA fragment and subsequent in vitro transcription using the respective phage polymerase (Sambrook et al., 1989).

In Situ Hybridization

In situ hybridization was carried out by adapting a previously described protocol (Schirmacher et al., 1992). Frozen tissues were embedded in OTC (Miles Inc., Elkhart, IN). 12-μm cryostat sections were postfixed in 4% paraformaldehyde, 5 mM $MgCl_2$, dehydrated in graded ethanol, acetylated (0.1 M trietholamin, pH 8, 0.25% [vol/vol] acetic anhydride), and prehybridized (50% formamide, 2.5× Denhardt's solution, 150 μg/ml salmon sperm DNA, 0.6 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8, 0.1% SDS, and 0.05 ng/ml wheat germ RNA). Hybridization was performed at 45°C in humid chambers using [^{35}S]UTP-labeled rat HGF sense and antisense probes (10^6 cpm/section). The hybridization buffer consisted of prehybridization buffer with 10% dextrane sulfate and 10 mM dithiotreitol. Subsequently, the sections were washed several times under stringent conditions (0.2 × SSC at 50°C), treated with RNaseA (20 μg/ml; Behring Diagnostics, Somerville NJ), dehydrated, and exposed to films (X-OMAT-AR; Eastman Kodak, Rochester, NY) at −70°C overnight. Afterwards, the slides were coated with emulsion (NTB-2; Eastman Kodak), and the exposure time, typically between 1 and 2 wk, was evaluated based on the signal intensity on the autoradiography film. After development and fixation, the sections were stained with Harris's hematoxylin and eosin, and were then mounted afterwards. The sections were observed under bright and dark field, and photomicrographs were taken with a microscope (Labophot; Nikon Corp., Tokyo, Japan).

Northern Hybridization

Tissue RNA was prepared by guanidium isothiocyanate extraction and cesium chloride ultracentrifugation (Chirgwin et al., 1979), while total RNA from primary cultures of fetal central nervous system neurons was obtained according to a method described by Chomczynski and Sacchi (1987). 10 μg of total RNA were separated on denaturing 1.2% agarose gels and transferred onto nylon membranes (Amersham Buchler, Braunschweig, Germany) by capillary transfer using 20 × SSC and immobilized by 5 min of shortwave UV treatment (Sambrook et al., 1989). The filters were prehybridized for ≥2 h and then hybridized overnight with 2×10^7 cpm of the respective [^{32}P]dCTP-labeled cDNA fragment in 20 ml hybridization buffer. The hybridization buffer consisted of 50% formamide, 12.5% dextrane sulfate, 5 × SSC, 1 × Denhardt's solution, 50 mM NaH_2PO_4, and 0.25 mg/ml salmon sperm DNA. After the hybridization reaction, the filters were washed under stringent conditions (0.1 × SSC at 65°C) and exposed to Kodak X-OMAT-AR films at −70°C using intensifying screens.

Reverse Transcriptase Polymerase Chain Reaction (rtPCR)

rtPCR was essentially carried out under conditions described by Gilliland et al. (1990) using the following primers:

HGF-SF: sense 5′-GGGGAATGAGAAATGCAGTCAG-3′ (1920-1941)
antisense 5′-CCTGTATCCATGGATGCTTC-3′ (2215-2234)
(sequence and numbers according to Tashiro et al. [1990])

c-*met*: sense 5′-ACAGTGGCATGTCAACATCGCT-3′ (2413-2434)
antisense 5′-GCTCGGTAGTCTACAGATTC-3′ (3049-3068)
(sequence and numbers according to Park et al. [1987])

In all cases, the amplification products were separated in 1.8% agarose gels, transferred onto nylon membranes (Amersham Buchler) after documentation, hybridized to digoxygenin-labeled HGF-SF or c-*met* cDNA fragments spanning the amplified sequence, washed under stringent conditions, and exposed to Kodak X-OMAT-AR films using a chemiluminescent detection kit (Boehringer Mannheim, Mannheim, Germany). Expected sizes of the amplified fragments were 314 bp in the case of rat HGF-SF and 655 bp in the case of human c-*met*. In addition, bands of the expected size from the different species were eluted from the gels, subcloned into pSK(+) (Stratagene) or pCR1000 (Clontech, Palo Alto, CA), and submitted to automated dideoxy chain termination sequencing (PRISM Ready Reaction Dyedeoxy Termination Cycle Sequencing Kit; Applied Biosystems, Inc., Foster City, CA) on a sequencer (373A; Applied Biosystems, Inc.).

Cell Cultures and Effect of HGF-SF

Neurons from septum and hippocampus of fetal rats (E17) were prepared as previously described (Zafra et al., 1990; Lindholm et al., 1994). The cells were taken up in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and plated on poly-DL-ornithine-precoated 24-well dishes (Costar Corp., Cambridge, MA) at a density of 0.5×10^6 cells/dish. 3 h after plating, the cultures were switched over to a serum-free medium as described previously (Zafra et al., 1990). Cultures were maintained for 7 d

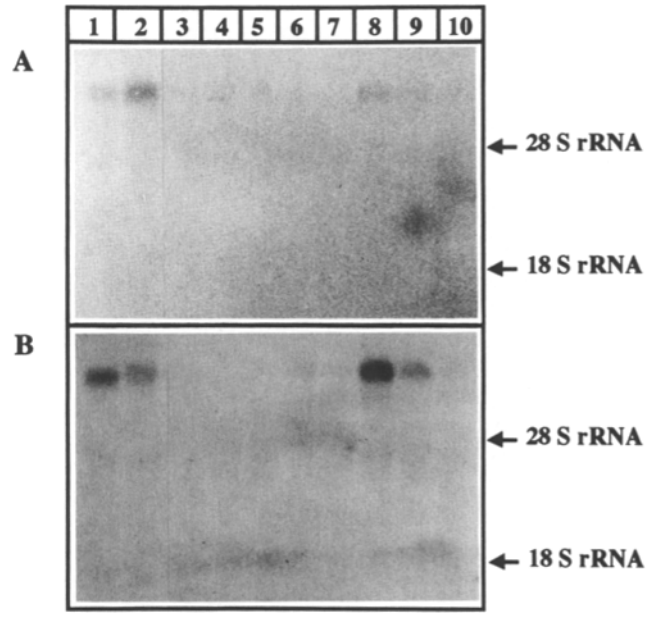

Figure 1. Northern hybridization analysis of HGF-SF (*A*) and c-*met* (*B*) expression in the adult and developing rat brain. 10 μg of total RNA obtained from different regions of adult (lanes *2–5*) and P4 rat brain (lanes *6–9*) were analyzed: (lane *1*) adult rat liver; (lanes *2* and *9*) hippocampus; (lanes *3* and *8*) cortex; (lanes *4* and *7*) cerebellum; (lanes *5* and *6*) midbrain; (lane *10*) liver from P4 rat. The positions of 18S and 28S ribosomal RNA are indicated (shade between the positions of 18S and 28S rRNA of lane *9* in *A* is a non-reproducible artifact).

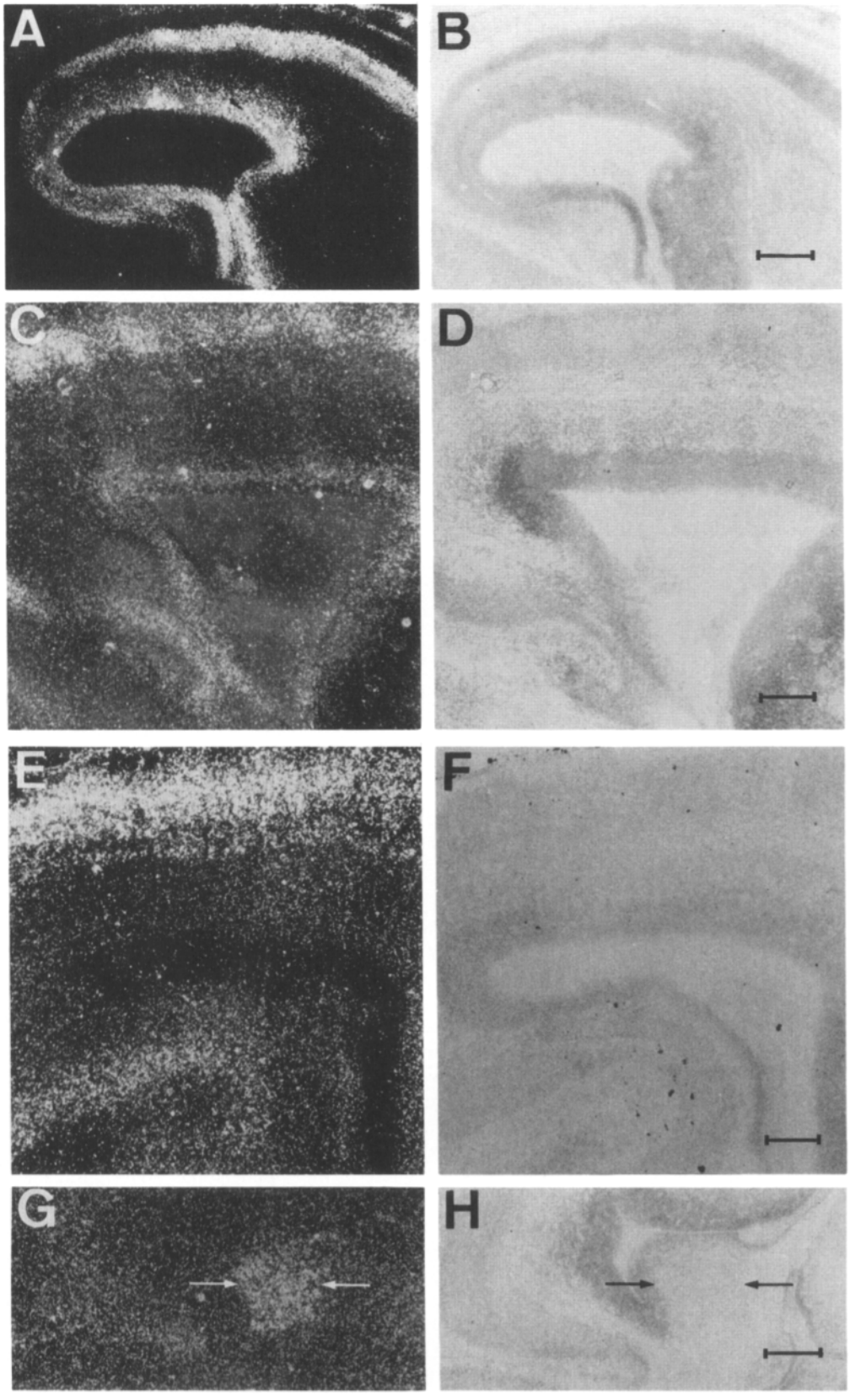

Figure 2. Expression of HGF/SF and c-*met* mRNAs in embryonic mouse brain. Dark fields images (*left panels*) show the hybridization signal and bright field pictures (*right panels*) the histology. Bars, 200 μm. (*A* and *B*) HGF-SF expression in the E14 telencephalon (parasagittal). Note expression in the periventricular neuroepithelium and in the preferentially frontal part of the cortical plate. (*C* and *D*) HGF-SF expression in the E17 telencephalon (parasagittal). Expression is still present in the neuroepithelium and the cortical plate. (*E* and *F*) c-*met* expression in the E17 telencephalon (parasagittal). Expression is located in the cortical plate and in the cortical subplate, preferentially in the posterior areas. (*G* and *H*) c-*met* expression in the E17 septum (*arrows*, horizontal section).

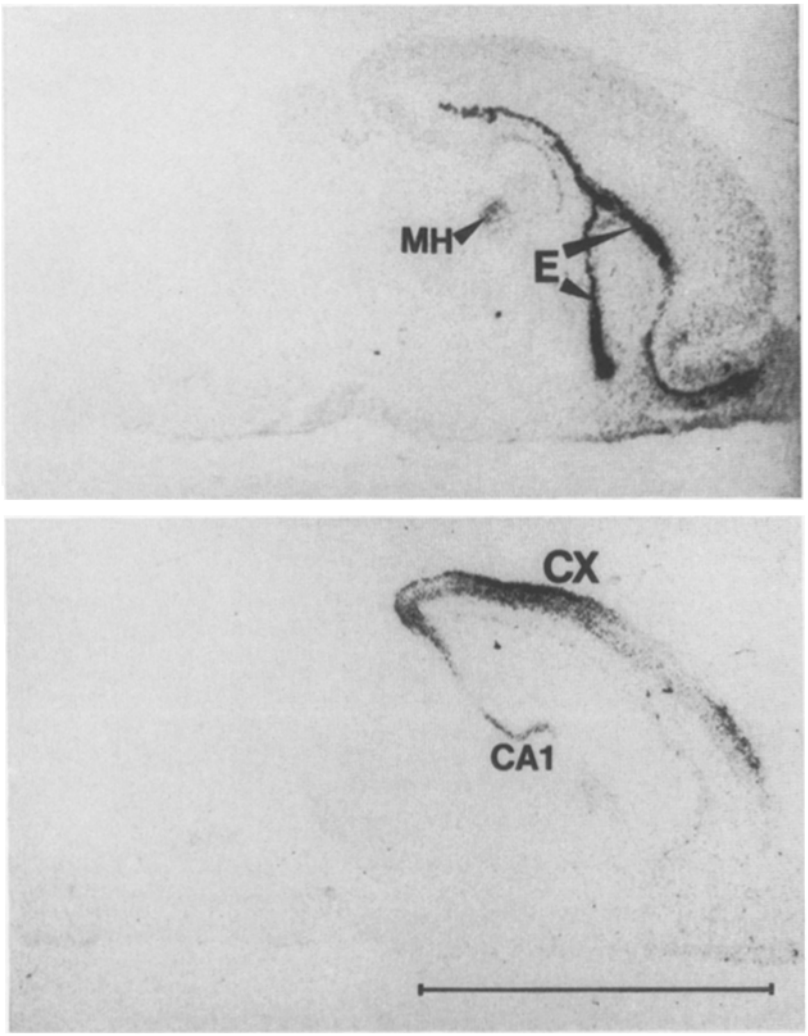

Figure 3. Expression of HGF-SF and c-*met* mRNAs in developing rat brain (P4). Film autoradiography analysis of parasagittal sections of whole brain hybridized for HGF-SF (above) and c-*met* transcripts (below). *MH*, medial habenula; *E*, ependymal lining; *CA1*, CA-1 area of the hippocampus; *CX*, cerebral cortex; Bar, 1 cm.

at 37°C in a 95% air/5% CO_2 humidified atmosphere. Human recombinant HGF (30 ng/ml; Yoshiyama et al., 1991) was then added to the cultures for 30 min and total cellular RNA was extracted by the method of Chomczynski and Sacchi (1987). Control cultures received either 30 ng/ml of nerve growth factor (NGF) purified from mouse submandibular gland (Berninger et al., 1993) or an equal volume of bovine serum albumin. RNA electrophoresis and hybridization were performed as described earlier (Lindholm et al., 1993) using a v-*fos* probe (Lindholm et al., 1988) labeled by random priming. For control purposes, all filters were also hybridized with a rat β-actin cDNA probe as described previously (Lindholm et al., 1988). After washing, the filters were exposed to x-ray films, and the relative intensity of the c-*fos* mRNA signals were determined using a laser densitometer (Ultro Scan XL; Pharmacia-LKB, Uppsala, Sweden). Four independent stimulation experiments were analyzed using autoradiography films with signal intensities in the linear range. Signal intensities at the site of specific c-*fos* hybridization were determined and normalized for the intensity of the respective β-actin hybridization signals. Relative signal intensities of the different stimulation experiments were obtained by arbitrarily setting the c-*fos* signal intensity of the respective nonstimulated neurons at 1, and afterwards determining mean and standard deviation of the four independent experiments.

Results

HGF-SF and c-met Transcripts Are Expressed in the Mammalian Brain

In a preliminary screening of rat tissues using rtPCR, we have detected expression of HGF-SF and c-*met* in developing and adult rat brain (data not shown). To further localize these transcripts, microdissection of adult and developing (P4) rat brain was performed, and total RNA from hippocampus, cortex, cerebellum, and midbrain was analyzed by Northern hybridization.

HGF-SF transcripts were present in all fractions from adult and developing brain (Fig. 1 *A*). The transcript size of ~6 kb corresponds well to the known, major HGF-SF mRNA species coding for full-length HGF-SF (Tashiro et al., 1990). In the adult brain, HGF-SF transcript levels were consistently highest in the hippocampus and significantly lower in cortex, cerebellum, and midbrain. In developing rat brain (P4), the signal was also present in the hippocampus, but the levels were higher in the cortex fraction.

Northern hybridization analysis for c-*met* transcripts demonstrated the presence of a transcript of ~7 kb in all fractions from adult and developing rat brain (Fig. 1 *B*). The expression was highest in the hippocampus of the adult and in the cortex and hippocampus of the developing brain (P4).

In Situ Localization of HGF-SF and c-met Transcripts in Adult and Developing Brains

To exactly localize the HGF-SF– and c-*met*–expressing cells in the brain, in situ hybridization with specific [^{35}S]UTP-

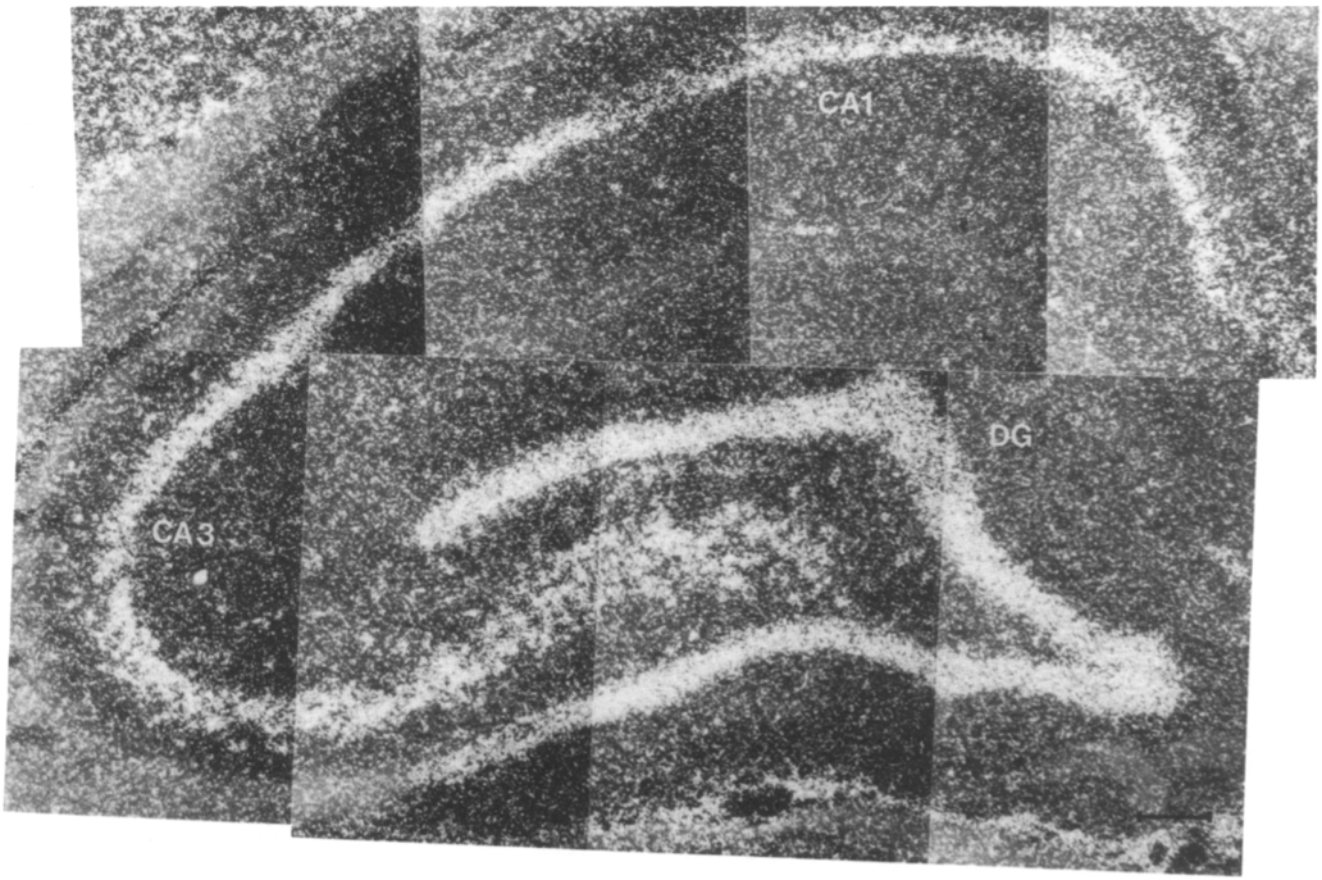

Figure 4. HGF-SF mRNA expression in adult rat hippocampus (*DG*, dentate gyrus; *CA1* and *CA3*, CA-1 and CA-3 areas; Bar, 200 μm).

labeled riboprobes was performed and analyzed by film autoradiography (see Fig. 3), as well as by dark and bright field microscopy (see Figs. 2, 4–7). As demonstrated before by rtPCR and Northern hybridization, expression of both genes was found in both adult and developing brains.

During the development, HGF-SF signals were first detected in E12 mouse brain. At that time and throughout further development, HGF-SF mRNA was prominently expressed in the neuroepithelial layer of the telencephalic vesicle. Furthermore, expression was seen in the developing cortical plate, most prominently in the frontal part (Fig. 2). At P4, there was prominent expression in the ependymal layer with a weaker expression in the hippocampus, the outer layers of the cortex, and the medial habenula (Fig. 3).

In the adult, low levels of HGF-SF mRNA were detected widespread in neurons of many brain areas. The strongest signals were found in the hippocampus (Fig. 4), where they were present in all hippocampal areas with highest levels in the dentate gyrus. Lower signals were detected in all cortical areas, most prominently in the superficial layers II–III and layer V. As in the developing brain, a strong signal was seen in the ependymal cells lining the brain ventricles, as well as in the chorioid plexus (Fig. 5). A strong signal was also present in the pineal body (not shown). In the hindbrain, HGF-SF mRNA was expressed in the pontine nuclei and in the deep cerebellar nuclei. A relatively strong signal was seen in cerebellar granule neurons in the adult (Fig. 5), where the expression was very low at P4. No signal above background was present in Purkinje cells.

c-*met* expression in the developing brain was detected from E13 forward, and was low up to E14, but E17 (Fig. 2), a relatively strong signal was detected in the developing cortical plate, as well as in the neurons of the cortical subplate. Posterior parts of the cortex, such as occipital and temporal areas, expressed higher levels of c-*met* mRNA compared to the frontal cortex. In addition, a clear signal was found in septal neurons at this age. At P4 (Fig. 3), there was a relatively strong expression in the outer layers of the cortex, especially in the parietal, occipital, and temporal areas. In the hippocampus, the c-*met* expression was confined to the CA-1 area, while no signal was detected in the dentate gyrus or the CA-3 area. Neurons in the septum continued to express c-*met* mRNA, and a specific signal was also detected in the developing facial nucleus. In the adult brain, the distribution of c-*met* mRNA resembled that found in P4. Thus, expression was found in the posterior cortical areas and in the CA-1 area of the hippocampus. A clear signal was again present in septal neurons (Fig. 6; see Table I).

Expression of HGF-SF in the Brain is Evolutionary Conserved

If brain HGF-SF is involved in important biological functions, its expression should have been conserved during evolution. Therefore, we analyzed HGF expression in the central nervous system of different mammalian species, as well as in avian brain.

rtPCR amplifications using specific primers derived from

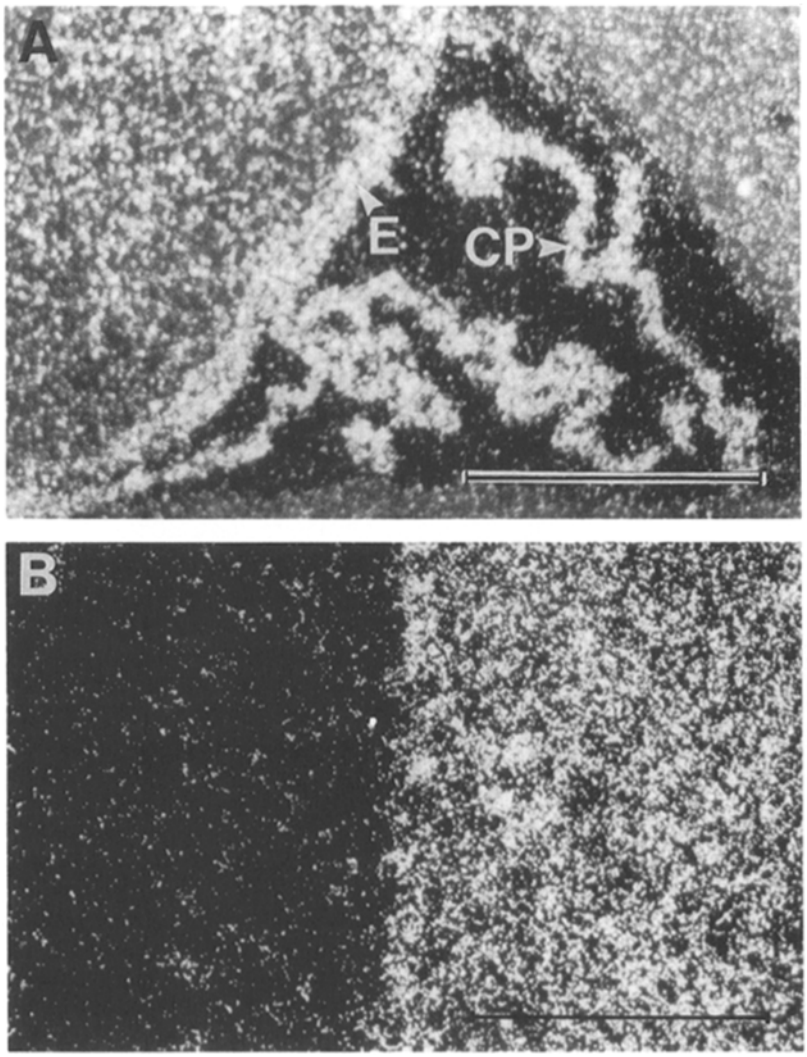

Figure 5. Expression of HGF-SF mRNA in adult rat brain. Bars, 200 μm. (*A*) HGF-SF expression in the lateral ventricle. *E*, ependymal lining; *CP*, chorioid plexus. (*B*) HGF-SF expression in the cerebellum (*right*, granule cell layer; *left*, molecular layer).

conserved regions of known HGF-SF and c-*met* cDNA sequences (Tashiro et al., 1990; Park et al., 1987) demonstrated that both transcripts are present in mouse and human brains. The identity of the amplification products was characterized by Southern blot hybridization and partial sequencing (data not shown). In situ hybridization analysis using [^{35}S]UTP-labeled rat HGF-SF riboprobes (Fig. 7) detected specific signals in hippocampal neurons of mouse and human brains. Furthermore, HGF-SF expression was demonstrated in cortical neurons of duck brain.

c-*met* transcripts were found in hippocampal neurons of adult human and mouse brains, but could not be detected in avian brain by in situ hybridization using human c-*met* probes, possibly because of lower homology of the different c-*met* mRNA sequences compared to HGF-SF (data not shown).

HGF-SF Stimulates c-fos Expression in Cultured Septal Neurons

To further investigate whether c-*met*–expressing neurons are able to respond to stimulation with HGF-SF, fetal septal neurons (E17) expressing c-*met* (see Fig. 2) were prepared and cultured for 1 wk (Lindholm et al., 1994). Since the activity of HGF-SF is conventionally assayed by functional effects elicited by epithelial cells, we decided to monitor for a potential effect of HGF-SF on neurons by analyzing early gene response. It has been demonstrated in rat fetal hepatocytes that stimulation with HGF-SF leads to an immediate activation of c-*fos* expression (Fabregat et al., 1992). As shown in Fig. 8, the addition of 30 ng/ml recombinant human HGF-SF (Yoshiyama et al., 1991) to fetal neuronal cultures from the septum induced reproducibly an approximately fourfold increase in c-*fos* mRNA levels in these cultures. Control experiments using recombinant NGF also led to a comparable increase of c-*fos* mRNA levels (sixfold) in the septal neurons. An increase in c-*fos* mRNA levels after stimulation with HGF-SF was also observed in cultured hippocampal neurons (E17), although the response varied between different primary cultures (data not shown). This was probably caused by differences in cell sampling because c-*met*–expressing neurons in the hippocampus are restricted to the CA-1 area (see Figs. 3 and 6; Table I). Taken together, the data demonstrate that stimulation with HGF-SF activates an immediate early gene response in c-*met*–expressing neurons.

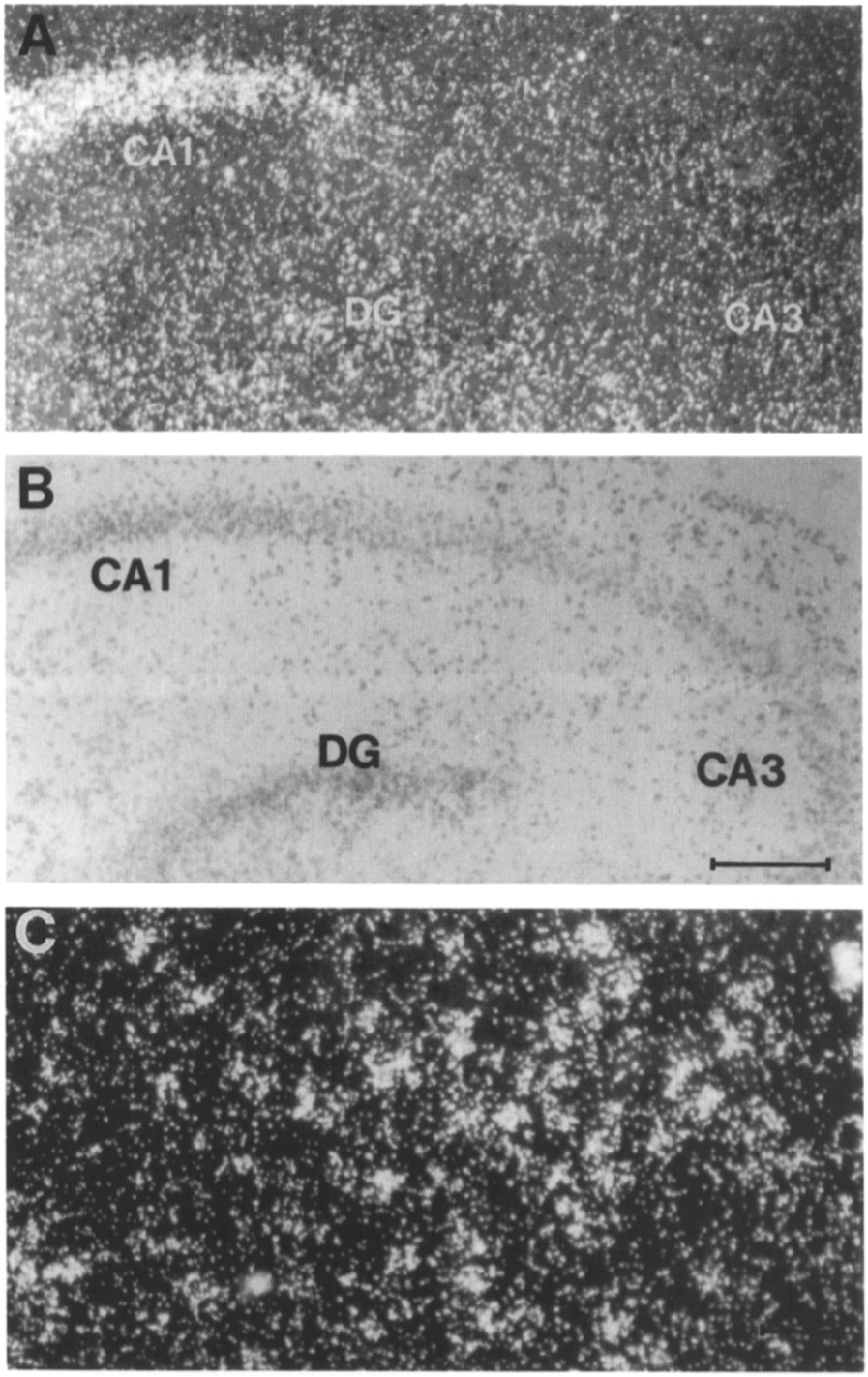

Figure 6. Expression of c-*met* mRNA in adult rat brain. (*A*) Hippocampus (*CA1/CA3*, CA-1 and CA-3 areas; *DG*, dentate gyrus) with c-*met* expression only in the CA-1 area. (*B*) Respective bright field (Bar, 200 μm). (*C*) c-*met*–positive cells in the medial septum. Bar, 100 μm.

Discussion

In this paper, we have demonstrated that HGF-SF and c-*met* are expressed in defined parts of the mammalian brain, and that, to a significant extent, both transcripts localize to neurons. HGF-SF immunolocalization to the central nervous system neurons and in the fetal brain has been described (Wolf et al., 1991; De Frances et al., 1992). Localization differences, especially in neuronal cell populations, are likely to result from the fact that previous studies could not distinguish between synthesis, uptake, and storage. While neuronal c-*met* expression has not been reported, immunolocalization of c-*met* to microglia has been reported (Di Renzo et al., 1993). So far, we have been unable to demonstrate c-*met* transcripts above background in microglia. Although this does not exclude expression, c-*met* mRNA levels in microglia must be very low. Taken together, HGF-SF and c-*met* have to be added to the family of neurotrophins and growth factors that are expressed together with their receptor tyrosine kinases in the developing and/or adult brain. These include the neurotrophic factors NGF/*trk* (Martin-Zanca et al., 1986), BDNF/*trk* (Leibrock et al.,

Table I. Semiquantitative Analysis of HGF-SF and c-met Transcripts in Adult and Developing Rat Brain

	HGF, adult	HGF, P4	c-*met*, adult	c-*met*, P4
Cortex	++ (II, III, V)	+	+ (II, III)	++
Hippocampus				
DG	++++	(+)	–	–
CA-1	+++	+	++	++
CA-3	+++	(+)	–	–
Olfactory bulb	++	–	+	–
Septum	+	–	+	++
Thalamus	+	++ (Medial habenula)	–	–
Pons	+ (Pontine nuclei, deep cerebellar nuclei)	(+)	–	–
Cerebellum				
Granule cells	++++	(+)	–	–
Purkinje cells	–	–	–	–
Pineal body	+++++	n.d.	–	n.d.
Ependyma	+++++	+++++	–	–

DG, dentate gyrus; n.d., not done.

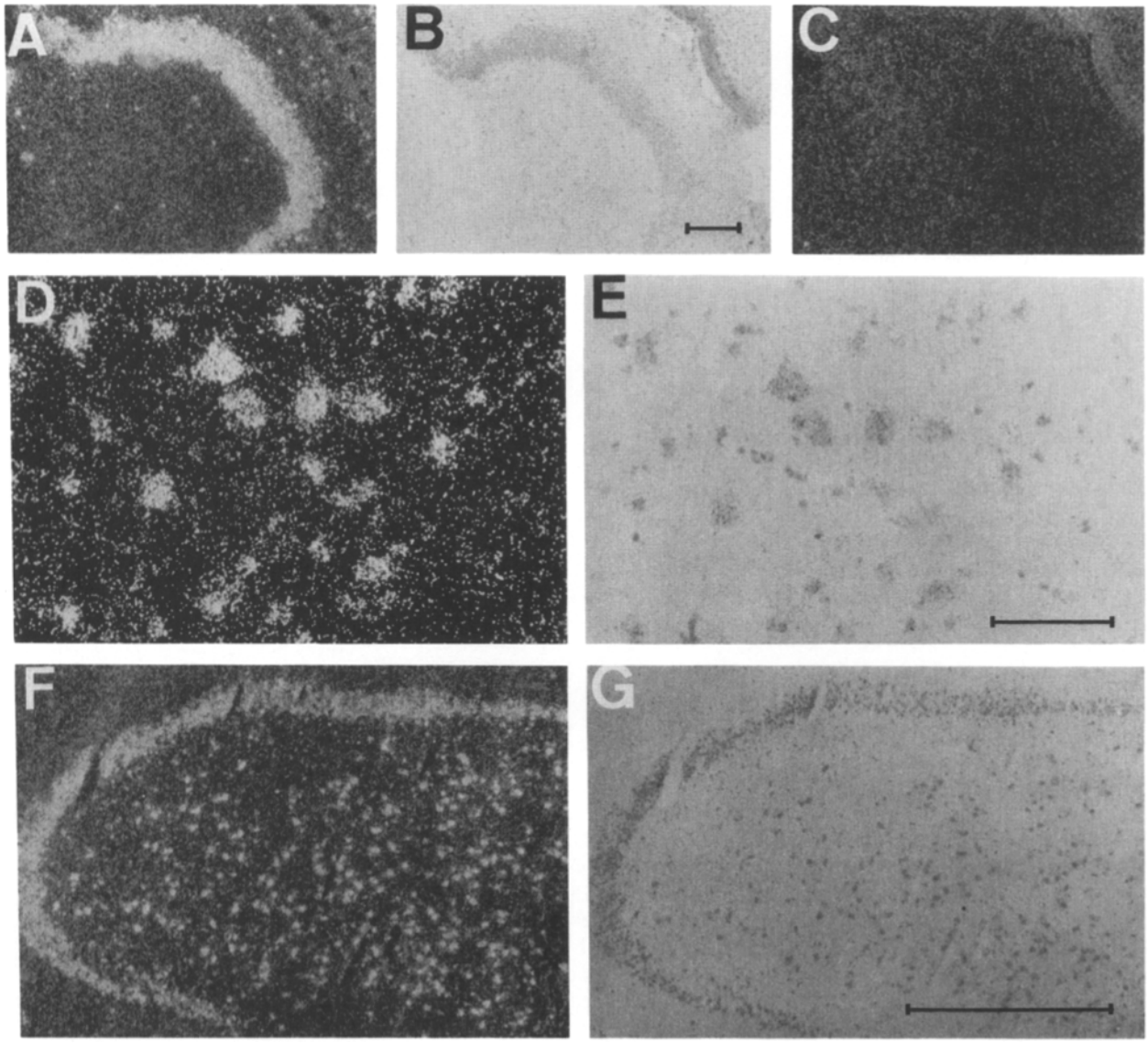

Figure 7. Evolutionary conservation of HGF-SF expression in neurons of adult brain. (*A–C*) HGF-SF expression in adult mouse hippocampus (*A*, antisense probe, dark field; *B*, corresponding bright field; *C*, control hybridization with respective HGF-SF sense probe). (*D* and *E*) HGF-SF expression in cortical neurons of adult duck brain (*D*, dark field; *E*, corresponding bright field). (*F* and *G*) HGF-SF expression in human hippocampus (*F*, dark field; *G*, corresponding bright field) (Bars, 200 μm in *B*, 100 μm in *E*, and 1 mm in *G*).

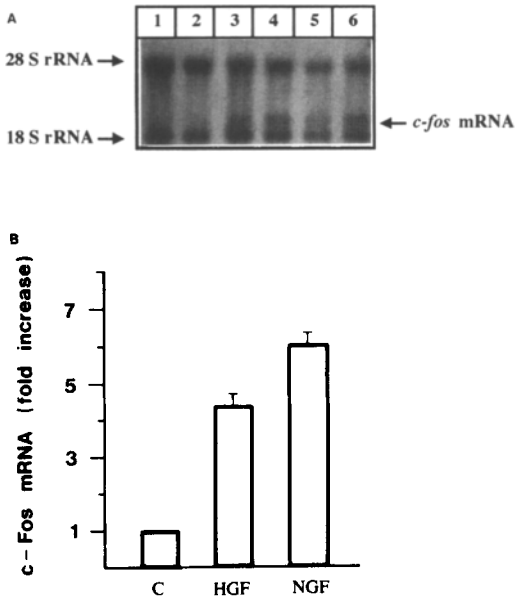

Figure 8. Effect of HGF-SF and NGF on c-*fos* mRNA levels in septal neurons. (*A*) Northern hybridization for c-*fos* of total cellular RNA from septal neurons incubated with 30 ng/ml of recombinant HGF-SF (lanes *3* and *4*), 30 ng/ml of recombinant NGF (lanes *5* and *6*), or just serum albumin (lanes *1* and *2*) for 30 min. Positions of the c-*fos* transcripts and 28S and 18S ribosomal RNAs are indicated. (*B*) Semiquantitative analysis of c-*fos* mRNA levels determined by densitometry analysis of Northern hybridization signals normalized for respective signal intensities of rehybridizations for β-actin mRNA. The values represent the mean and standard deviation of four different experiments. *C*, control; $P < 0.01$ for NGF and HGF-SF vs *C*.

1989; Klein et al., 1990), FGF/*flg* (Gospodarowicz et al., 1984; Wanaka et al., 1990), as well as the growth factors steel/c-*kit* (Keshet et al., 1991) and EGF/EGFR (Fallon et al., 1984; Rall et al., 1985; Gomez-Pinilla et al., 1988). Recent evidence suggests that of those, at least BDNF partly acts in a paracrine and/or autocrine manner on hippocampal (Collazo et al., 1992; Berninger et al., 1993) and developing cerebellar neurons (Lindholm et al., 1993; Leingärtner et al., 1994). To a significant extent, brain HGF-SF is also expressed in the ependyma and the chorioid plexus, indicating that HGF/SF may be secreted into the cerebrospinal fluid.

c-*met* represents the high affinity HGF-SF receptor (Naldini et al., 1991; Bottaro et al., 1991), and it is able to mediate all the known effects of HGF-SF in epithelial cells (Weidner et al., 1993). We have also demonstrated that HGF/SF induces a physiological response in c-*met*–expressing neurons. Therefore, it is reasonable to assume that expression of both genes in the brain leads to a functional interaction.

The high conservation of HGF-SF and c-*met* expression in the brain during evolution suggests that they are likely to mediate an important function. Known HGF-SF coding and even promoter sequences show extremely little variation between species (Nakamura et al., 1989; Tashiro et al., 1990; Miyazawa et al., 1991; Jung, W., and P. Schirmacher, unpublished results), and at least neuronal HGF-SF expression is conserved even beyond the mammalian kingdom.

The function of HGF-SF in the brain differs from the conventional HGF-SF–induced, c-*met*–mediated mesenchymal–epithelial and –endothelial interaction, at least with respect to the cell types involved. Interestingly, melanocytes, cells of neural crest origin, are stimulated to grow by HGF-SF (Matsumoto et al., 1991). In the adult, both genes are expressed in neurons in a way that paracrine, interneuronal signaling is possible. Furthermore, in some parts of the brain (CA-1 area of the hippocampus and cortical layers II–III), both HGF-SF and c-*met* mRNAs, are expressed, which suggests the potential for an autocrine cycle, although we currently cannot provide direct evidence for colocalization of both messages in single neurons.

In analogy to the neurotrophins, such as NGF and BDNF, both of which are highly expressed in the hippocampus, HGF-SF may possibly represent a target-derived neurotrophic factor. For example, HGF-SF–expressing neurons in the hippocampus may either act on c-*met*–expressing neurons in the CA-1 area of the hippocampus or c-*met*–positive neurons in the septum that project into the hippocampus. This hypothesis is further supported by the fact that HGF-SF can induce an immediate early gene response in c-*met*–expressing neurons derived from the septum to an extent comparable to the known stimulatory effect of NGF. In addition activation of hybrid c-*met* receptors in PC12 cells induces neurite outgrowth (Litzenburger, T., N. Inagaki, P. Schirmacher, and D. Lindholm, manuscript in preparation). Our data using septal neurons provide strong evidence for the HGF-SF/c-*met* interaction to constitute a novel signaling system active in the developing brain and possibly also in adult mammalian brain. The observed effects suggest a trophic role of HGF-SF for a subpopulation of central nervous system neurons. Whether HGF-SF has additional effects on responsive neurons in the brain remains to be analyzed.

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References

Berninger, B., D. E. Garcia, N. Inagaki, C. Hahnel, and D. Lindholm. 1993. BDNF and NT-3 induce intracellular Ca^{2+} elevation in hippocampal neurones. *Neuroreport.* 4:1303–1306.

Bottaro, D. P., J. S. Rubin, D. L. Faletto, A. M.-L. Chan, T. E. Kmiecik, G. F. Vande Woude, and S. A. Aaronson. 1991. Identification of the hepatocyte growth factor receptor as the c-*met* proto-oncogene product. *Science (Wash. DC).* 251:802–804.

Bussolino, F., M. F. Di Renzo, M. Ziche, E. Bocchielto, M. Olivero, L. Naldini, G. Gandino, L. Tamagnone, A. Coffer, and P. M. Comoglio. 1992. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J. Cell Biol.* 119:629–641.

Chirgwin, J. M., A. E. Przbyla, R. J. MacDonald, and W. J. Rutter. 1979.

Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidium-thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

Collazo, D., H. Takahashi, and R. D. G. McKay. 1992. Cellular targets and trophic functions of neurotrophin-3 in the developing rat hippocampus. *Neuron.* 9:643–656.

De Frances, M., H. Wolf, G. K. Michalopoulos, and R. Zarnegar. 1992. The presence of hepatocyte growth factor in the developing rat. *Development.* 116:387–395.

Di Renzo, M. F., A. Bertolotto, M. Olivero, T. Crepaldi, C. A. Pagni, and P. M. Comoglio. 1993. Selective expression of the met/HGF-receptor in human central nervous system microglia. *Oncogene.* 8:219–222.

Di Renzo, M. F., R. P. Narsimhan, M. Olivero, S. Bretti, S. Giordano, E. Medico, P. Gaglia, P. Zara, and P. M. Comoglio. 1991. Expression of the c-met/HGF receptor in normal and neoplastic human tissues. *Oncogene.* 6:1997–2003.

Fabregat, I., C. De Juan, T. Nakamura, and M. Benito. 1992. Growth stimulation of rat fetal hepatocytes in response to hepatocyte growth factor: modulation of c-myc and c-fos expression. *Biochem. Biophys. Res. Commun.* 189:684–690.

Fallon, J. H., K. B. Serrogy, S. E. Loughlin, R. S. Morrison, R. A. Bradshaw, D. J. Knauer, and D. D. Cunningham. 1984. Epidermal growth factor immunoreactive material in the central nervous system: location and development. *Science (Wash. DC).* 224:1107–1109.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.

Gilliland, G., S. Perrin, and H. F. Bunn. 1990. Competitive PCR for quantitation of mRNA. *In* PCR Protocols: A Guide to Methods and Application. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, editors. Academic Press, San Diego, CA. pp. 60–69.

Gomez-Pinilla, F., D. J. Knazer, and M. Nieto-Sampredo. 1988. Epidermal growth factor receptor immunoreactivity in rat brain development and cellular localization. *Brain Res.* 438:385–390.

Gospodarowicz, D., J. Cheng, G. Lui, A. Baird, and P. Böhlen. 1984. Isolation of brain fibroblast growth factor by heparin-sepharose affinity chromotography: identity with pituitary fibroblast growth factor. *Proc. Natl. Acad. Sci. USA.* 81:6963–6967.

Ishiki, Y., H. Ohnishi, Y. Muto, K. Matsumoto, and T. Nakamura. 1992. Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo. *Hepatology.* 16:1227–1235.

Keshet, E., S. D. Lyman, D. E. Williams, D. M. Anderson, N. A. Jenkins, N. G. Copeland, and L. F. Parada. 1991. Embryonic RNA expression patterns of the c-*kit* receptor and its cognate ligand suggest multiple functional roles in mouse development. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2425–2435.

Klein, R., D. Martin-Zanca, M. Barbacid, and L. F. Parada. 1990. Expression of tyrosine kinase receptor gene trkB is confined to the murine embryonic and adult nervous system. *Development.* 109:845–850.

Leibrock, J., F. Lottspeich, A. Hohn, M. Hofer, B. Hengerer, P. Masiakowski, H. Thoenen, and Y.-A. Barde. 1989. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature (Lond.).* 341:149–152.

Leingärtner, A., C.-P. Heisenberg, R. Kohlbeck, H. Thoenen, and D. Lindholm. 1994. Brain-derived neurotrophic factor increases neurotrophin-3 expression in cerebellar granule neurons. *J. Biol. Chem.* 269:828–830.

Lindholm, D., R. Heumann, B. Hengerer, and H. Thoenen. 1988. Interleukin 1 increases stability and transcription of mRNA encoding nerve growth factor in cultured rat fibroblasts. *J. Biol. Chem.* 263:16348–16351.

Lindholm, D., G. Dechant, C.-P. Heisenberg, and H. Thoenen. 1993. Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. *Eur. J. Neurosci.* 5:1455–1465.

Lindholm, D., J. Hartikka, M. de Penha Berzaghi, E. Castren, G. Tzimargiorgis, R. A. Hughes, and H. Thoenen. 1994. Fibroblast growth factor-5 promotes differentiation of cultured rat septal cholinergic and raphe serotonergic neurons: comparison with the effects of neurotrophins. *Eur. J. Neurosci.* In press.

Matsumoto, K., H. Tajima, and T. Nakamura. 1991. Hepatocyte growth factor is a potent stimulator of human melanocyte DNA synthesis and growth. *Biochem. Biophys. Res. Commun.* 176:45–51.

Martin-Zanca, D., S. H. Hughes, and M. Barbacid. 1986. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature (Lond.).* 319:743–748.

Michalopoulos, G. K., W. Bowen, A. K. Nussler, M. J. Beich, and T. A. Howard. 1993. Comparative analysis of mitogenic and morphogenic effects of HGF and EGF on rat and human hepatocytes maintained in collagen gels. *Am. J. Physiol.* 156:443–452.

Miyazawa, K., A. Kitamura, and N. Kitamura. 1991. Structural organization and the transcription initiation site of the human hepatocyte growth factor gene. *Biochemistry.* 30:9170–9176.

Montesano, R., K. Matsumoto, T. Nakamura, and L. Orci. 1991. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell.* 67:901–908.

Nakamura, T., T. Nishizawa, M. Hagiya, T. Seki, M. Shimonishi, A. Sugimura, K. Tashiro, and S. Shimizu. 1989. Molecular cloning and expression of human hepatocyte growth factor. *Nature (Lond.).* 342:440–443.

Naldini, R., K. M. Weidner, E. Vigna, G. Gandino, A. Bardelli, C. Ponzetto, R. P. Narsimhan, G. Hartmann, R. Zarnegar, G. K. Michalopoulos et al. 1991. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2867–2878.

Park, M., M. Dean, K. Kaul, M. J. Braun, M. A. Gouda, and G. Vande Woude. 1987. Sequence of MET protooncogene cDNA has features of the tyrosine kinase family of growth-factor receptors. *Proc. Natl. Acad. Sci. USA.* 84:6379–6383.

Rall, L. B., J. Scott, G. I. Bell, R. J. Crawford, J. D. Penschow, H. D. Niall, and J. P. Coghlan. 1985. Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. *Nature (Lond.).* 313:228–231.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schirmacher, P., A. Geerts, A. Pietrangelo, H. P. Dienes, and C. E. Rogler. 1992. Hepatocyte Growth Factor/Hepatopoietin A is expressed in fat-storing cells from rat liver but not myofibroblast-like cells derived from fat-storing cells. *Hepatology.* 15:5–11.

Sonnenberg, E., D. Meyer, K. M. Weidner, W. Birchmeier, and C. Birchmeier. 1993. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.* 123:223–235.

Stern, C. D., G. W. Ireland, S. E. Herrick, E. Gherardi, J. Gray, M. Perryman, and M. Stoker. 1990. Epithelial scatter factor and development of the chick embryonic axis. *Development.* 110:1271–1284.

Stoker, M., E. Gherardi, M. Perryman, and J. Gray. 1987. Scatter factor is a fibroblast derived modulator of epithelial cell mobility. *Nature (Lond.).* 327:239–242.

Tashiro, K., M. Hagiya, T. Nishizawa, T. Seki, M. Shimonishi, S. Shimizu, and T. Nakamura. 1990. Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissue. *Proc. Natl. Acad. Sci. USA.* 87:3200–3204.

Theiler, K. 1972. The House Mouse. Development and Normal Stages from Fertilization to 4 Weeks Age. Springer-Verlag, Berlin. 168 pp.

Wanaka, A., E. M. Johnson, Jr., and J. Millbrandt. 1990. Localization of FGF receptors mRNA in the adult rat central nervous system by in situ hybridization. *Neuron.* 5:267–281.

Weidner, K. M., J. Behrens, J. Vandekerckhove, and W. Birchmeier. 1990. Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J. Cell Biol.* 11:2097–2108.

Weidner, K. M., M. Sachs, and W. Birchmeier. 1993. The c-*met* receptor tyrosine kinase transduces motility, proliferation, and morphogenic signals of Scatter Factor/Hepatocyte Growth factor in epithelial cells. *J. Cell Biol.* 111:145–154.

Wolf, H. K., R. Zarnegar, and G. K. Michalopoulos. 1991. Localization of hepatocyte growth factor in human and rat tissues: an imunohistochemical study. *Hepatology.* 14:1035–1043.

Yoshiyama, Y., N. Arakaki, D. Naki, K. Takahashi, S. Hirono, J. Kondo, H. Nakayama, E. Gohda, N. Kitamura, H. Tsubouchi et al. 1991. Identification of the N-terminal residue of the heavy chain of both native and recombinant human hepatocyte growth factor. *Biochem. Biophys. Res. Commun.* 175:660–667.

Zafra, F., B. Hengerer, J. Leibrock, H. Thoenen, and D. Lindholm. 1990. Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3545–3550.

Zarnegar, R., M. C. De Frances, D. P. Kost, P. Lindroos, and G. K. Michalopoulos. 1991. Expression of hepatocyte growth factor mRNA in regenerating rat liver after partial hepatectomy. *Biochem. Biophys. Res. Commun.* 177:559–565.

Zarnegar, R., and G. Michalopoulos. 1989. Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. *Cancer Res.* 49:3314–3320.

Zarnegar, R., S. Muga, R. Raija, and G. Michalopoulos. 1990. Tissue distribution of hepatopoietin A: a heparin binding polypeptide growth factor for hepatocytes. *Proc. Natl. Acad. Sci. USA.* 87:1252–1256.