General Discussion

In the Part 1 and 2, I observed that mouse tongue myogenesis and synaptogenesis were almost completed at birth. Because suckling and swallowing are required for feeding and must begin immediately after birth, the completion of myogenesis and synaptogenesis in the mouse tongue, which is essential for suckling and swallowing, must be completed by birth. Recently, the myogenesis and synaptogenesis in masseter muscle are reported to finish 2 ~ 3 weeks after birth (Yamane *et al.*, 2001; Yamane *et al.*, in press). Because the masseter muscle mainly functions in the biting movement of the jaw and because the feeding behavior of the mouse begins to switch from suckling to biting approximately 2 ~ 3 weeks after birth (Kubota *et al.*, 1988), the completion of masseter myogenesis and synaptogenesis may not be necessary until the biting movement begins. I assume that the completion of myogenesis and synaptogenesis is closely related to the initiation of the functional requirement of each muscle.

In the Part 4, mouse tongue organ culture system for studying the differentiation of myoblasts was established. This organ culture system seems to have three main advantages in comparison with cell culture systems as follows. First, it is easy to maintain the original characteristics of tongue tissues. Second, it is possible to study the interactions among several tissues such as epithelial and muscle tissues. Third, it is potential to observe effects of cytokines such as growth factors without serum which contains a variety of cytokines. Thus this organ culture system seems to be a very useful tool for studying the roles of cytokines such as growth factors in the embryonic development of moue tongue including the myoblast differentiation and myofiber maturation.

In vivo results of IGF and their receptor expressions in the Part 3 and *in*

vitro results of endogenous and exogenous IGF-I in the tongue organ culture system in the Part 4 indicate that IGFs positively regulate the differentiation of tongue myoblasts through IGFR1. These results accord the previous results of myogenic cell lines such as C2 and L6 (Florini *et al.*, 1994, 1996). In the Part 4, however, stimulatory effect of exogenous IGFBP4 on the tongue myoblast differentiation was observed. On the other hand, in L6 myogenic cell line, IGFBP4 is reported to function only as an inhibitor of the differentiation induced by IGFs (Ewton and Florini, 1995; Ewton *et al.*, 1998). Furthermore, the inhibitory effect of des(1-3)IGF-I with reduced affinity for IGFBPs on the differentiation of tongue myoblasts was observed. All of the IGF-I analogue with reduced affinity for IGFBPs including des(1-3)IGF-I are reported to stimulate approximately 100 times more potent than native IGF-I in stimulating L6 myoblast differentiation and there is no report on inhibitory effect of des(1-3)IGF-I on the myoblast differentiation (Florini *et al.*, 1994, 1996). Thus the stimulatory and inhibitory effects of IGFBP4 and des(1-3)IGF-I, respectively, on the differentiation of tongue myoblasts may be unique characteristics of IGF-IGFBP regulatory mechanism in tongue striated muscles, although there are differences between cell and organ culture systems.

In the Part 4, exogenous IGF-I stimulated the myoblast differentiation and induced the expression of IGFBP4, 5 and 6 in the cultured tongue. Low concentration of des(1-3)IGF-I promoted tongue myoblast differentiation, whereas high concentrations of this analogue inhibited it due to toxic reactions such as the abnormal shape of tongue, low cell density and low staining intensity with hematoxylin and eosin. From these results, I propose one hypothesis on the function of IGFBPs in the differentiation of mouse tongue myoblasts as follows. IGFBPs

function to control the concentration of free IGFs within an optimal or normal range for the differentiation. In the case that the concentration of free IGFs becomes lower than the optimal concentration, IGFBP-IGF complex releases IGFs to raise the concentration of free IGFs. In the case that the concentration of free IGFs becomes higher than the optimal concentration, IGFBPs bind to free IGFs to decrease the concentration of free IGFs. Since this mechanism functioned to control the concentration of exogenous IGF-I within the normal or optimal range, only the stimulatory effects of exogenous IGF-I were observed. However, this mechanism was not able to control the concentration of des(1-3)IGF-I with the reduced affinity to IGFBPs. Since the present low concentration of des(1-3)IGF-I was within the normal or optimal range for the differentiation, it stimulated the tongue myoblast differentiation. On the other hand, the present high concentration of this analogue exceeded the normal range, thus it inhibited the differentiation by inducing toxic reactions.

In conclusion, the myogenesis and synaptogenesis in the mouse tongue striated muscle are almost competed at birth. They are controlled and progressed normally by the IGF-IGFBP regulatory mechanism and, thereby, are able to finish at birth to meet early functional demands such as suckling, swallowing and respiration immediately after birth.