



The Expression of Insulin-like Growth Factor-I, II and Their Cognate Receptor 1 and 2 during Mouse Tongue Embryonic and Neonatal Development

Authors: Yamane, Akira, Mayo, Mark L., and Shuler, Chuck

Source: Zoological Science, 17(7) : 935-945

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.17.935>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

The Expression of Insulin-like Growth Factor-I, II and Their Cognate Receptor 1 and 2 during Mouse Tongue Embryonic and Neonatal Development

Akira Yamane^{1*}, Mark L. Mayo² and Chuck Shuler²

¹*Department of Pharmacology, School of Dental Medicine, Tsurumi University, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, Japan*

²*Center for Craniofacial Molecular Biology, University of Southern California, 2250 Alcazar Street, Los Angeles, CA*

ABSTRACT—While the signal of insulin-like growth factors (IGF) in skeletal muscle cells is known to be mediated by IGF receptor (IGFR) 1, not IGFR 2, there is no report even on the expression of IGFRs during tongue myogenesis. Here we examined changes in the mRNA level of IGFR 1 and 2, in addition to IGF-I and II, during mouse tongue myogenesis between embryonic day (E) 11 and newborn by competitive reverse-transcriptase polymerase chain reaction. Immunolocalization of IGF-I, II, IGFR 1 and 2 was examined using confocal laser scanning microscope at E13, E15 and newborn stages. Immunolocalization of fast myosin heavy chain was also examined to detect differentiating myoblasts, myotubes and myofibers. IGF-I, II and IGFR 1 mRNAs were highly expressed between E13 and E15 during differentiation of myoblasts and formation of myotubes. IGF-I and II proteins were co-localized to differentiating myoblasts, myotubes and myofibers with IGFR 1 protein. High level expression of IGFR 2 mRNA was also observed between E13 and E15. However, the expression of IGFR 2 protein was sparsely observed throughout the whole tongue tissues and not restricted to the striated muscle tissue. These data suggest that IGFR 1 is related to the IGF signal transduction and the differentiation of mouse tongue striated muscle, whereas IGFR 2 is not directly involved in them.

INTRODUCTION

The roles of insulin-like growth factor (IGF)-I and II in myogenesis of cultured myoblasts have been well evaluated (reviewed by Florini *et al.*, 1994; 1996). The autocrine secretions of IGF-I and II stimulate proliferation and subsequently differentiation of cultured myoblasts (Engert *et al.*, 1996; Ewton *et al.*, 1994; Florini *et al.*, 1991; Rosenthal and Cheng, 1995; Yoshiko *et al.*, 1996). The mitogenic action of IGFs utilizes mitogen-activated protein (MAP) kinase signaling pathway, while phosphatidylinositol 3-kinase/p70^{S6k} signaling pathway is essential for the IGF-stimulated differentiation (Coolican *et al.*, 1997). Recently it has been reported that IGF-I is involved in the regulation of skeletal muscle hypertrophy and a shift in myofiber phenotypes through Ca²⁺-calcineurin signaling pathway (Semsarian *et al.*, 1999).

It is known that both the IGF-I and II can bind to IGF receptor (IGFR) 1, 2, and insulin receptor (reviewed by Florini *et al.*, 1996). However, the IGF signalings during skeletal

myogenesis are shown to be mediated only by IGFR 1 (Liu *et al.*, 1993; Navarro *et al.*, 1997). It appears that IGFR 2 serves IGF-II turnover in skeletal muscle tissue (Ewton *et al.*, 1987; Kiess *et al.*, 1987; Lau *et al.*, 1994; Ludwig *et al.*, 1996; Wang *et al.*, 1994).

The actions of the IGFs appear to be modulated by a family six high-affinity IGF binding proteins (IGFBP), designated to IGFBP-1 to 6 (reviewed by Jones and Clemmons, 1995). The IGFBPs function not only as modulators of the IGF actions, but also as carrier proteins for the IGFs in the circulation. It has been reported that five IGFBPs except for IGFBP-3 are expressed and modulate the IGF actions in skeletal muscles (Ferguson *et al.*, 1992; Florini *et al.*, 1996).

It has been already reported that several unique developmental characteristics differentiate tongue striated muscle from other skeletal muscles such as limb and trunk muscles. The maturation of myofibers in tongue striated muscle occurs earlier than in other skeletal muscles (Prigozy *et al.*, 1997; Yamane *et al.*, 2000). Transforming growth factor α promotes the early myogenesis in mouse tongue (Yamane *et al.*, 1997; 1998a; b), while it inhibits myogenesis in mouse hind limb muscle (Luetteke *et al.*, 1993). Hepatocyte growth factor is not involved in the migration of tongue precursor cells

* Corresponding author: Tel. +81-45-581-1001;
FAX. +81-45-573-9599.
E-mail: GAH03667@nifty.ne.jp

(Mackenzie *et al.*, 1998), but is involved in the migration of hind limb muscle precursor cells (Bladt *et al.*, 1995).

In vivo expressions of IGFs, IGFRs and IGFBPs during the development of skeletal muscles have not been examined intensively (Ferguson *et al.*, 1992; Ishii, 1989; Kleffens *et al.*, 1999). Particularly, despite the unique developmental characteristics, there are only a few reports on the expression of IGFs and IGFBPs (Ferguson *et al.*, 1992; Kleffens *et al.*, 1999) and no report on the expression of IGFRs during the development of tongue striated muscle. We thus examined changes in the mRNA level and the immunolocalization of IGFR 1 and 2, in addition to IGF-I and II, during the development of tongue striated muscle. The immunolocalization of fast myosin heavy chain was also examined to characterize differentiating myoblasts, myotubes and myofibers (Dalrymple *et al.*, 1999).

MATERIALS AND METHODS

Tissue

Pregnant ICR mice were purchased (Nippon Clea, Tokyo, Japan). Tongues were dissected from embryos at E11, 13, 15, and 17, and from newborn mice. The tissues for PCR analysis were immediately frozen and stored at -80°C until use. Five or six samples were collected at each developmental stage. Four preparations at E11 and two preparations at E13 were pooled due to their small amounts of RNA. The tissues obtained from E13, E15 and newborn mice for immunohistochemistry were immediately fixed in Bouin's solution.

RNA extraction and competitive RT-PCR amplification

Total RNA extraction and competitive RT-PCR amplification were performed as previously described (Yamane *et al.*, 2000; Yamane *et al.*, 1998b). Briefly, total RNA extraction was performed according to the manufacturer's specifications of Rapid total RNA isolation kit (5 Prime-3 Prime Inc., Boulder, CO, USA). The RNA was treated with 2 units of ribonuclease-free deoxyribonuclease I (Life Technologies, Gaithersburg, MD, USA), and then was transcribed with 200 units of reverse transcriptase (SuperScript II, Life Technologies, Gaithersburg, MD, USA).

In conventional PCR technique, due to the exponential nature of PCR, any variations affecting amplification efficiency could result in dramatic changes in product yield and the plateau effect after many cycles could lead to inaccurate estimate of product yield. To overcome these problems, the competitors (internal standard), DNA fragment with the same sequences as each gene-specific primer at 3' and 5' ends, was used for the competitive RT-PCR amplification (Gilliland *et al.*, 1990; Siebert and Larrick, 1992; Yamane *et al.*, 1998b; 2000). The competitors constructed according to the manufacturer's instructions of PCR MIMIC Construction Kit (Clontech Laboratory Inc., Palo Alto, CA, USA) were amplified with 50 ng of the total cDNA in the presence of primer pair specific to target gene in a thermal cycler (TP3000, TaKaRa Biochemicals, Shiga, Japan). The sequences and product size for target gene-specific primers except for glyceraldehyde-phosphate dehydrogenase (GAPDH) are shown in Table 1 and those for GAPDH were previously reported (Zhao *et al.*, 1995). To determine the specificities of primers for IGFR 1 and 2, the sequences of the resultant PCR products were analyzed by an automated sequencer. The specificities of primers for IGF-I and II were previously confirmed by Hannon *et al.* (1992). Amplification products were separated by electrophoresis on an agarose gel containing ethidium bromide. Fluorescent intensities of the bands of the target genes and their respective competitors were measured by an image analyzer (Argus-100, Hamamatsu Photonics K.K., Hamamatsu, Japan). We

Table 1. Sequences of PCR primers and product sizes for the target genes

IGF-I	
Forward	5' -GCT CTT CAG TTC GTG TGT GG-3'
Reverse	5' -TTG GGCATG TCA GTG TGG-3'
Product size	221 bp
IGF-II	
Forward	5'-CGT GGA AGA GTG CTG CTT CC-3'
Reverse	5'-GAC ATC TCC GAA GAG GCT CC-3' *
Product size	329 bp
IGFR 1	
Forward	5'-TCT TGG ATG CGGTGT CCA ATA AC-3'
Reverse	5'-GCA GCA CTC ATT GTT CTC GTT GC-3' **
Product size	215 bp
IGFR 2	
Forward	5'-TGC ACA CTC TTC TTC TCC TGG CA-3'
Reverse	5'-GCA GAT GTT GAT ATA GAA GTC AGG-3' #
Product size	186 bp

* Hannon *et al.*, 1992

** Wada *et al.*, 1993

Ludwig *et al.*, 1994

bp, base pairs

then calculated the ratios of fluorescent intensities of the target gene bands to those of their respective competitor bands.

To calculate the cDNA quantity of each target gene from the calculated ratio of fluorescent intensity, we generated a standard curve for each target gene by following ways. Each target gene was amplified by PCR in the presence of primers specific to the target genes without competitor. The amplified PCR product, identified as a single band on the electrophoretic gel, was purified by passing it through a spin column (CHROMA SPIN+TE 100, Clontech Laboratory Inc., Palo Alto, CA, USA). The quantity of cDNA in the purified product was measured by a spectrophotometer at a wavelength of 260 nm and 1:2 serial dilutions of the cDNA were made. The diluted cDNA (cDNA standard) and the respective competitor, the concentration of which was identical to that used for the experimental samples, were co-amplified by PCR and analyzed in the same manner as the experimental samples. The logarithmic values of the ratios of fluorescent intensities in the bands of known quantities of diluted cDNA to those in the bands of the respective competitors were plotted against the logarithmic values of the known quantities of the diluted cDNA. From this relation (i.e. standard curve), the quantity of cDNA for target genes was estimated in the experimental samples and divided by the quantity of GAPDH to normalize the variations in the yield of mRNA and efficiency of reverse transcription. The cDNA quantity seems to be equivalent to the mRNA quantity, because the cDNA was obtained by reverse transcription of the mRNA. The resulting value was expressed as a percent relative to the mean value of each target gene at the newborn stage.

Immunohistochemistry

Specimens for immunohistochemistry were fixed in Bouin's fixative for two hours at 4°C , immersed in a graduated series of sucrose solutions (5–40% w/v) in phosphate buffered saline (PBS) at 4°C , embedded in Tissue-Tek Oct Compound (Miles Laboratory, Elkhart, IN, USA) and frozen. Sagittal sections of tongues were prepared at a $10\ \mu\text{m}$ thickness in a cryostat and air-dried for 1 hr at room temperature. The frozen sections were post-fixed in acetone at -20°C , rehydrated in PBS, and incubated with 5% of normal goat serum for 30 min to block non-specific immunostaining. For double immunostaining, the sections were incubated with a mixture of the rabbit polyclonal antibodies against IGF-I, II (AUSTRAL Biologicals, San Ramon, CA, USA), IGFR 1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA)

or IGF2 (a kind gift from Dr. S.D. Scott, Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW, Australia; Scott and Baxter, 1987), and the mouse monoclonal antibody against fast skeletal muscle myosin heavy chain (Sigma-Aldrich Inc.). After washing 3 times in PBS, the sections were incubated with a mixture of the FITC-conjugated goat antibody against rabbit IgG and the rhodamine-conjugated goat antibody against mouse IgG (Sigma-Aldrich Inc.). After washing in PBS and subsequent incubating with Equilibration buffer of SlowFade-Light Antifade Kit (Molecular Probes Inc., Eugene, OR, USA), the stained sections were mounted in SlowFade-Light Antifade reagent and visualized with a confocal laser

scanning microscope (PCM2000, Nikon, Tokyo, Japan). For control staining, the primary antibodies were replaced with normal rabbit, mouse IgG or PBS; all controls showed no staining.

Statistical Analyses

Scheffe's method was used to compare the mean values between two groups.

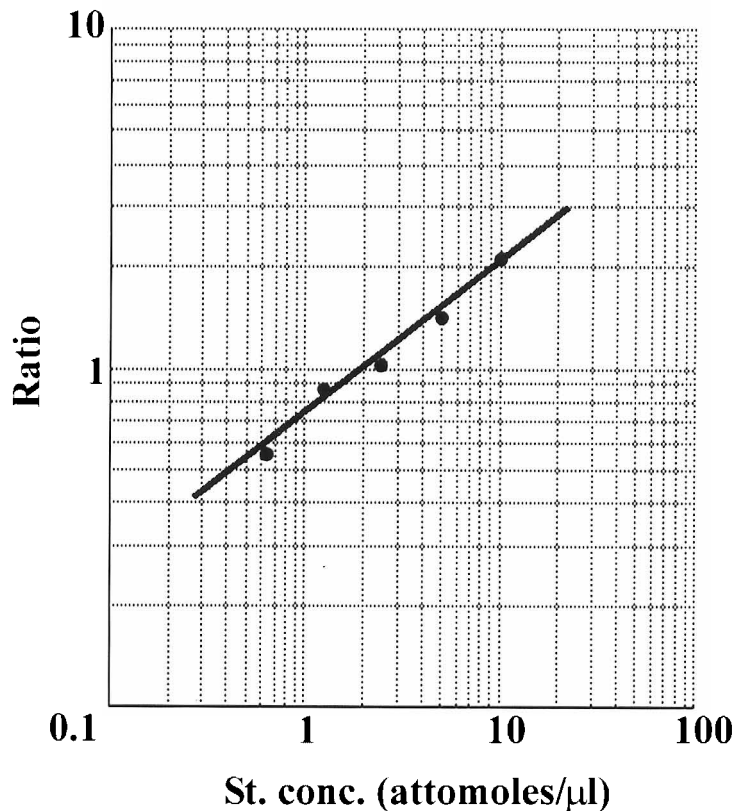
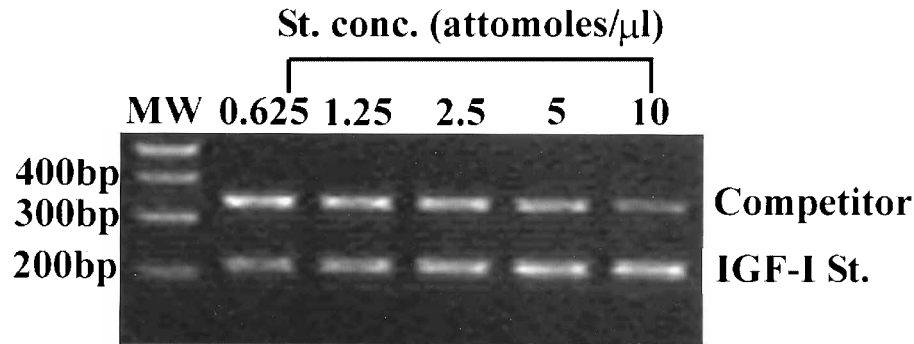


Fig. 1. Electrophoretic gel pattern of IGF-I cDNA standard and its competitor (upper panel) after competitive PCR and the standard curve (lower panel). The standard curve in the lower panel was generated from the result of image analysis of electrophoretic bands in the upper panel. The formula of the regression line is represented by $y = 0.46X - 0.15$, where y is the logarithmic value of the ratio of the fluorescent intensity in the IGF-I cDNA standard band to that in its competitor band and x is the logarithmic value of the concentration of the cDNA standard. St. conc.; Standard concentrations. MW; molecular weight markers. bp; base pairs.

RESULTS

Standard curves

Fig. 1 shows the electrophoretic gel pattern (upper panel) of IGF-I cDNA standard and its competitor after competitive PCR to generate a standard curve (lower panel). The sizes of PCR products for IGF-I cDNA standard and its competitor were 221 and 338 bp, respectively. The fluorescent intensities of IGF-I cDNA standard and competitor bands on the gel appeared to be inversely related. The formula of the regression line is represented by $y=0.46X-0.15$.

The formulae for the regression lines and correlation

Table 2. Formulae for the regression lines and correlation coefficients for IGF-I, II, IGFR 1 and 2.

Target gene	Formula	r	significance
IGF-I	$y = 0.46x - 0.15$	0.99	$p < 0.001$
IGF-II	$y = 0.38x - 0.39$	0.99	$p < 0.001$
IGFR 1	$y = 0.52x - 0.64$	0.99	$p < 0.001$
IGFR 2	$y = 1.45x - 1.57$	0.99	$p < 0.001$

y, the logarithmic value of the ratio of the fluorescent intensity in the target gene band to that in its respective competitor band.
x, the logarithmic value of the concentration of the cDNA standard.
r, correlation coefficient.

coefficients for IGF-I, II, IGFR 1 and 2 are included in Table 2. The correlation coefficients were greater than 0.99 for all the genes and were statistically significant from zero ($p < 0.001$). This result indicated that the quantities of the target gene cDNAs could be reliably determined from these standard curves.

Changes in the mRNA level of IGF-I, II, IGFR 1 and 2 during the development of mouse tongue

The mRNAs of IGF-I (Fig. 2A), II (2B), IGFR 1 (2C) and IGFR 2 (2D) were actively expressed between E13 and E15 stages in the mouse tongues. This time period corresponds with differentiation of myoblasts and formation of myotubes in tongue striated muscle. IGF-I mRNA was highly expressed between E11 and E15, then decreased in the level of expression until birth. Its quantity at the newborn stage was less than 40% ($p < 0.0001$) of the E15 value. The quantity of IGF-II mRNA increased by 43% ($p < 0.05$) between E11 and E15 and showed a peak value at E15. After E15, the quantity decreased and became 53% ($p < 0.001$) of the E15 value at the newborn stage. IGFR 1 mRNA was expressed at E11 and the expression decreased throughout all subsequent developmental stages. However, the quantities at E13 and E15, during which

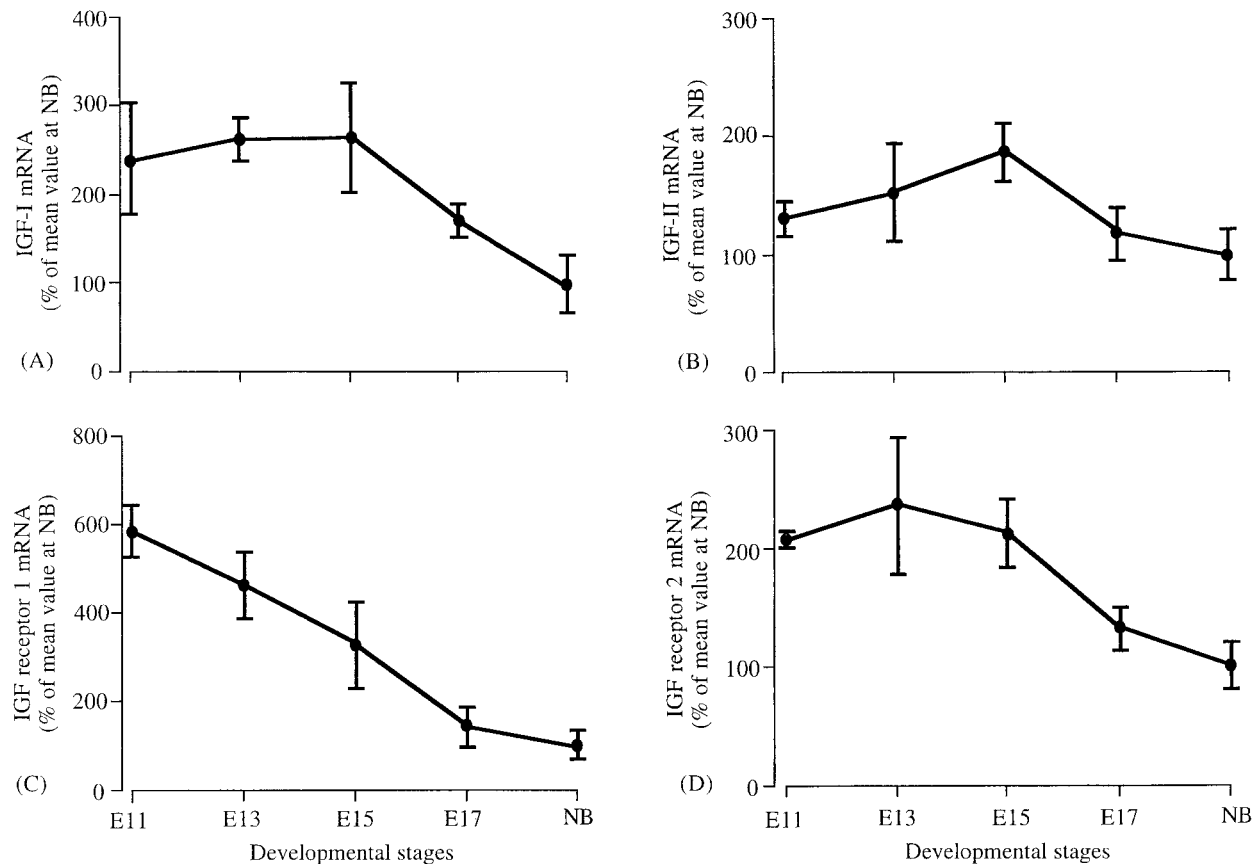


Fig. 2. Relative changes in the mRNA level for IGF-I (A), II (B), IGFR 1 (C) and IGFR 2 (D) in mouse tongues at stages E11, 13, 15 and 17, and at birth assessed by using competitive RT-PCR. Each point with its vertical bar represents the mean \pm 1 SD of five or six samples. The vertical axis is expressed as a percentage of the mean value at the newborn stage. All the four mRNAs studied were highly expressed between E13 and E15 during which differentiation of myoblasts and formation of myotubes actively occurred. NB, newborn.

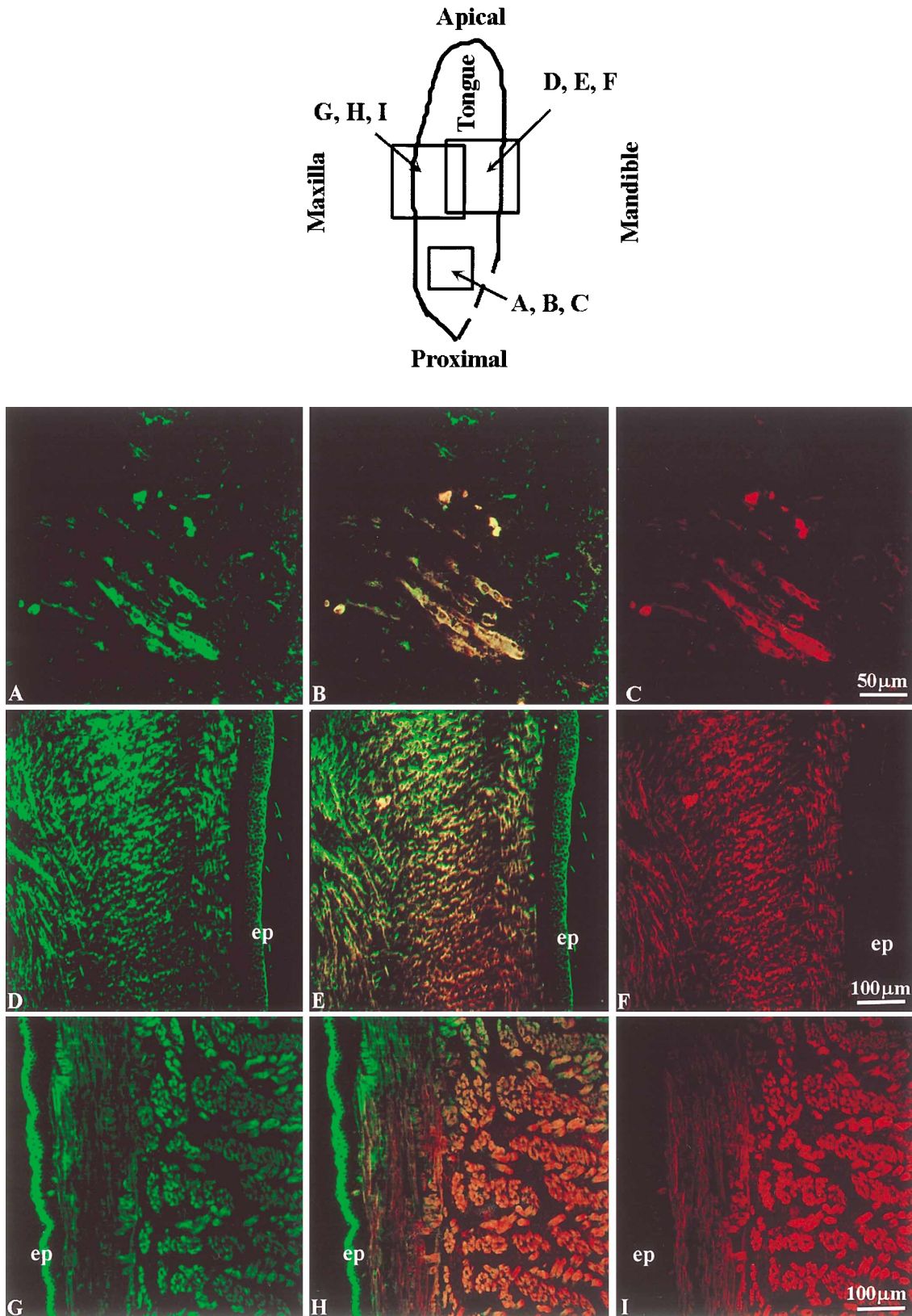


Fig. 3. Confocal microscopic images of sagittal sections of tongues obtained from E13 (A, B, C) and E15 (D, E, F) mouse embryos, and newborn mice (G, H, I). A, D and G show immunostaining for IGF-I; C, F and I show immunostaining for fast myosin heavy chain; B, E and H show double-staining. Immunostaining for IGF-I was observed in differentiating myoblasts, myotubes and myofibers. The epithelial tissue (ep) displayed strong immunostaining for IGF-I at E15 (D) and newborn (G) stages. The top diagrammatic representation shows a sagittal section of tongue viewed from the buccal side. The squares indicate the regions shown in A~I.

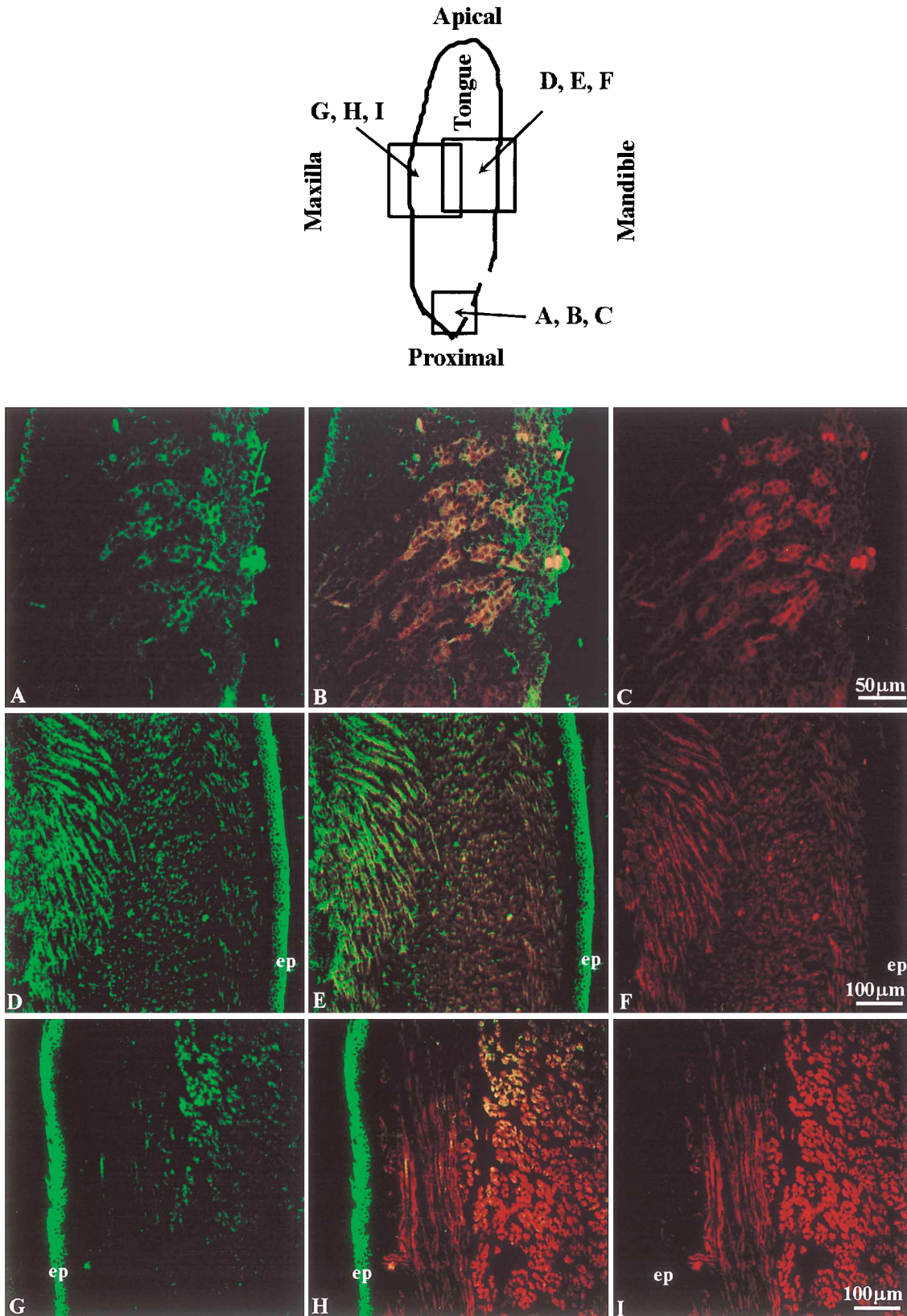


Fig. 4. Confocal microscopic images of sagittal sections of tongues obtained from E13 (A, B, C) and E15 (D, E, F) mouse embryos, and newborn mice (G, H, I). A, D and G show immunostaining for IGF-II; C, F and I show immunostaining for fast myosin heavy chain; B, E and H show double-staining. Differentiating myoblasts, myotubes and myofibers were immunostained for IGF-II. The epithelial tissue (ep) displayed strong immunostaining for IGF-II at E15 (D) and newborn (G) stages. The top diagrammatic representation shows a sagittal section of tongue viewed from the buccal side. The squares indicate the regions shown in A ~ I.

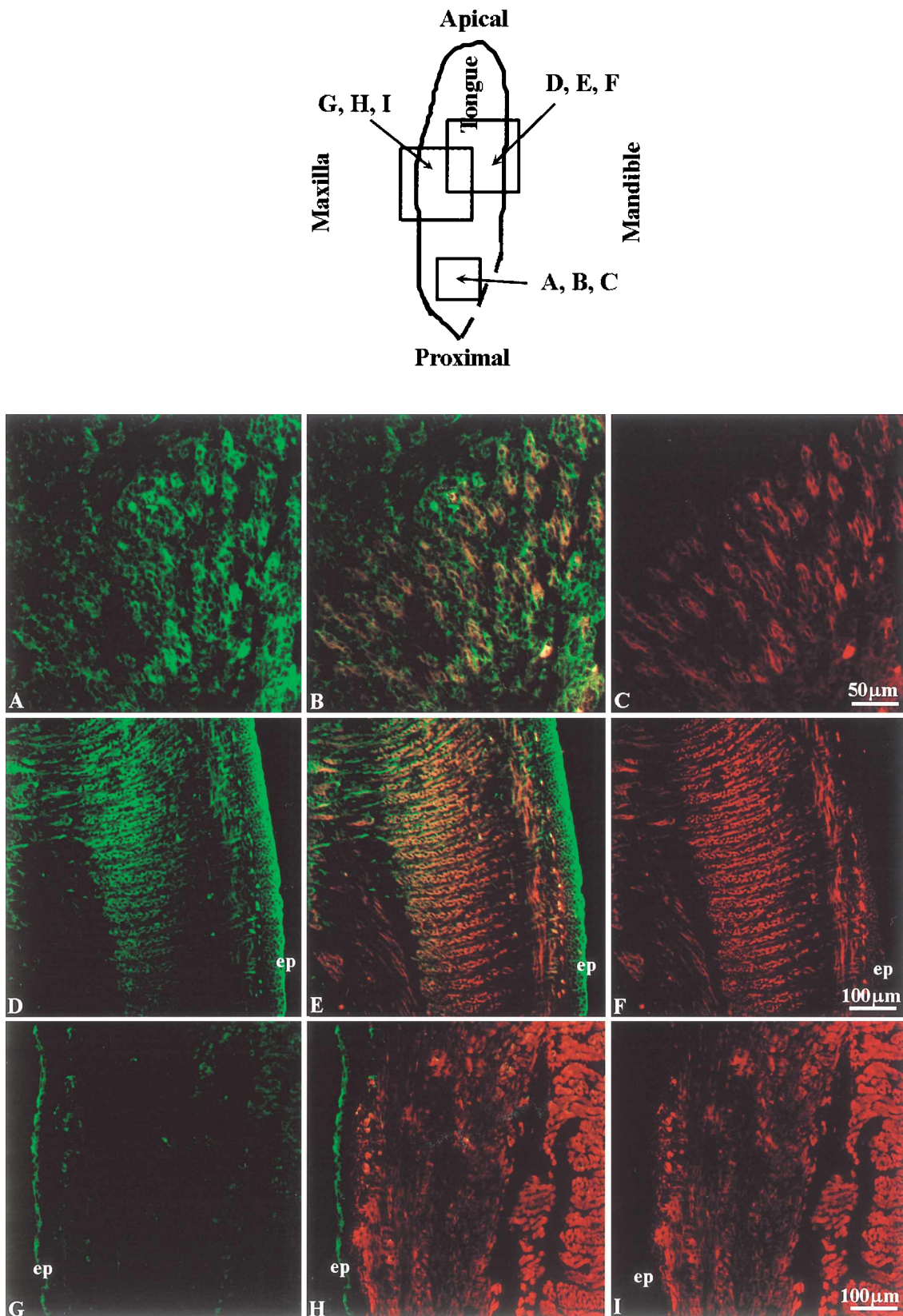


Fig. 5. Confocal microscopic images of sagittal sections of tongues obtained from E13 (A, B, C) and E15 (D, E, F) mouse embryos, and newborn mice (G, H, I). A, D and G show immunostaining for IGFR 1; C, F and I show immunostaining for fast myosin heavy chain; B, E and H show double-staining. Immunostaining for IGFR 1 was also observed in differentiating myoblasts, myotubes and myofibers. ep, epithelial tissue. The top diagrammatic representation shows a sagittal section of tongue viewed from the buccal side. The squares indicate the regions shown in A~I.

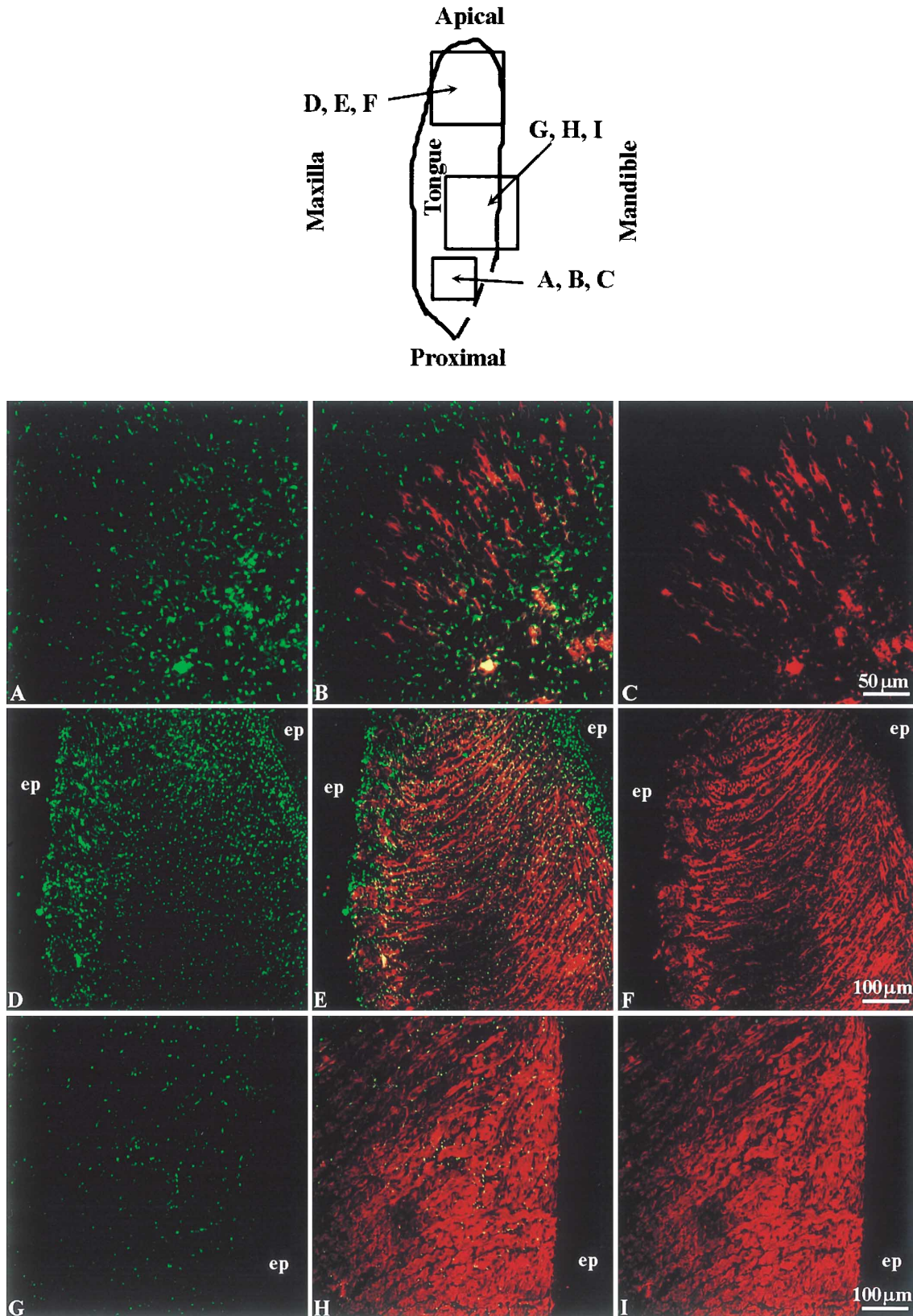


Fig. 6. Confocal microscopic images of sagittal sections of tongues obtained from E13 (A, B, C) and E15 (D, E, F) mouse embryos, and newborn mice (G, H, I). A, D and G show immunostaining for IGFR 2; C, F and I show immunostaining for fast myosin heavy chain; B, E and H show double-staining. Immunostaining for IGFR 2 was sparsely distributed in the whole tongue and not restricted to differentiating striated muscle. ep, epithelial tissue. The top diagrammatic representation shows a sagittal section of tongue viewed from the buccal side. The squares indicate the regions shown in A–I.

the tongue muscle differentiation actively occurred, were over 2-fold greater than those at E17 and newborn stages ($p < .01 \sim 0.0001$). High level expression of IGFR 2 mRNA was observed between E11 and E15. After E15, the expression decreased and ultimately became less than 50% ($p < 0.0001$) of the E15 value at the newborn stage.

Localization of IGF-I, II, IGFR 1 and 2 during the development of mouse tongue

IGF-I (Fig. 3) and II (Fig. 4) displayed similar immunostaining patterns and the proteins were present in differentiating myoblasts, myotube and myofibers in the developing mouse tongue. At E13, several cells with strong immunostaining for IGF-I and II were found in the proximal region of the developing tongue (Figs. 3A and 4A). These cells were spindle-shaped and displayed strong immunostaining for fast myosin heavy chain, indicating that these cells were differentiating myoblasts (Figs. 3B, 3C, 4B and 4C). At E15, strong immunostaining for IGF-I and II was observed in the myotubes and myofibers which displayed strong immunostaining for fast myosin heavy chain (Figs. 3D, 3E, 3F, 4D, 4E and 4F). At the newborn stage, immunostaining for both IGF-I and II was observed in well-developed muscle fibers (Figs. 3G and 4G), but was weak in comparison with that in the myotubes at E15 (Figs. 3D and 4D). The epithelial tissue of the developing tongue had a strong immunopositive reaction for both IGF-I and II at E15 and newborn stages (Figs. 3D, 3G, 4D and 4G).

The immunostaining pattern for IGFR 1 was very similar to those for IGF-I and II in the developing mouse tongue (Fig. 5). At E13, immunopositive cells for both IGFR 1 and fast myosin heavy chain were observed in the proximal region (Fig. 5A, 5B and 5C). At both the E15 and newborn stages, the immunostaining for IGFR 1 was observed in myotubes and myofibers, which also contained fast myosin heavy chain (Fig. 5D ~ 5I). The immunoreaction for IGFR 1 at the newborn stage was very weak (Fig. 5G) in comparison with that at the E13 and E15 stages (Fig. 5A and 5D). The epithelial tissue in the developing tongue was immunopositive for IGFR 1 at both the E15 and newborn stages (Fig. 5D and 5G).

The immunostaining pattern for IGFR 2 in the developing mouse tongue differed from the other three proteins studied (Fig. 6). Dot-shaped immunostaining for IGFR 2 was distributed sparsely throughout the developing tongue except for the epithelial tissue at all stages studied (Fig. 6A, 6D and 6G). The immunostaining was not restricted to differentiating myoblasts, myotube and myofibers (Fig. 6B, 6E and 6H). Immunostaining at the newborn stage (Fig. 6G) was less than at the E13 (Fig. 6A) and E15 (Fig. 6D) stages.

DISCUSSION

We observed that mRNA and protein of IGF-I and II were highly expressed in differentiating myoblasts and myotubes of mouse tongue (Figs. 2, 3 and 4). Previously, few studies examined *in vivo* expression of IGF-I and II in skeletal muscle tissues (Ferguson *et al.*, 1992; Ishii, 1989). Ferguson *et al.*

(1992) detected IGF-I and II gene expression in developing mouse tongue between E12 and E15, which is consistent with our present observation. Ishii (1989) reported that IGF-II mRNA increased with accumulation of polyneuronal innervation and decreased with elimination of superfluous synapses in rat hind limb muscle. Polyneuronal innervation accumulated between E11 and E15, and elimination of superfluous synapses occurred after E15 in mouse tongue striated muscle (Yamane *et al.*, unpublished data). The present results seem to accord with the results in rat hind limb muscle (Ishii, 1989).

It has been already reported that autocrine secretion of IGF-I and II stimulates differentiation of cultured myoblasts such as C2C12 and L6 (Florini *et al.*, 1991; Yoshiko *et al.*, 1996). Thus the expression of IGF-I and II in differentiating myoblasts and myotubes (Figs. 3 and 4) suggests that the autocrine signals of IGF-I and II regulate differentiation of mouse tongue myoblast and formation of myotube, too. IGF-I and II gene expression persisted in the mouse tongue myofibers at the newborn stage (Figs. 3 and 4). Recently, it has been reported that stable expression of IGF-I in C2C12 myogenic cells results in a switch to glycolytic metabolism, suggesting that IGF-I may be related to a change in myofiber phenotypes (Semsarian *et al.*, 1999). Since mouse tongue myofibers mature to be fast-twitch glycolytic between E15 and newborn stages (Prigozy *et al.*, 1997; Yamane *et al.*, 2000), IGF-I and II expression in tongue striated muscle at the newborn stage may be involved in maturation into fast-twitch glycolytic fibers.

Strong immunostaining for IGF-I and II was observed in the mouse tongue epithelial tissue adjacent to the striated muscle tissue at E15 and newborn stages (Figs. 3 and 4). The development of craniofacial organs such as tooth and Meckel's cartilage is thought to depend on inductive interactions between epithelium and mesenchyme (reviewed by Hall 1992 and Slavkin, 1988). The present data suggest the potential that paracrine secretion of IGF-I and II from the tongue epithelial tissue may play a role in differentiation and maturation of mouse tongue striated muscle. Since there has been no report on the involvement of interactions between epithelial and muscle tissues in the development of mouse tongue striated muscle, further studies seem to be needed.

IGFR 1 was co-localized to differentiating myoblasts, myotubes and myofibers with IGF-I and II (Figs. 3, 4 and 5). Since many studies have shown that IGF-I and II control differentiation of myoblasts *in vitro* (reviewed by Florini *et al.*, 1994; 1996), the co-localization suggests that IGFR1 is closely involved in the autocrine signal transduction of IGFs, differentiation of myoblasts and formation of myotubes in the developing mouse tongue. It has been reported that null mutants for the IGFR 1 gene exhibit a severe growth deficiency with poor skeletal muscle formation (45% of normal size) (Liu *et al.*, 1993); overexpression of IGFR 1 affects proliferation and differentiation (Quinn and Roh, 1993; Quinn *et al.*, 1993; 1994), and abolishes the exogenous IGF requirement for differentiation of cultured myoblasts (Navarro *et al.*, 1997). These results support our conclusion.

IGFR2 displayed a different localization pattern from IGFs and IGFR 1 in the developing mouse tongue (Fig. 6). IGFR 2 protein was expressed sparsely throughout the developing tongue. This expression pattern suggests that IGFR 2 is not directly related to the signal transduction of IGFs, differentiation of myoblasts and formation of myotubes in the developing mouse tongue. There are several reports that provide supportive evidence for this conclusion. Blocking antiserum against IGFR 2 does not inhibit IGF-induced myogenesis in L6 cells (Kiess *et al.*, 1987) and IGF-I analogs with a low affinity for IGFR 2 exhibit the identical activity to native IGF-I for myogenesis in L6 cells (Ewton *et al.*, 1987).

ACKNOWLEDGEMENTS

We would like to thank Professor M. Chiba (Tsurumi University) for his support and encouragement throughout the present study. We also would like to thank Dr. S.D. Scott (Kolling Institute of Medical Research, Royal North Shore Hospital) for providing us with the antibody against IGFR 2. The part of the present study was performed by grants-in aid from the Ministry of Education, Science, Sports and Culture of Japan (No. 08672146, 10671757), and the Foundation of Pharmacodynamics to A.Y. in the Tsurumi University High-Technology Research Center.

REFERENCES

- Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C (1995) Essential role for the *c-met* receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 376: 768–771
- Coolican SA, Samuel DS, Ewton DZ, McWade FJ, Florini JR (1997) The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. *J Biol Chem* 272: 6653–6662
- Dalrymple KR, Prigozy TI, Mayo M, Kedes L, Shuler C (1999) Murine tongue muscle displays a distinct developmental profile of MRF and contractile gene expression. *Int J Dev Biol* 43: 27–37
- Engert JC, Berglund EB, Rosenthal N (1996) Proliferation precedes differentiation in IGF-I-stimulated myogenesis. *J Cell Biol* 135: 431–440
- Ewton DZ, Falen SL, Florini JR (1987) The type II insulin-like growth factor (IGF) receptor has low affinity for IGF-I analogs: Pleiotypic actions of IGFs on myoblasts are apparently mediated by type I receptor. *Endocrinology* 120: 115–123
- Ewton DZ, Roof SL, Magri KA, McWade FJ, Florini JR (1994) IGF-II is more active than IGF-I in stimulating L6A1 myogenesis: Greater mitogenic actions of IGF-I delay differentiation. *J Cell Physiol* 16: 277–284
- Ferguson MWJ, Sharpe PM, Thomas BL, Beck F (1992) Differential expression of insulin-like growth factors I and II (IGF I and II), mRNA, peptide and binding protein 1 during mouse palate development: comparison with TGF β peptide distribution. *J Anat* 181: 219–238
- Florini JR, Ewton DZ, Coolican SA (1996) Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev* 17: 481–517
- Florini JR, Ewton DZ, Magri KA, Mangiacapra FJ (1994) IGFs and muscle differentiation. *Adv Exp Med Biol* 343: 319–326
- Florini JR, Magri KA, Ewton DZ, James PL, Grindstaff K, Rotwein PS (1991) "Spontaneous" differentiation of skeletal myoblasts is dependent upon autocrine secretion of insulin-like growth factor-II. *J Biol Chem* 266: 15917–15923
- Gilliland G, Perrin S, Blanchard K, Bunn HF (1990) Analysis of cytokine mRNA and DNA: Detection and quantitation by competitive polymerase chain reaction. *Proc Natl Acad Sci USA* 87: 2725–2729
- Hall BK (1992) Cell-cell interactions in craniofacial growth and development. In "The Biological Mechanisms of Tooth Movement and Craniofacial Adaptation" Ed by Z Davidovitch, The Ohio State University, Ohio, pp 11–17
- Hannon K, Smith II CK, Bales KR, Santerre RF (1992) Temporal and quantitative analysis of myogenic regulatory and growth factor gene expression in the developing mouse embryo. *Dev Biol* 151: 137–144
- Ishii DN (1989) Relationship of insulin-like growth factor II gene expression in muscle to synaptogenesis. *Proc Natl Acad Sci USA* 86: 2898–2902
- Jones JL, Clemmons DR (1995) Insulin-like growth factors and their binding proteins: Biological actions. *Endocr Rev* 16: 3–34
- Kiess W, Haskell JF, Lee L, Greenstein LA, Miller BE, Aarons AL, Rechler MM, Nissley SP (1987) An antibody that blocks insulin-like growth factor (IGF) binding to the type II IGF receptor is neither an agonist nor an inhibitor of IGF-stimulated biologic responses in L6 myoblasts. *J Biol Chem* 262: 12745–12751
- Kleffens MV, Groffen CAH, Dits NFJ, Linderbergh-Kortleve DJ, Schuller AGP, Bradshaw SL, Pintar JE, Zwarthoff EC, Drop SLS, Neck JWV (1999) Generation of antisera to mouse insulin-like growth factor binding proteins (IGFBP)-1 to -6: Comparison of IGFBP protein and messenger ribonucleic acid localization in the mouse embryo. *Endocrinology* 140: 5944–5952
- Lau MMH, Stewart CEH, Liu Z, Bhatt H, Rotwein P, Stewart CL (1994) Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes Dev* 8: 2953–2963
- Liu J-P, Baker J, Perkins AS, Robertson EJ, Efstratiadis A (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor (*igf-1*) and type 1 IGF receptor (*igf1r*). *Cell* 75: 59–72
- Ludwig T, Tenscher K, Remmler J, Hoflack B, Lobel P (1994) Cloning and sequencing of cDNAs encoding the full-length mouse mannose 6-phosphate/insulin-like growth factor II receptor. *Gene* 142:311–312
- Ludwig T, Eggenschwiler J, Fisher P, D'Ercole AJ, Davenport ML, Efstratiadis A (1996) Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in *Igf2* and *Igf1r* null backgrounds. *Dev Biol* 177: 517–535
- Luetke NC, Lee DC, Palmiter RD, Brinster RL, Sandgren EP (1993) Regulation of fat and muscle development by transforming growth factor α in transgenic mice and in cultured cells. *Cell Growth and Differ* 4: 203–213
- Mackenzie S, Walsh FS, Graham A (1998) Migration of hypoglossal myoblast precursors. *Dev Dyn* 213: 349–358
- Navarro M, Barenton B, Garandel V, Schneckeburger J, Bernardi H (1997) Insulin-like growth factor I (IGF-I) receptor overexpression abolishes the IGF requirement for differentiation and induces a ligand-dependent transformed phenotype in C2 inducible myoblasts. *Endocrinology* 138: 5210–5219
- Prigozy TI, Dalrymple K, Shuler C, Kedes L (1997) Differential expression of troponin C genes during tongue myogenesis. *Dev Dyn* 209: 36–44
- Quinn LS, Ehsan M, Steinmetz B, Kaleko M (1993) Ligand-dependent inhibition of myoblast differentiation by overexpression of the type-1 insulin-like growth factor receptor. *J Cell Physiol* 156: 453–461
- Quinn LS, Roh JS (1993) Overexpression of the human type-1 insulin-like growth factor receptor in rat L6 myoblasts induces ligand-dependent cell proliferation and inhibition of differentiation. *Exp Cell Res* 208: 504–508
- Quinn LS, Steinmetz B, Maas A, Ong L, Kaleko M (1994) Type-1 insulin-like growth factor receptor overexpression produces dual

- effects on myoblast proliferation and differentiation. *J Cell Physiol* 159: 387–398
- Rosenthal SM, Cheng Z-Q (1995) Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal myoblasts. *Proc Natl Acad Sci USA* 92: 10307–10311
- Scott CD, Baxter RC (1987) Purification and immunological characterization of the rat liver insulin-like growth factor-II receptor. *Endocrinology* 120: 1–9
- Semsarian C, Wu M-J, Ju Y-K, Marciniak T, Yeoh T, Allen DG, Harvey RP, Graham RM (1999) Skeletal muscle hypertrophy is mediated by a Ca^{2+} -dependent calcineurin signalling pathway. *Nature* 400: 576–581
- Siebert PD, Larrick JW (1992) Competitive PCR. *Nature* 359: 557–558
- Slavkin HC (1988) Gene regulation in the development of oral tissues. *J Dent Res* 67: 1142–1149
- Wada J, Liu ZZ, Alvares K, Kumar A, Wallner E, Makino H, Kanwar YS (1993) Cloning of cDNA for the α subunit of mouse insulin-like growth factor I receptor and the role of the receptor in nephric development. *Proc Natl Acad Sci USA* 90: 10360–10364
- Wang Z-Q, Fung MR, Barlow DP, Wagner EF (1994) Regulation of embryonic growth and lysosomal targeting by the imprinted *igf2/Mpr* gene. *Nature* 372: 464–467
- Yamane A, Mayo ML, Bringas Jr P, Chen L, Huynh M, Thai K, Shum L, Slavkin HC (1997) TGF- α , EGF, and their cognate receptor are co-expressed with desmin during embryonic, fetal, and neonatal myogenesis in mouse tongue development. *Dev Dyn* 209: 353–366
- Yamane A, Bringas Jr P, Mayo ML, Amano O, Takahashi K, Vo H, Shum L, Slavkin HC (1998a) Transforming growth factor α up-regulates desmin expression during embryonic mouse tongue myogenesis. *Dev Dyn* 213: 71–81
- Yamane A, Takahashi K, Mayo M, Vo H, Shum L, Zeichner-David M, Slavkin HC (1998b) Induced expression of MyoD, myogenin and desmin during myoblast differentiation in embryonic mouse tongue development. *Archs Oral Biol* 43: 407–416
- Yamane A, Mayo M, Shuler C, Crowe D, Ohnuki Y, Dalrymple K, Saeki Y (2000) Expression of myogenic regulatory factors during the development of mouse tongue striated muscle. *Archs Oral Biol* 45: 71–78
- Yoshiko Y, Hirao K, Sakabe K, Seiki K, Takezawa J, Maeda N (1996) Autonomous control of expression of genes for insulin-like growth factors during the proliferation and differentiation of C2C12 mouse myoblasts in serum-free culture. *Life Sci* 59: 1961–1968
- Zhao J, Araki N, Nishimoto SK (1995) Quantitation of matrix Gla protein mRNA by competitive polymerase chain reaction using glyceraldehyde-3-phosphate dehydrogenase as an internal standard. *Gene* 155: 159–165

(Received February 23, 2000 / Accepted April 21, 2000)