D111 The Cloning and Expression Pattern of Cathepsin D During Salamander(*Hynobius leechii*) Limb Regeneration

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During salamander limb regeneration, various kinds of lysosomal hydrolases and proteases are known to be involved in the process of tissue demolition and liberation of dedifferentiating stump cells from their surroundings. In regenerating salamander limbs, we have found that cathepsin D, a lysosomal aspartic protease, activity was significantly elevated during dedifferentiation. Here, we report the cloning of a cathepsin D cDNA in the Korean salamander, *Hynobius leechii* and the expression profile of cathepsin D mRNA in the course of limb regeneration. By whole mount *in situ* hybridization technique, the signal of cathepsin D transcript was shown to be localized at the vicinity of amputation surface under the wound epidermis at 2 days after amputation. The intensity of signal increased at dedifferentiation stage (4-8 days after amputation), but it declined thereafter when blastema forms. RA(retinoic acid) treatment caused the elevation of expression and, interestingly, the signal was also detected at the posterior junction of stump and blastema.
In conclusion, increased level of cathepsin D expression appears to be responsible for the elevation of cathepsin D activity during dedifferentiation period in the regeneration process of salamander limbs.

D112 Role of Hyperpolarization Attained by Activation of the Potassium Channels in Chick Myoblast Fusion

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Calcium influx is known to be a prerequisite for myoblast fusion. However, little is known about the channels that are responsible for calcium influx in early myoblasts. Previously, we showed that the hyperpolarization generated by reciprocal activation of stretch-activated channels and K_{Ca} channels is involved in the calcium influx that triggers myoblast fusion (Shin *et al.* (1996) *Dev. Biol.* 175:14-23). We also showed that a high level of K_{Ca} channels is expressed in early stage of myogenesis and the activation of K_{Ca} channels hyperpolarizes membrane potential. In this study, we examined whether the increase in membrane fluidity, that is known to occur prior to myoblast fusion, promotes calcium influx by hyperpolarization of membrane potential. When linoleic acid, an agent to increase membrane fluidity, was treated to myoblasts, the membrane potential of myoblasts was dramatically hyperpolarized. This hyperpolarization was most likely mediated by activation of K_{Ca} channels and other tetraethylammonium (TEA)-resistant potassium channels since the hyperpolarization was reversed upon treatment of TEA. Moreover, the treatment of linoleic acid increased the intracellular calcium level and promoted the myoblast fusion. These results suggest that the increase in membrane fluidity observed prior to myoblast fusion provides a driving force for calcium influx by attaining hyperpolarization of membrane potential.